3rd Iberoamerican Congress
4th Latin American Congress
2nd International Symposium on Lignocellulosic Materials

Biorefineries

Science, Technology and Innovation for the Bioeconomy

November 23 to 25, 2015, Concepción-Chile

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Organization

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Sunday 22th

18:30 Welcome cocktail (at the premises of UDT)

Monday 23th

09.00 – 09:30 Opening ceremony
Welcome words: Alex Berg, Chairman

09:30 – 10:15 **Plenary session: Piergiuseppe Morone, University of Rome, Italy**
Making the transition towards a biobased economy

10:15 – 10:45 Coffee break

Economic, environmental and social sustainability

10:45 – 11:20 Keynote Speaker
Orlando Rojas, Aalto University, Finland
Valorization of Biorefinery Streams by the Development of Advanced Materials from Lignin and Nano/micro-celluloses

11:20 – 11:40 **Sustainability analysis of lignocellulosic bioethanol production and electricity generation. Western Mexico case study**
Arturo Sánchez, National Polytechnic Institute - IPN, Mexico

11:40 – 12:00 **Techno-economical feasibility assessment of butanol production from lignocellulosic biomass**
Julián Quintero, Pontifical Catholic University of Valparaíso, Chile

12:00 – 12:20 **Kinetic model for the oxidation of hazardous compounds in an industrial effluent from forest biomass processing**
Fernando E. Felissia, National University of Misiones - UNaM, Argentina

12:20 – 12:40 **Contribution of chemurgy to the advancement of biorefinery in the context of circular economy - a polish perspective**
Janusz Golaszewski, University of Warmia and Mazury in Olsztyn, Poland

12:40 – 13:40 Lunch

13:40 – 14:15 Keynote Speaker
Ronalds Gonzalez, NC State University, USA
Challenges and opportunities in the bio-refinery economy

14:15 – 14:35 **Systemic view of bio refineries linked to the production of food and energy**
Jorge Antonio Hilbert, National Agricultural Technology Institute - INTA, Argentina

14:35 – 14:55 **Productivity and costs of two low-investment biomass harvesting systems applied in a situation of mixed forest of semi-natural origin**
Patricio Carey, Austral University of Chile - UACh, Chile

14:55 – 15:15 **Assessment of supply chains for pre-treatment of forest residues in Chile**
Tobias Zimmer Karlsruhe Institute of Technology - KIT, Germany

15:15 – 15:35 **Lignocellulosic biofuels co-production and co-generation using integrated biorefineries. A solution to the treatment of agro-industrial wastes**
Arturo Sánchez Carmona, National Polytechnic Institute - IPN, Mexico

15:35 – 16:05 Coffe Break

Chemical conversion

Keynote Speaker
Franck Dumeignil, University of Lille, France
EuroBioRef: Designing next generation biorefineries

2G Bioethanol Biorefinery using sugarcane lignocellulosic biomass residues
Francisco Girio, National Laboratory of Energy and Geology - LNEG, Portugal

Where are we with green Biorefineries?
Rafal Bogel-Lukasik, National Laboratory of Energy and Geology - LNEG, Portugal

Conversion of inulin-containing and lignocellulosic biomass to the platform chemical 5-hydroxymethylfurfural in water
David Steinbach, Karlsruhe Institute of Technology - KIT, Germany

Lipid extraction from *Chlorella vulgaris* using electromagnetic field
Catalina Bernal López, University of Valle, Colombia

Keynote Speaker
Johanna Buchert, Natural Resources Institute, Finland
Research as tool to create value-addedforest- based bioeconomy

Development of selective fractionation methods for the integrated upgrade of corn cobs
Florbela Carvalheiro, National Laboratory of Energy and Geology - LNEG, Portugal

Using magnetic resonance imaging to monitor process flows of multiphase systems
Robert Powell, University of California, Davis, USA

Solid state fermentation of chemically untreated sugarcane bagasse for fungal production of single cell oil as biodiesel feedstock
Mahesh Khot, Savitribai Phule Pune University, India

Bioethanol

16:05 – 16:40 Keynote Speaker
Valdeir Arantes, University of Sao Paulo, Brazil
The potential for the fractionation of lignocellulose for integrated production of biofuel and value-added products: A case of bioethanol, food additives and nanocellulose

16:40 – 17:00 **Improvement of the lignocellulose hydrolysis by use of auxiliary enzymes**
Oriana Salazar, University of Chile, Chile

17:00 – 17:20 **The influence of sono-assisted alkaline pretreatment of sugarcane bagasse in enzymatic hydrolysis for cellulosic ethanol production**
Luiz Pereira Ramos, Federal University of Parana, Brazil

17:20 – 17:40 **Comparison between microwave and conduction-convection heating for autohydrolysis processing in the production of high added-value compounds and substrates for biofuel under the biorefinery concept**
Héctor A. Ruiz, Autonomus University of Coahuila, Mexico

17:40 – 18:00 **Different strategies for lignocellulose sugars conversion into ethanol from phosphoric acid steam exploded olive tree pruning**
Mercedes Ballesteros, Centre for Energy, Environment and Technology - CIEMAT, Spain

Thermochemical conversion

Keynote Speaker
Jaap Kiel, University of Twente, The Netherlands
The role of thermochemical conversion in biorefinery concepts: not just combustion

Effects of biomass source on the composition and reactivity of thermochemical reaction products
Steve Kelley, North Carolina State University, USA

Upgrading of low-grade biogenic feedstock by innovative screw pyrolysis
Marco Tomasi Morgano, Karlsruhe Institute of Technology - KIT, Germany

Pyrolysis of mixtures of concentrated spent pulping liquor and sodium formate to produce a phenolic bio-oil
Adriaan van Heiningen, University of Maine, USA

Selective production of formic acid from aqueous phase bio-oil by catalytic oxidation using heteropoly acids (for bio-oil hydrodeoxygenation)
Mauricio Escobar, Technological Development Unit - UDT, Chile

Tuesday 24th

9:00 – 9:20 **Second generation bioethanol from *Eucalyptus globules labill* and *Nothofagus pumilio* using ionic liquids**
Maria Cristina Ravanal Espinosa, University of Chile, Chile

9:20 – 9:40 **Ethanol production from CMC and Avicel using ethanologenic *Escherichia coli* expressing a novel endoglucanase**
Inés Loaces, Biological Research Institute Clemente Stable - IIBCE, Uruguay

9:40 – 10:00 **Sequential thermochemical hydrolysis, enzymatic saccharification and fermentation to ethanol of stover from white corn with ethanologenic bacteria**
Alfredo Martinez, National Autonomous University of Mexico - UNAM, Mexico

10:00 – 10:40 Coffe Break

Catalytic hydrodeoxygenation of pyrolysis oil over nickel-based catalysts under H_2/CO_2 atmosphere
Wolfgang Olbrich, Karlsruhe Institute of Technology - KIT, Germany

Concept for combined heat and power production from wood via gasification followed by catalytic gas cleaning
Tim Schulzke, Fraunhofer UMSICHT, Germany

Activated biochar derived from agricultural residual biomass pretreated with alcaline agent
Luis Sebastian Romero-Hermoso Osorio, Scientific and Technological Bio-resource Nucleus - BIOREN, Chile

Microalgae

10:40 – 11:20 Keynote Speaker
Gabriel Acién, University of Almería, Spain
Integral utilization of microalgae: Production of biofertilizers

11:20 – 11:40 **Chilean Technological Consortium "Desert Bioenergy S.A."**
Laura Azócar, Scientific and Technological Bio-resource Nucleus - BIOREN, Chile

11:40 – 12:00 **Multi-scenario economic evaluation for a biorefinery based on microalgae biomass with application of anaerobic digestion**
Cristian P. Bravo-Fritz, Pontifical Catholic University of Chile, Chile

12:00 – 12:20 **High pressure biomass conversion processes for biofuels and chemicals production**
Pablo E. Hegel, National Council of Scientific and Technical Research - CONICET, Argentina

12:20 – 12:40 **Energy recovery through the anaerobic digestion of the residual microalgae biomass from a biodiesel production process**
Alvaro Torres, Scientific and Technological Bio-resource Nucleus - BIOREN, Chile

12:40 – 13:00 **Evaluation of technical feasibility of biogas upgrading using microalgae**
Leslie Meier Figueroa, Scientific and Technological Bio-resource Nu4cleus - BIOREN, Chile

13:00 – 14:00 Lunch

14:00 – 14:20 **Production of PHB production from glycerol waste by *B. xenovorans* LB400**
Pamela Villegas Pizarro, Federico Santa María Technical University - UTFSM, Chile

14:20 – 14:40 **Medium chain length production by *Pseudomonas fluorescens* and unrelated carbon source**
Diana Marcela Vanegas Hernández, Pontificia Bolivariana University, Colombia

14:40 – 15:00 **Life cycle assessment of biomethane from waste water algae: The All-Gas approach**
Daniel Maga, Fraunhofer Institute for Environmental, Safety and Energy Technology UMSICHT, Germany

15:00 – 15:40 Coffe Break

15:40 – 18:00 Poster presentation

20:00 Official dinner

Cellulose fibers and microfibers

Keynote Speaker
Gary Chinga-Carrasco, Paper and Fibre Research Institute - PFI, Norway
Kraft pulps from Eucalyptus and Pinus radiata - raw materials for nanocellulose production and novel bio-applications

Comparative analysis of commercial cellulases cocktails for the production of nanocrystalline cellulose
Germano Andrade Siqueira, University of Sao Paulo, Brazil

Biocomposites from microfibrillated cellulose and biodegradable polymers
Patricia Eisenberg, National Institute of Technology - INTI, Argentina

Cellulose nanofibrils from agro-industrial waste: Production and characterization
Fabiola Valdebenito, Scientific and Technological Bio-resource Nucleus - BIOREN, Chile

Characterization of *Eucalyptus* bark and its potencial use for fiber and cellulose nanofibrils
Miguel Pereira, University of Concepción, Chile

Biomass valorisation by heterogeneous catalysis: Ethylene glycol production via hydrogenolysis of cellulose using Pd-WXC/C catalyst
Antonio Aprigio da Silva Curvelo, Universidade de Sao Paulo, Brazil

Affibody functionalized bacterial cellulose tubes for bioseparation applications
Ilari Filpponen, Aalto University, Finland

The role of ligno-nanocellulosics in the biorefinery concept
Maria Soledad Peresin, VTT, Finland

Wednesday 25th

	Natural polyphenols	Carbohydrates and cellulosic fibers applications
9:00 – 9:40	Keynote Speaker **Antje Potthast, University of Natural Resources and Life Sciences - BOKU, Austria** Analyzing biorefinery product streams - challenges, requirements and (some) solutions	Keynote Speaker **Frank Miletzky, Papiertechnische Stiftung, Germany** Between two stools: the paper industry in a change
9:40 – 10:00	**Mild chemical modification of acetosolv lignin from several Chilean sources** Danny Eugenio García Marrero, Technological Development Unit - UDT, Chile	**Ethanol-water fractionation of wheat straw and saccharification of the cellulosic residue** Juan Carlos Villar, National Research Institute and Agricultural and Food Technology - INIA, Spain
10:00 – 10:20	**Use of organosolv lignin modified by alkaline catalyst in adhesive resins: Evaluation of the behavior** Marcela Norambuena, Biotechnology Center, Chile	**Determination of hemicellulose extraction conditions from alkaline-sulfite pretreated sugar cane bagasse with a crude enzymatic extract from *Bacillus pumilus*** Maiara Paparele dos Santos, Universidade de São Paulo, Brazil
10:20 – 10:40	**The potential of cork-containing barks for biorefineries: structural and chemical features, fractionation and conversion routes for materials and chemicals** Helena Pereira, University of Lisbon, Portugal	**Addition of poly-electrolytes on recycled fiber for paper sheet formation** José Turrado Saucedo, University of Guadalajara, Mexico
10:40 – 11:20	Coffee break	
11:20 – 11:40	**Novel synthetic and natural adhesives: performance evaluation using ABES** Cecilia Fuentealba, Technological Development Unit - UDT, Chile	**Biodegradable films formed by polyelectrolyte complexes of xylan and chitosan** Paulina Mocchiutti, National University of Litoral, Argentina
11:40 – 12:00	**Biochar-based materials for the sustainable catalysis and photocatalysis** Juan Matos Lale, Technological Development Unit - UDT, Chile	**Foamed packaging made from cellulose acetate** Rafael Erdmann, Fraunhofer UMSICHT, Germany
12:00 – 12:20	**Poster award ceremony**	

Making the transition towards a biobased economy

Piergiuseppe Morone, University of Rome, Italy

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1. Introduction

Significant changes are expected to occur in the near future. Most notably, the world population which stands on 7.2 billion in mid-2014 is projected to increase by almost one billion people within the next decade, and to reach 9.6 billion by 2050 (FAO, 2015). At the same time, large and fast-growing economies (mainly China and India, which in spite the holdback due to the financial crises, are still growing at significant rates of 6.8 and 7.4, respectively) will experience increasing wealth. A major effect of these two trends is higher consumption and demand for food, manufactured goods and energy sources – all of which add pressure to the world economic system and the environment. Overarching these issues are the threat of climate change and the mounting concern on managing sustainably the increasing amount of waste produced worldwide.

Bearing these facts in mind, a transition from a society heavily based on fossil-fuels towards a bio-based one, seems a desirable and much needed feat. Hence, the question to be addressed is whether socio-technological conditions are mature to embrace such a sustainable transition, or not.

2. A history of changes: long waves and technological revolutions

With this background in mind, the discussion developed in this short essay takes a neo-Schumpeterian perspective which is grounded in the long wave theory first proposed by the Russian economist Nikolaj Dmitrievič Kondratiev (1925). Kondratiev waves (named after the Russian economist by Schumpeter) are cycle-like phenomena in the modern world economy. Each long wave ranges from forty to sixty years and the cycles consist of an upswing phase (characterised by high sectoral growth) and a downswing phase (characterised by a significant slow down in growth). Specifically, Kondratiev identified three phases in the cycle - expansion, stagnation, recession - and focused on prices and interest rates, seeing the ascendant phase as characterized by an increase in prices and low interest rates, while the descendent phase as characterised by a decrease in prices and higher interest rates.

Kondratiev's ideas were taken up by Joseph Schumpeter at the end of the 1930s. In his seminal contribution on Business Cycles, Schumpeter (1939) suggested that these waves arise from the clustering of basic innovations that are used by skilled individuals to create neuer Kombinationen. This initiates a technological revolution that creates a new and fast growing leading economic sector which, in turn, will facilitate an upswing in the long wave. Innovators who have successful applications are considered able to exploit a temporary monopoly. In contrast, radical innovations are discouraged during the upswing because existing technologies already generate ample earnings. Next, after some time, a swarm of imitators saturates the market, and consequently, margins will erode and earnings will diminish. By then, economic development will slow down and the long wave will enter the downswing phase (Castellacci, 2006).

Following this line of reasoning, neo-Schumpeterian long wave theory flourished in the 1980s developing a representation of the capitalist system as constituted by two related sub-systems, the techno-economic and the socio-institutional. It is the joint evolution of these sub-systems that determines the 'mode of development', and consequently the rise and fall of long waves in the long run. In particular, neo-Schumpeterian long wave theory explains countries' long run macroeconomic performance in terms of the diffusion of interrelated radical innovations, which is the technological paradigm, to the whole economic system of families. When a new technological paradigm emerges, it gives a big impulse to the techno-economic sub-system to adopt the new best practice technology with high profit prospects (Castellacci, 2006).

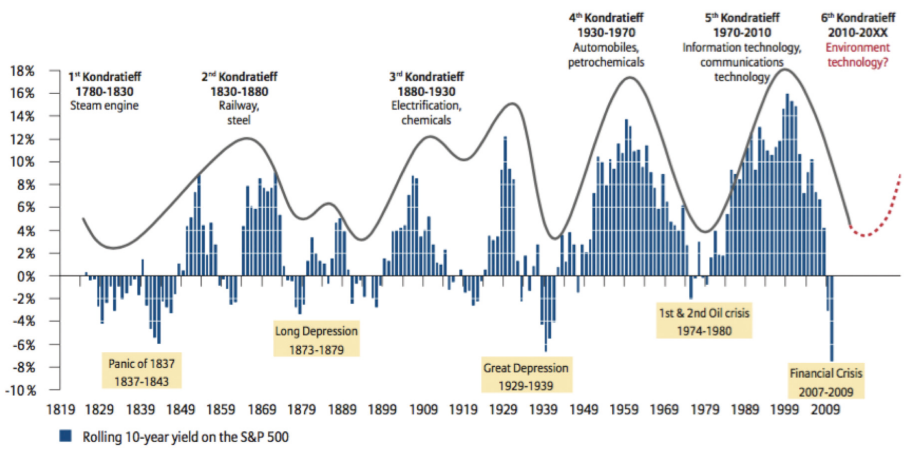
Over the last decade there has been considerable progress in historical economics (Freeman and Louçã, 2001) and in the history of technology (Perez, 2002), and numerous investigations of the relationship between technological innovation and economic cycles led to the identification of the following five technological waves: (1) The Industrial Revolution (1780-1830); (2) The Age of Steam and Railways (1830-1880); (3) The Age of Steel and Heavy Engineering (1880-1930); (4) The Age of Oil, Electricity, the Automobile and Mass Production (1930-1970), and; The Age of Information and Telecommunications (1970-2010).

3. The transition towards sustainability

Following this taxonomy, each new wave initiates with a major economic crisis and stagnation, which spurs from the exhaustion of an old dominant technological regime and leads to the emergence of a new paradigm[1]. In this perspective, the economic crisis in 2007–2010 might be a result of the coming end of the 'wave of the Information and telecommunications technological revolution'. Some authors have started to predict what the sixth wave might be (e.g. Moody and Nogrady, 2010; Allianz, 2013) forecasting that it will be driven by resource efficiency and clean technology (figure 1).

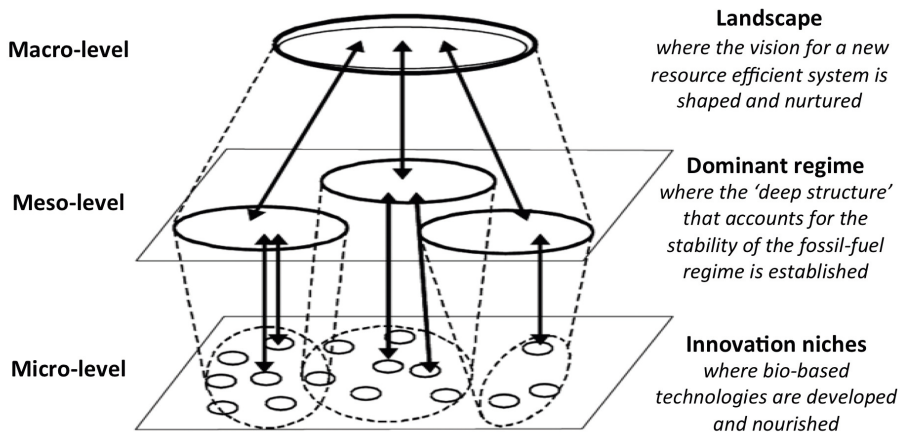
This brings us back to the beginning of this short essay; in fact, there is a growing consensus around the idea that a revolution spurred by expanded use of renewable bio-based products and bioenergy might significantly reshape the world economy over the coming years. In this respect, present and future technological achievements in green chemistry and biotechnologies might represent the cluster of radical innovations needed to start the upswing phase of a sixth long wave. This would mark a transition from a fossil-fuel based economy to a bio-based one, thus providing new opportunities for sustained (and sustainable) growth across the world.

Following this neo-Schumpeterian perspective, the fundamental question which still needs to be addressed is what could make this paradigm shift happen. To answer this question we shall refer to the multi-level perspective to the socio-technical transition theory (Geels, 2004; Geels, 2011). In a nutshell, a transition from a fossil-fuel regime to one based on biomasses will occurs if innovation niches (i.e. the 'micro level' where new green technologies are developed and nourished) and the socio-technical landscape (i.e. the 'macro level' where the vision for a new resource efficient system is shaped and nurtured) simultaneously exert pressure on the incumbent technological regime (i.e. the 'meso level' forming the 'deep structure' that accounts for the stability of the existing fossil-fuel system and referring to the semi-coherent set of rules that orient and coordinate the activities of the social groups that reproduce the various elements of such socio-technical system). The alignment of micro and macro sources of pressure might ultimately determine a socio-technical transition where a new bio-based technology replaces the incumbent fossil-fuel regime (figure 2).



Source: Adapted from Allianz (2010)
Figure 1 Kondratieff cycles – long waves of prosperity
Rolling 10-year yield on the S&P 500 since 1814 till March 2009 (in %, p. a.)

[1] This was the case in the Panic of 1837, the Long Depression of 1873, the Great Depression triggered in 1929 and the oil crises of 1974 and 1980.



Source: Adapted from Geels (2002)

Figure 2 Multi Level Perspective

4. Conclusions

It is widely agreed that materials and energy sources are fast approaching their physical limits. At the same time, the amount of waste produced under the current system seems to be reaching a climax. This is all magnified by the demographic and economic trends briefly discussed in the introduction. Whether the period of turmoil following the financial crisis has reached an end is still an open question, yet long-term perspective seems to militate in favour of the beginning of a new long wave of sustained (and sustainable) growth and, although the main and supporting roles in this 6th Kondratieff cycle have not been clearly assigned yet, the roots seem already to have taken hold. In this short essay we took the view that the new long wave will be characterised by a transition towards a sustainable bio-based economy. For this to happen technological niches (i.e. the locus where new radical innovations are developed) must reach maturity and sufficient pressure must be exerted from landscape actors (i.e. all those stakeholders acting, at various levels and through different channels, in the society) towards the fossil-fuel socio-technical regime. This, in turn, will outline a shared view for the change to occur.

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Oral Presentations

Monday 23th

Economic, environmental and social sustainability

Valorization of biorefinery streams by the development of advanced materials from lignin and nano/micro-celluloses

Orlando Rojas, Aalto University, Finland

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Abstract
Biopolymers cleaved from the fiber cell wall, including lignin and cellulose in various assembled forms, such as micro/nanofibrils (MNFC) as well cellulose nanocrystals (CNC), are available as main or side streams in biorefinery platforms. They can be used in the development of advanced materials by directing and engineering their re-assembly into multifunctional systems. This includes MNFC/CNC films with strength and barrier capabilities, coatings, etc. as well as composites with outstanding mechanical and thermal performance. Such composites may display functionalities such as high surface area, porosity and chemical and mechanical tunnability. Biorefinery biopolymers can be integrated with other components to spun filaments for novel applications and thus to valorize its impact. Here we introduce some examples highlighting their prospects.

Introduction
Nature organizes fibers in a highly hierarchical structure, encompassing a wide range of size domains, from the nano to the meter scales. Such structures can be utilized in advanced 2D and 3D materials. One example is that of cellulose films and nano/micro-papers, which have been extensively studied over the past decade. The use of cellulose nanocrystals (CNC) in high performance coatings is very attractive not only because of their properties but also because they may provide a platform for novel, functional assemblies. Likewise, nonwoven mats formed by ultra-thin fibers with diameters in the micron and nano-scales have considerable high surface area-to-volume ratio, superior mechanical properties and are very versatile in terms of surface functionality. Spinning into fibers and filaments has opened several opportunities for the manufacture of related materials, which will find applications in the fields of medicine, pharmacy, tissue engineering and nanocomposites.
Highly competitive compared to typical inorganic fillers such as carbon nanotubes, hydroxyapatite, gold, silver, clay, or silica, CNCs are strong and highly resistant to heat, wear, erosion and corrosion. It is also light weighted and relatively inexpensive. The aspect ratio of the nanocrystals (L/d, where L = length and d = diameter) can vary from 1 to 100, depending on the source and strongly influence their structuring and thus the properties of composite systems that use CNC as reinforcing phase. The aspect ratio also defines the values of the percolation threshold which markedly influences the mechanical properties of the final composites. This is attributed to the fact that a high interfacial area and a high degree of dispersion are reached when the nanocrystals physically interact with the continuous phase. However, a key issue in order to gain the benefits of the extraordinary properties of CNC in highly ordered structures, is a well-controlled alignment and distribution of CNC within the matrix. This aspect is usually challenge since CNC tends to agglomerate in non-polar matrices, and what complicates the scene even more is that low interfacial compatibility between the phases leads to poor composite mechanical properties.

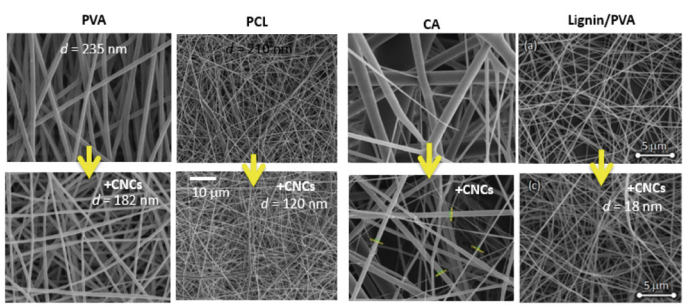

Figure 1. Composite fibers spun with or without reinforcing nanocellulose. Different polymer matrices are shown: PVA, PCL, CA and lignin/PVA.

Many efforts have been devoted to mimic bio-composites by blending cellulose from different sources with polymer matrices such as polyolefins, polyamides, polyesters, polyurethanes, polypeptides, polysaccharides. In the case of incorporation of hydrophilic nanocellulose to non-polar matrices, several surface chemical modification techniques have been applied. The effect of nanocellulose to reinforce different polymeric matrices in fibers is presented here (**Figure 1**). Finally, cellulose and its derivatives are presented as viable biointerface, due to their inherent non-toxicity, hydrophilicity, and chemical resistivity. In fact, the surface chemistry of MNFC can be appropriately adjusted to reduce non-specific adsorption.

Experimental
Cellulose nanocrystals were obtained by acid hydrolysis of different natural fibers, producing CNCs with differences in charge density, aspect ratio, and crystallinity. Thin films of CNCs were produced by film casting, spin coating and also by the Langmuir–Schaeffer (LS, surface lifting) (Habibi et al., 2010) methods. Ultrathin films of aligned CNCs were deposited on solid supports by using convective and shear-assisted techniques, with or without application of external electric fields (Hoeger et al., 2011, Csoka et al., 2011, 2012). A schematic illustration of the convective-shear assembly setup used for the production of coating films of CNCs is shown in **Figure 2** (Hoeger et al., 2011). It included a solid support and a withdrawal plate. The solid support was treated with PEI solution, which was placed in the horizontal stage of the assembly device; CNC suspension was added between the inclined plate and the substrate. Mono or multilayers of CNCs were formed upon shearing. Optionally, low-strength external AC electric fields were applied in order to improve the degree of alignment of the CNCs in the coating layer. The morphology and chemical composition of the CNC films were characterized by using atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS). Also, their swelling behavior and stability after treatment with aqueous and alkaline solutions were studied using quartz crystal microgravimetry (QCM). Ellipsometry and nanoindentation were used to measure key features of the films produced. Piezoelectric measurements were performed in contact mode by measuring the deflection of the film with a diamond AFM tip. In this case a 10 Hz sin frequency signal was employed using a signal generator and the peak-to-peak voltage was varied by 2.5 V units with a maximum value of 20 V (Csoka et al., 2014).

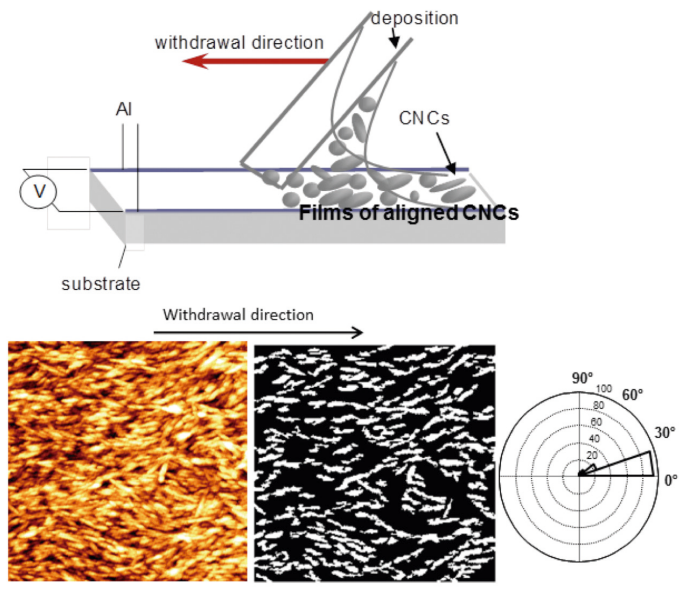

Figure 2. The shear/convective assembly technique (top) and resultant coating layers of CNC with high degree of alignment according to image analyses and respective polar plots of angle directors (bottom).

CNCs were used to reinforce the polymer matrix produced in the form of a fiber. Polymeric ultrathin fibers were produced by spinning upon application of electrostatic fields to the respective viscoelastic solution. To achieve high quality, continuous and defect-free electrospun composite fibers, several variables of the process were carefully controlled. This included the properties of the polymeric solution (viscosity, conductivity, surface tension, and elasticity), operational parameters (electric field strength, flow rate and pressure on the nozzle) as well

as environmental conditions (temperature, humidity, and surrounding gas/air velocity). The given polymeric solution was pumped through the syringe, forming at first a drop at the tip of the capillary which is solely held by surface tension of the solution. Once the electrical field was applied, free charges were generated in the solution, which responded to the electrical potential by migrating from the tip of the capillary to the collector, in opposite polarity direction. With increasing intensity of the electrical field the drop stretched forming a cone, known as Taylor cone. At a critical applied voltage value, the balance of forces between surface tension of the solution and electrostatic forces was disrupted and a jet of liquid was produced towards the opposite electrode, forming the composite mat of micro- or nano-fibers.

In the utilization of MNFC for biosensing platforms, we first tested the interactions of biomocules with MNFC with preadsorbed polymers (chitosan and CMC) (Orelma et al., 2011a). Following, we developed a platform for selective binding of biomolecules by using a random copolymer rich in primary amines, poly(2-aminoethylmethacrylate hydrochloride-co-2-hydroxyethyl methacrylate) (poly(AMA-co-HEMA)) that was first grafted from initiator-coated MNFC substrates via activators regenerated by electron transfer atom transfer radical polymerization (ARGET-ATRP). Finally, we carried out immobilization of acetylated-HWRGWVA peptide, which has specific binding affinity with IgG. In an additional approach with used MNFC-avidin biointerfaces to scavenge biotinylated molecules from solution as demonstrated by successful surface complexation of biotinylated anti-human immunoglobulin G (Biotin-anti-hIgG). The respective ligands covalently linked to the soft copolymer layers were characterized by X-ray photoelectron spectroscopy (XPS), water contact angle, ellipsometry, and atomic force microscopy (AFM). The extent of binding, binding affinity, and selectivity for target IgG molecules as well as the capability to minimize nonspecific interactions with other proteins were examined by fluorescence imaging, surface plasmon resonance (SPR), and quartz crystal microgravimetry (QCM).

Results and discussion

In order to produce films of MNFC, the convective assembly process was used. The degree of orientation in these films was comparable with those obtained after application of high electric fields. High CNC alignment was explained to depend on a balance of forces that included hydrodynamic (shear and drag), surface tension (capillary forces), and electrostatic interactions. Best alignment was obtained in cases where the shear force was dominant. A transverse Young's modulus, hardness and coefficient of friction of ca. 8.3, 0.38 and 0.51 GPa, respectively, were measured. Notably, the transverse Young's modulus was found to be in agreement with reported values predicted by molecular modeling and measured for single CNCs by using atomic force microscopy (Hoeger et al., 2011). Upon application of shear combined with an external electric field, the calculated dipole moments and Clausius–Mossotti factors allowed the determination of the critical frequencies, the peak dielectrophoresis as well as the principal orientation of the CNCs in the ultrathin films. As a result of the combination of shear forces and low electric field highly ultrathin films with controlled, unprecedented CNC alignment were achieved (**Figure 2**) (Csoka et al., 2011).

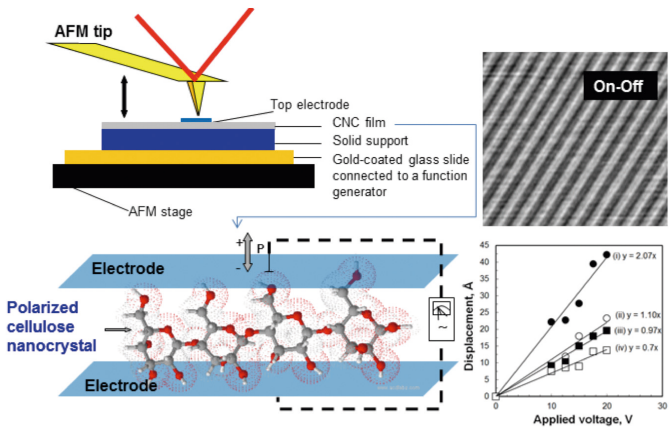

Figure 3. Schematic illustration of piezoelectric measurements using an AFM tip to determine deflection of CNC film upon application of cyclic voltages (on/off) and respective plots of displacements as a function of the applied voltage.

The relationship between polarization gradients and strain mechanics of the obtained films was examined. The piezoelectric response of the films was ascribed to the collective contribution of the asymmetric crystalline structure of the cellulose crystals. The magnitude of the effective shear piezoelectric constant (d25) of highly ordered CNC films was determined to be 2.1 Å/V, which is comparable to that of a reference film of a piezoelectric metal oxide (**Figure 3**) (Csoka et al., 2014).

Composite microfibers from polystyrene and CNCs were produced by electrospinning (Rojas et al., 2009). Surface porosity, unique ribbon-shapes, and the presence of twists along the fiber axis were observed in such composite microfibers. The reinforcing effect of CNCs was confirmed as the glassy modulus of electrospun microfibers increased with CNC load. This effect is explained to be the result of mechanical percolation of CNCs forming a stiff and continuous network held by hydrogen bonding (Rojas et al., 2009).

Nanofiber web composites based on biodegradable poly(ε-caprolactone) (PCL) and CNCs were also produced successfully via electrospinning. Chemical grafting of CNCs with short PCL chains was used in an attempt to obtain better compatibility between the hydrophobic PCL matrix and the hydrophilic CNC disperse phases. A significant increase in the storage modulus at all temperatures tested and nonlinear deformation strength properties were observed when CNCs were incorporated in the PCL nanofibers. The reinforcement effect of CNCs was demonstrated and explained in terms of differences in the fiber diameter, CNC loading, and crystallization processes (Zoppe et al., 2009).

When using hydrophilic matrices, such as polyvinyl alcohol (PVA), similar results were obtained: Reinforcing CNCs induced a 3-fold increase of the storage modulus of fully hydrolyzed PVA (Peresin et al., 2010a)(**Figure 4**). More relevant, however, was the observation of a stabilizing effect of the CNCs in the PVA matrix which could be otherwise compromised by water absorption, disrupting the hydrogen bonding within the structure. The reduction in tensile strength of neat PVA fiber mats as they were conditioned from low relative humidity (10% RH) to high relative humidity (70% RH) was found to be about 80%, from 1.5 to 0.4 MPa. When the structure was reinforced with CNCs, the reduction in strength was limited to 40%, from 2 to 0.8 MPa over the same range in relative humidity. More importantly, the CNC-loaded PVA fiber mats showed a reversible recovery in mechanical strength after cycling the relative humidity (**Figure 4**). Overall, humidity treatments of the composite PVA fiber mats induced significant enhancement of their strength as a result of the adhesion between the continuous matrix and the CNCs (Peresin et al., 2010b).

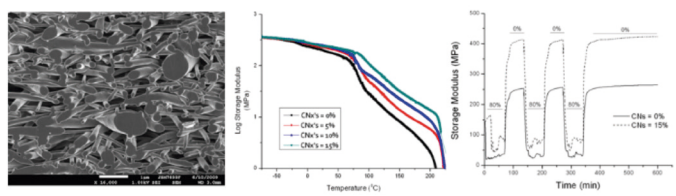

Figure 4. SEM image of cross-section of electrospun PVA/CNC (15%) nonwoven (left). The resultant storage moduli of electrospun nanofiber mats as a function of temperature for fully hydrolyzed PVA-98 is shown for different CNC loadings (0, 5, 10 and 15%) (center). The variation of storage modulus of unfilled and PVA fiber webs loaded with 15% CNC after cycling environmental relative humidity between 80 and 0% (at T = 25 oC) is also included (right).

Lignin-based fibers were produced by electrospinning aqueous dispersions of lignin, PVA and CNCs. Defect-free nanofibers with up to 90 wt % lignin and 15% CNCs were achieved. The properties of the aqueous dispersions, including viscosity, electrical conductivity, and surface tension, were examined and correlated to the electrospinnability and resulting morphology of the composite fibers (**Figure 5**). A ternary lignin–PVA–water phase diagram was constructed as a tool to rationalize the effect of mixing ratios on the dispersion electrospinnability and morphology of the resulting fibers. The thermal stability of the system was observed to increase with the addition of CNCs owing to a strong interaction of the lignin–PVA matrix with the dispersed CNCs, mainly via hydrogen bonding (Ago et al., 2012a).

Importantly, the size of the phase separated (lignin-PVA) domains was reduced by the addition of CNCs (**Figure 6**). When electrospun fiber surfaces were lignin-rich, the addition of CNCs affected their surfaces. In contrast, no surface effects were observed with the addition of CNCs in PVA-rich fibers. Here, the importance

Valorization of biorefinery streams by the development of advanced materials from lignin and nano/micro-celluloses

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of molecular interactions and phase separation on the surface properties of fibers from lignin and CNCs for the fabrication of new functional materials was highlighted.

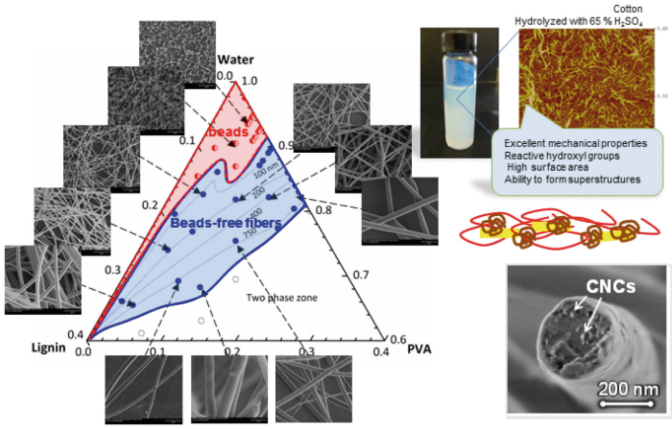

Figure 5. Ternary diagram indicating spinnability domains according to the composition of the precursor solutions (lignin, PVA, and water). The respective morphology after electrospinning is represented by half-filled circles to indicate beading, filled circles for bead-free fibers, and unfilled circles for a phase separation zone (not suitable for electrospinning). Lines are added as guides to the eye to identify the interfaces between the different domains and iso-radius contour lines are also included according to SEM images of spun fibers (representative SEM images are added around the ternary diagram). An illustration of a cross section of a composite single fiber with CNC (see AFM image and precursor aqueous suspension) is also included (Ago et al., 2012 a,b)

All-cellulose composite fibers were produced by electrospinning dispersions containing cellulose acetate (CA) and CNCs. The obtained fibers had typical widths in the nano- and micro-scale and presented a glass transition temperature of 145 ºC. The CA component was converted to cellulose by using alkaline hydrolysis to yield all-cellulose composite fibers that preserved the original morphology of the precursor system. Noticeable changes in the thermal, surface and chemical properties were observed upon deacetylation. Not only the thermal transitions of cellulose acetate disappeared but the initial water contact angle of the web was reduced drastically (Vallejos et al., 2012).

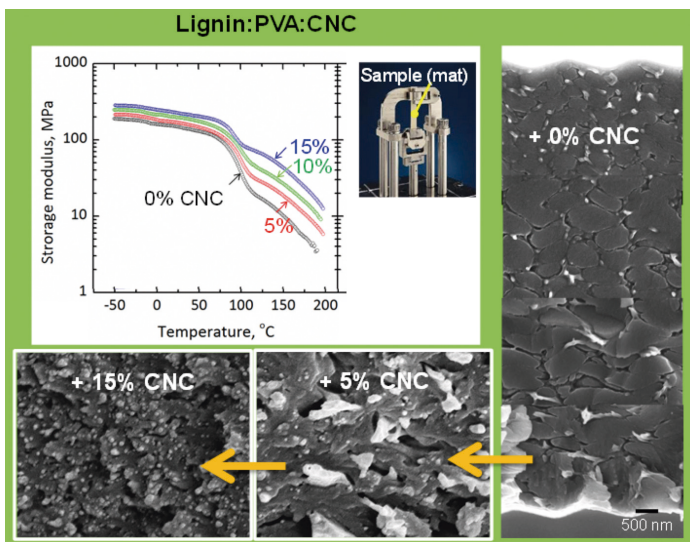

Figure 6. SEM images of cross sections of lignin/PVA films to understand phase separation in fibers. The addition of CNC to the aqueous dispersion used as precursor is observed to reduce the characteristic size of the phase-separated domains. Also included are the DMA profiles for the storage modulus of the fiber mats as a function of temperature that are shown for various amounts of CNC loading.

As far as the utilization of MNFC bio-interfaces, we installed amine groups by adsorption of chitosan from aqueous solution, which allowed for hIgG to physisorb from acid media and produce a functionalized substrate with high surface density (10 mg/m^2). Alternative installation of the carboxyl groups on cellulose substrate via carboxymethylated cellulose (CMC) adsorption from aqueous solution enhanced the physisorption of hIgG at acidic (adsorbed amount of 5.6 mg/m^2) and neutral conditions. hIgG adsorption from alkaline conditions reduced the surface density (Orelma et al., 2011a).

Further developments consisted on highly selective, novel biosensors based on MNFC carrying random poly(AMA-co-HEMA) copolymers with immobilized Ac-HWRGWVA peptides. The biosensors were found to have specific binding affinity with IgG while maintaining excellent nonspecific protein resistance. Several factors influence IgG binding including layer molecular composition and IgG concentration (**Figure 7**). Layers carrying short peptides with an AMA/HEMA molar ratio of 20:80 displayed excellent IgG specific binding while very low nonspecific BSA adsorption. Detection of IgG in PBSS buffer at concentrations as low as 0.05 mg/mL was achieved via QCM. A high affinity constants for the binding of Ac- HWRGWVA peptides to IgG were determined.

In a different approach, adsorption and chemical conjugation of avidin and its deglycosylated form, neutravidin, on films of MNFC were carried out. The installation of carboxyl groups on cellulose after modification with carboxymethylated cellulose (CMC) or TEMPO-oxidation significantly increase physisorption of avidins, which can be then covalently conjugated by using EDS/NHS coupling chemistries. The developed cellulose-avidin biointerfaces were able to scavenge biotinylated molecules from solution as demonstrated by successful surface complexation of biotinylated bovine serum albumin (Biotin-BSA) and anti-human immunoglobulin G (Biotin-anti-hIgG). Finally, we show that MNFC substrates carrying immobilized anti-hIgG are effective in detecting human immunoglobulin G (hIgG) from fluid matrices (Orelma et al., 2012a,b). The results highlight the potential on MNFC for detection and bioseparation.

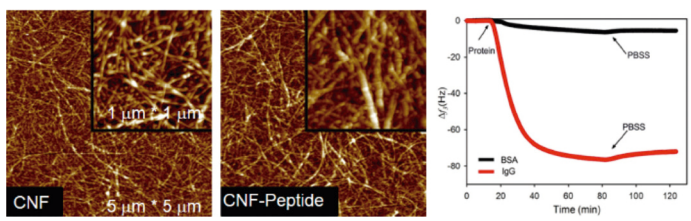

Figure 7. MNFC films carrying peptides. The QCM profiles on the right indicate the high selective of the functionalized MNFC towards IgG compared to BSA.

Conclusions

Nanocellulose films can be used as a coating technology to modify the surface of other materials to attain unique properties. Besides their unique mechanical strength, CNC films have a large piezoelectric response. CNCs induce a high electromechanical actuation and strain which changes as a function of CNC alignment. Such structures can result in high mechano-electrical energy transfer. Thus, the electromechanical properties of ultrathin films of CNC can be considered in potential applications given their flexoelectric behavior, biodegradability, and renewability. Moreover, defect-free nonwoven mats of composite nano/micro-fibers reinforced with CNC were produced. The produced composite nonwovens were very porous forming interconnected structures. Compared to fibers from the polymer matrix, the composite fibers displayed enhanced thermo-mechanical properties. Reinforcing CNC induces an increase of the storage modulus, which can be ascribed to the efficient stress transfer between CNC and the polymer in the fibers. It is demonstrated that nanocellulose can be used effectively to reinforce hydrophobic and hydrophilic fiber matrices and to produce unique structural properties, enabling new functionalities and properties. Such high porosity and surface-to-volume ratio of composite fiber mats can be of great potential in high performance applications, including separation membranes, scaffolds for tissue engineering, wound dressing materials, and nano-sensors. Finally, we demonstrate the use of activated MNFC films as solid support in biotechnologies. The developed MNFC-based biointerfaces can be used for the installation of peptide ligands or antihuman IgG. The proposed platform based on chemical conjugation of antibodies and peptides on MNFC is expected to open new venues for the development of diagnostics, immunoassays and bioseparation.

Acknowledgements

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Full mill model for dissolving pulp based biorefinery

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Abstract

Material and energy balances of a modern theoretical dissolving softwood kraft pulp mill, using pre-hydrolysis in water, have been established. The mill produces 1000 ADt/day and there is an energy excess in the mill that could be used to produce power. If lignin is removed with the LignoBoost process the recovery boiler is unloaded enabling an increased pulp production. With a lignin removal of 0.2 ton/ADt the pulp production could increase 8.5%. According to a sensitivity analysis the lignin price has the largest impact on the economic performance.

Introduction

Full mill simulation models for market kraft pulp production have been developed in different projects for European softwood, birch and eucalyptus [1]. The models have been used during a number of years both in research activities and in mill specific projects for trouble shooting and as a basis for evaluation of new process concepts. Recently, many mills have shown an interest in upgrading their process to production of dissolving pulp, which traditionally has a higher market price than the market pulp. To meet this trend a simulation model for dissolving pulp production using pre-hydrolysis kraft process was developed at Innventia. Material and energy balances have been established using the simulation program WinGEMS 5.0 and all data presented are for steady state operation. The model has been used to evaluate effects of a lignin removal combined with an increased pulp production.

Process description

The model reflects a greenfield dissolving softwood kraft pulp mill producing 1000 ADt/day. The design of the mill should consider high energy efficiency and pulp quality, low specific consumption of wood, chemicals and water, maximized production of energy from biomass, as well as low emissions and cost efficient solutions. The dissolving pulp mill model is based on data obtained from major pulp companies, equipment suppliers and laboratory trials, representing best available, commercially proven, technology. The process layout is shown in Figure 1.

Pre-hydrolysis stage

The fibre line consists of a separate pre-hydrolysis stage before the impregnation stage. Condensate from black liquor evaporation and steam are added to the pre-hydrolysis stage. After the pre-hydrolysis stage the liquor is removed from the chips and led to the evaporation plant. This is done to enable the use of the pre-hydrolysate for production of additional bio-refinery products which has not been evaluated in this study. In a real mill internal circulation of pre-hydrolysate would be used to decrease the amount of extracted pre-hydrolysate significantly. The pH in the pre-hydrolysis liquor remaining in the chip is 3-4 and if the temperature is increased rapidly, lignin condensation may cause high amounts of reject. To prevent this, cold white liquor is charged to the pre-hydrolysed chips at a temperature below 120°C.

Fibre line

The pre-hydrolysed chips are thereafter subjected to impregnation, cooking and oxygen delignification in double stages. The oxygen delignified pulp is bleached in a four stage sequence D1(EOP)D2P. EOP filtrate is recycled back to brown stock washing. With the use of wash presses both COD to effluent and effluent volume are kept low. Yield, charges etc. are presented in Table 1.

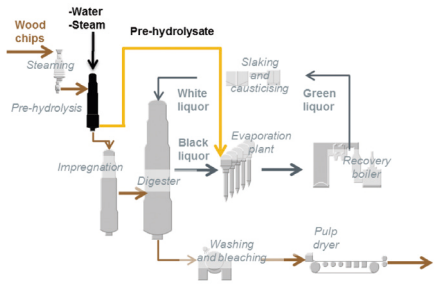

Figure 1 Schematic process layout for the dissolving pulp mill using pre-hydrolysis in water, the pre-hydrolysate taken to the evaporation plant.

Table 1. Summary of some key operating data for the fibre line compared with the market pulp mill model.

	Unit	Dissolving pulp	Market pulp
Pre-hydrolysis yield	%	82	-
Kappa number after cook		25	30
Combined cooking and pre-hydrolysis yield	%	37	47
Alkali charge on wood as effective alkali (NaOH)	%	22	20
Sulphidity (white liquor)	%	35	35
Kappa number after oxygen stage		5	12.5
Alkali charge as NaOH in oxygen delignification	kg/ADt	25	25

Results and discussion

Evaporation

With the pre-hydrolysate recycled back to the evaporation plant the load on the evaporation increases significantly. The pre-hydrolysate stands for approximately 30% of the total flow to the evaporation. Flow and dry solid content etc. are listed in Table 2.

Table 2. Flow and dry solid content in black liquor and pre-hydrolysis liquor to the evaporation plant.

	Unit	Dissolving pulp	Market pulp
Weak black liquor to evap., excl. spill	t/ADt	15	10
ditto dry solids content	%	15.3	16.4
Pre-hydrolysate to evap	t/ADt	6	-
ditto dry solids content	%	4.4	-
Strong black liquor, DS content incl. ash	%	80	80
Total evaporated (condensate)	t/ADt	18	9.1

Energy, steam and power production

The process is very energy efficient and the black liquor alone produces enough steam to satisfy the process consumption in the mill. Steam use is listed in Table 3.

Table 3. Steam use in process GJ/ADt.

	Dissolving pulp	Market pulp
Recovery and power boiler (including soot blowing)	1.5	0.91
Evaporation	6.8	3.5
Pre-hydrolysis stage	3.7	-
Other process (pulping, bleaching, pulp machine, chem. prep. etc.)	4.9	4.3
Total process consumption	17.0	8.6
Back pressure turbine	5.4	3.0
Condensing turbine	6.3	5.8
- Of which is cooled away in turbine condenser	4.0	3.6

There is also an excess of steam from the recovery boiler and power boiler that is utilized in a condensing turbine to produce “green” power, Table 4. Compared to the market kraft pulp mill the process steam consumption is much higher for the dissolving pulp mill. However, the possibility to produce power is higher due to the much lower overall yield.

Table 4. Power production and consumption in kWh/ADt.

	Dissolving pulp	Market pulp
Back-pressure part of the turbine	1451	801
Condensing part of the turbine	644	588
Sum production	**2094**	**1389**
Process	808	716
Sold	1286	673
Sum consumption	**2094**	**1389**

Non-process elements, NPEs

When evaluating biorefinery concepts the effects on the process chemicals as well as the energy use need to be addressed. However, in order to fulfil both process availability and sustainability requirements, a special focus has to be put on NPEs from the beginning of the concept development, as they will have large influence in biomass-based processes. NPEs are elements not taking part in desired chemical reactions within a given process stage. Data for the NPE content in wood and pre-hydrolysate is presented in Table 5 and result from laboratory Figure 2.

Table 5. Wood composition and NPEs to the evaporation with the pre hydrolysate.

	Al	Ba	Ca	K	Mg	Mn	P	Si
Wood composition (mg/kg dry wood)	9	6	630	373	126	50	55	15
% of incoming with wood to evaporation with pre-hydrolysate	22	43	42	53	45	43	48	43

In the pre-hydrolysis NPEs are dissolved and will follow the pre-hydrolysate to the recovery cycle. This decreases the content of NPEs in the dissolving pulp. For some elements, i.e. P and Si, the increased level in the recovery area will have an impact on the chemical balance in the mill. Silicon and phosphorous are elements accumulating in the lime cycle [2] and with the dissolving pulp concept the total amount to the lime cycle increases compared to a kraft market pulp mill. The free CaO had to be decreased to 85% (in kraft market pulp model is 90%) to not increase the lime make up need. As a consequence, the lime kiln load is higher compared to a market pulp mill (385 ton/ADt for dissolving pulp and 261 ton/ADt for market kraft pulp as lime from kiln).

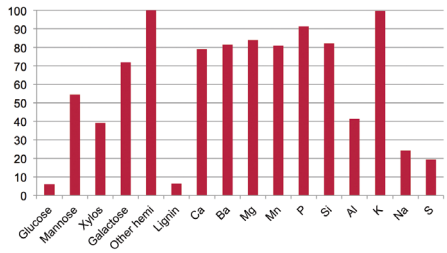

Figure 2. Dissolution of organic components and selected elements in the pre-hydrolysis stage in laboratory trials. The result is presented as percent dissolved of what was present in the wood. In case of more present in the pre-hydrolysate than in the wood a dissolution of 100% was assumed (other hemi and K).

Lignin removal

Lignin removal is an excellent way of unloading the recovery boiler that is often the limiting factor in a kraft pulp mill planning for an increased pulp production. In this model (a green field mill) the capacity of all unit operations is in balance and a pulp production increase, enabled by unloading the recovery boiler through the lignin removal, assumes an expansion of the other unit operations. This is in general less costly than a capacity increase in the recovery boiler.

Black liquor is acidified with carbon dioxide to pH 9-10 and lignin precipitates. The lignin is filtered and the lignin lean black liquor is returned to the evaporation plant. The lignin is thereafter re-slurried in an acidic stage with sulphuric acid at a low pH. Thereafter the lignin is displacement washed in a press filter. The wash liquor is returned to the evaporation plant. Process scheme of the concept is shown in Figure 3. [3]

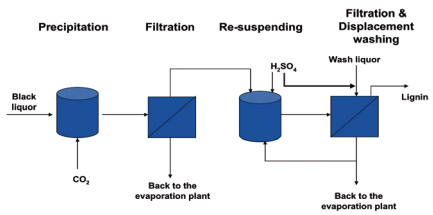

Figure 3. Schematic process layout of the LignoBoost concept.

The lignin removal was assumed to be 0.2 ton lignin /ADt. Lignin removal will have a large impact on the sodium sulphur balance, see Figure 4. The effect of lignin removal on the sulphur intake to the mill is significant. Some of the sulphur is removed with the lignin but the main part has to be taken out, normally with the ESP dust. The removal of ESP dust has to increase more than four times and the demand for sodium hydroxide make up increases consequently. In a real mill implementing a LignoBoost unit this is most likely considered as too high and the possibility to decrease other sulphur intake to the mill has to be investigated. The use of spent acid in the LignoBoost process other than elsewhere in the mill could decrease the overall intake of sulphur to the mill. There is also a lot of ongoing activity to decrease the need of sulphuric acid in the LignoBoost.Internal production of sulphuric acid is also an alternative. With the lignin removal the steam production decreases at the same time as the steam consumption in the process increases. However, as the pulp yield is relatively low for dissolving pulp production there is still an exccess of energy in the mill. The sold power decreases from 1 290 kWh/ADt to 700 kWh/ADt with the lignin removal.

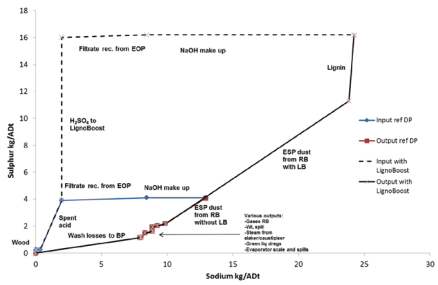

Figure 4. Sodium sulphur balance for dissolving pulp mill with and without lignin extraction (LB).

Conclusions

With a lignin production of 0.2 ton/ADt the pulp production could increase with 8.5%, see Table 6. The heat value of the black liquor will decrease but will still be high enough for stable and favourable combustion properties in the recovery boiler.

Table 6 Increased load on selected unit operations per hour (h), day (d) or year (y).

	Dissolving pulp	Dissolving pulp including lignin removal	Lignin removal, increased pulp production	% increase
Pulp production (ADt/d)	1000	1000	1085	8.5
Wood (t/d)	2563	2563	2780	8.5
Evaporated water (t/h)	747	774	839	12.4
Dry solids to recovery boiler (t/d)	2520	2324	2520	0.0
White liquor incl. makeup (m3/d)	5 150	5 151	5 586	8.5
Lime kiln load (t/d)	385	368	399	3.6
Lignin production (t/y)	0	65 387	70 912	

Yearly income from lignin and dissolving pulp minus variable production cost is shown in Figure 5. As a reference point the power price was 50$/MWh, dissolving pulp price 714€/ADt and the lignin price was 508€/ton lignin. Lignin is not a commodity product with a known market price and the number 750$(CAD)/ton lignin was taken from Browne [4] described as conservatively estimated at the plant gate. Lost income from power production due to lignin removal was considered as a production cost. The power price was varied from 50€/kWh ±100% where -100% corresponds to a mill without condensing turbine. The lignin price was varied ±50%. Dissolving pulp price was varied ±30% The additional income has to cover all necessary costs related to the lignin removal and increased pulp production i.e. equipment for lignin separation and increased pulp production, rebuilding the mill, land preparation, upgrading of the lignin to high value product if necessary etcetera.

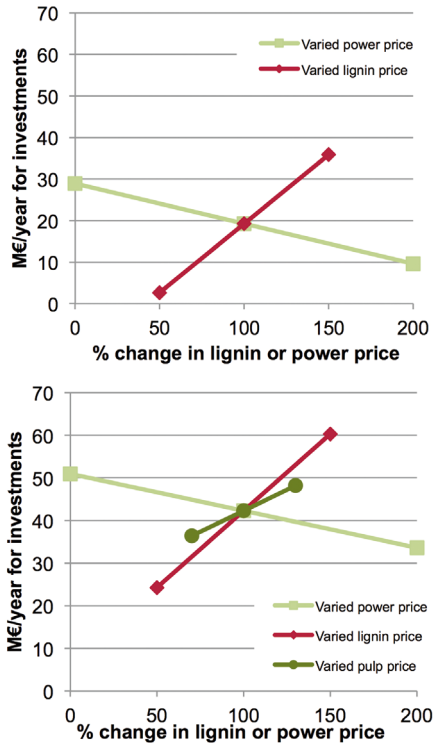

Figure 5. Effect of changes in lignin and electricity price on the economic capacity for investments after the variable costs has been covered. Left diagram is without increased pulp production, to the right with increased pulp production. Lost electricity production has been considered as a variable cost.

Of the studied parameters, the lignin price has the largest impact on the economic performance. An increased pulp production is enabled by lignin removal and is economic viable especially if the possibility to produce electricity is low.

Acknowledgements

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Lignocellulosic biofuels co-production and co-generation using integrated biorefineries. A solution to the treatment of agro-industrial wastes

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Abstract

This work presents a comparative analysis of an integrated biorefinery design (IBD) for biofuels (i.e. bioethanol, biogas and biohydrogen) co-production and electricity cogeneration against a conventional biorefinery design (CBD). The steady state mass and energy models as well as equipment capital were solved with state-of-the-art simulation tools for the 100 – 2,000 ton DB/day capacity interval and polysaccharides content (pc) (45% -60%) of the main feedstock. All other feedstocks were adjusted proportionally. The analysis is based on the total production cost (TPC) and the end-use energy ratio (EER) in mid-size agriculture sectors. The lowest TPC, 1.7 USD/L etOH, is obtained at 2,000 ton DB/day and 45% pc with 108% EER, in contrast with CBD's results at same feedstock conditions of $0.94 USD/L etOH and EER 87%.

Key words: integrated biorefinery design, conventional biorefinery design, end-use energy ratio, lignocellulosic bioethanol production, total production cost.

1. Introduction

Lignocellulosic materials are currently considered as an alternative for the production of biofuels that may alleviate the expected energy demands and contributing to the mitigation of the negative impacts caused by the production and use of first generation biofuels worldwide (e.g. [1]). Some other industrial wastes such as cheese whey and tequila vinasses, which are highly polluting, and produced in large quantities [2], can be considered as feedstock in a biorefinery scheme for producing co-products with high energetic potential (i.e. biohydrogen and biogas). This paper evaluates an integrated biorefinery design (IBD) from a techno-economic point of view, based on its total production cost (TPC) and its end-used energy ratio (EER), and then the comparison against those obtained for a conventional biorefinery plant (CBD) is presented.

2. Methodology

IBD is based on schemes already published in literature [3] employing wheat straw as the main feedstock. This design has seven processing stages: acid pretreatment, dark fermentation, enzymatic saccharification, alcoholic fermentation, separation, waste-water treatment and cogeneration. These stages are presented in Figure 1. In the acid pretreatment stage the wheat straw is depolymerized using an acid solution at a high pressure. After of this stage, two streams are derived: hydrolyzed and wet solids. Hydrolysates are sent to the dark fermentation stage where, after being neutralizing, are mixed with cheese whey to increase biohydrogen production [4]. The wet solids are sent to enzymatic saccharification where enzymes convert cellulose into glucose. The glucose rich stream is sent to alcoholic cofermentation stage where sugars are converted to ethanol, which is sent to the separation stage for purification. The residues from this stage are sent to the waste-water treatment stage together with the residues from the dark fermentation stage and a vinasses stream coming from a tequila factory. This stage produces biogas and some sediment that, together with the biohydrogen from dark fermentation are sent to the cogeneration stage in which high and low pressure steam and electricity are produced.

CBD is similar to IBD without the dark fermentation stage. CBD employs all C5 and C6 monosaccharides in the alcoholic cofermentation stage (See Figure 1). Note from Figure 1, that only red dotted lines show the differences of CBD with respect to IBD. The simulation software SuperPro Designer v8.5 was used to solve the mass and energy balances of the biorefineries, varying the capacity (2,000, 1,000, 500, 250 y 100 ton DB/day) and the polysaccharides content (pc) (60, 55, 50, 45%) of the main feedstock (i.e. wheat straw). All other feedstocks are adjusted proportionally to plant capacities. The Net Present Value (NPV) =0 is solved for TPC as a function of plant capacity against polysaccharides content and local financial conditions [5].

The EER defined as the ratio of energy produced (steam, electricity and chemical energy of ethanol) to the total energy consumed in the process [6], was calculated using data from the energy balances.

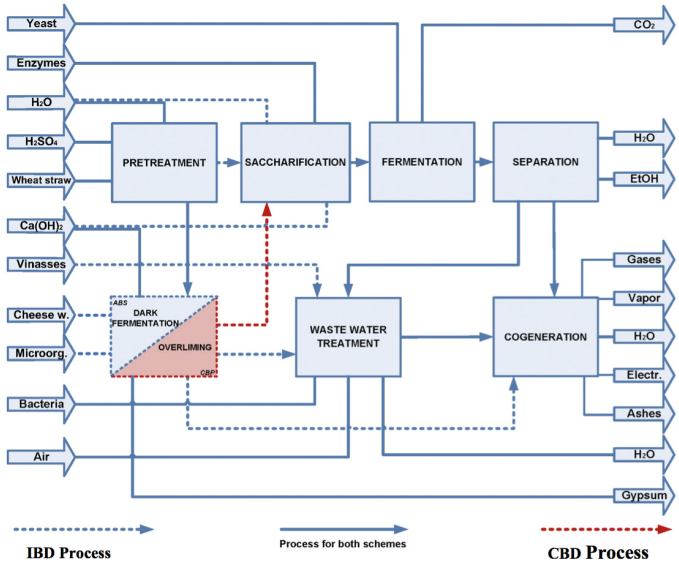

Figure 1. Process block diagrams for IBD and CBD.

3. Results and discussion

The sensitivity of TPC and EER values for IBD and CBD was explored for plant capacities of 100, 250, 500, 1,000 and 2,000 ton DB/day and feedstock polysaccharides content (i.e. wheat straw) of 45, 50, 55 and 60% w/w.

Table 1. Total production cost (USD/L etOH) for IBD scheme.

Plant capacity (ton DB/day)	Polysaccharides content (% w/w)			
	45%	50%	55%	60%
100	$4.69	$4.61	$4.59	$4.53
250	$2.91	$2.95	$3.01	$3.01
500	$2.18	$2.33	$2.41	$2.46
1000	$1.89	$2.00	$2.10	$2.17
1500	$1.76	$1.88	$1.97	$2.06
2000	$1.70	$1.81	$1.89	$1.97

Table 1 shows the TPC of IBD versus plant capacity and pc (%w/w). The smallest TPC value of $1.70 USD/L EtOH was obtained at 2,000 ton DB/day and 45% pc for IBD, and for CBD of $0.93 USD/L EtOH at the same conditions. A larger TPC for IBD is attributed mainly to larger capital expenditures to treat the cheese whey and vinasses in the hydrogen production process and waste-water treatment respectively. However, considerable smaller plants with different pc (% w/w) produce ethanol at relatively acceptable TPC.

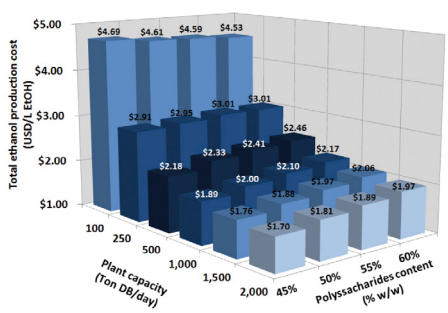

Figure 2. TPC versus plant capacity and polysaccharides content for IBD.

Figures 2 and 3 show that TPC has a quasi-linear section from 500 ton DB/day onwards and the expected linear dependence of total pc (% w/w). The region from 500 to 1,000 ton DB/day is a very attractive alternative to explore further since their technical and economic feasibility (see Table 1). Table 2 shows the EER versus plant capacity and pc (% w/w). The higher value obtained was 134% at 2,000 ton DB/day, 60% pc and for CBD of 159% at same conditions. Indicating energy self-sufficiency and a surplus, nevertheless bioethanol production is lower in IBD, thus profitability remained higher in CBD, due all its monosaccharides are used in alcoholic fermentation.

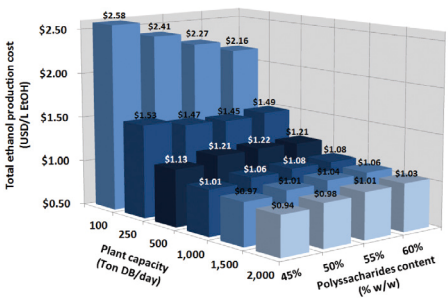

Figure 3. TPC versus plant capacity and polysaccharides content for CBD.

Table 2.End-use energy ratio for IBD scheme.

Plant capacity (ton DB/ day)	Polysaccharides content (% w/w)			
	45%	50%	55%	60%
100	72%	78%	84%	90%
250	88%	96%	103%	110%
500	96%	105%	112%	120%
1000	100%	109%	117%	125%
1500	103%	112%	119%	128%
2000	108%	117%	125%	134%

Figures 4 and 5 show that EER linearly increases, as it was expected at higher pc (%w/w). As a result of higher sugar concentrations, ethanol and co-products production increases, as well as LP and high HP steam, electricity and cogeneration. Equipment size and required amount of energy do not vary with pc (% w/w).

4. Conclusions

After mass and energy balances were solved and considering that co-products are sent to cogeneration stage to increase the electricity production, the minimum TPC and obtained for IBD was $1.70 USD/l EtOH with 108% of EER for the largest capacity considered (i.e. 2,000 ton DB/day) and cheapest feedstock (pc 40% w/w). A quasi-linear surface section was identified for plant capacities that may be suitable for mid-size economies. Therefore, the region from 500 to 1,000 ton DB/day is a very attractive alternative to explore further since their technical and economic feasibility. Although IBD TPC is 81% higher, it could be a solution for the treatment of agro-industrial residues since it reduces its high COD index and provides an income from it, which mitigates the investment of treatment equipment.

Nevertheless, the profitability does not consider environmental and social factors that could contribute to biorefinery sustainability; but the economic and energetic analysis could be a starting point for exploring new technologies and different process parameters that improve the relationship between environmental, social and economic impacts.

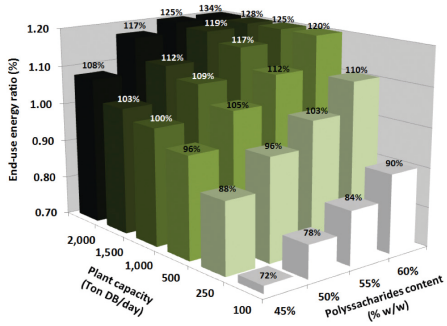

Figure 4. EER versus plant capacity and polysaccharides content for IBD.

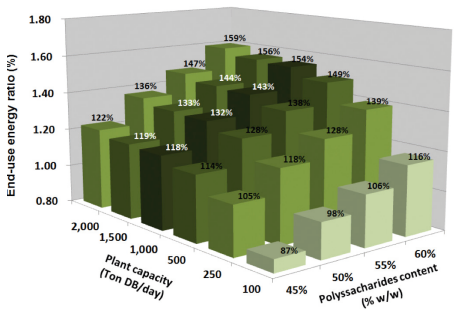

Figure 5. EER versus plant capacity and polysaccharides content for CBD.

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Contribution of chemurgy to the advancement of biorefinery in the context of circular economy – a polish perspective

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Abstract
The paper discusses the place of chemurgy within the framework of biorefinery and circular economy. Circular economy creates a sustainable and low-emission economy system, in which any marketable products will make a closed cycle of materials and energy flow. Chemurgy creates a link between chemistry and agricultural sectors by transforming agricultural raw materials to industrial bioproducts (biochemicals, biomaterials, biofuels, bioenergy). All processes involved in the conversion of agricultural raw material into industrial products tend to be channeled into the concept of a biorefinery, which seems to be a pivotal installation for the market of bioproducts, i.e. bioeconomy. The paper discusses R&D concepts of biorefinery processes in Poland, in which agricultural raw materials (SRWC, sugar beet) are processed, and lignocellulose and sugar refineries have been chosen as examples. In both cases, the biorefinery concepts correspond to the idea of circular economy, where multi-product processing is associated with the monitoring of matter and energy circulation in accord with the model 'from cradle to cradle'.

Introduction and objective
Chemurgy is a science on the industrial use of agricultural raw materials for chemicals. The relationship between agriculture and chemistry has long history that manifests itself in many aspects of agricultural production and processing. It began with the development of the first synthetic fertilizers. The Desfosses' research in 1828 proved that it was possible to conduct the reaction of atmospheric nitrogen with certain chemicals [1]. The artificial nitrogen fixation by the Haber-Bosch process, in which nitrogen (N2) from the atmosphere is converted into ammonium (NH4+) or nitrogen dioxide (NO_2), was implemented industrally in 1913 [2]. Further progress in the synthetic fertilizer industry led to the production of granulated, suspensed and time-release encapsulation fertilizers. Subsequently, the chemical industry introduced pesticides and fungicides, fuels and other chemicals for farm mechanization as well as vaccination in livestock production. Chemistry has also contributed to food industry providing such chemicals as saccharin, sweeteners, vitamins, minerals, and refrigerants and chlorofluorocarbons for food preservation. The efficient use of synthetic fertilizers by newly bred and highly productive cultivars of cereals and the application of other agrochemicals has improved agricultural output and in some regions of the world initialized the so called Green Revolution (1950s-1970s).
Currently, the connection between chemistry and agriculture is entering a new stage of development, which surpasses production of chemicals for use by agriculture and the food industry while placing more emphasis on a rational use of the chemical potential of agricultural raw products for the generation of industrial products. Consequently, it is necessary to re-evaluate both the production and agricultural spheres as well as the chemical and processing engineering. Hence, agriculture must enter into an alliance with the chemical industry in order to create a new economic sector, such as sustainable biomass industry. An alliance of this type is not new in economy. In the 1920s and 1030s, when the potential of fossil fuels for economic growth had not been fully uncovered and the market needed new industrial products, research was underway to study the chemical processing of agricultural raw materials into industrial products. This then new science was named chemurgy. Today, the principal objectives of chemurgy include (i) the development of new, non-food applications of agricultural raw materials, (ii) creation of a new market for agricultural products, which are produced in excessive amounts, and (iii) search for new ways of utilizing residue and waste from agricultural production, which all create the basis for developing bioeconomy. It is presumed that products generated from biological resources should gradually replace the products which are now made from fossil fuels, thus creating a new market of bioprocesses and consequently a new market of bioproducts and bioservices. At the moment, the bioeconomy turnover in the EU reaches 2 billion euro annually. This sector provides 22 million of work places, which corresponds to 9% of all work places in the EU [3]. According to OECD[1] data, biological raw material conversion processes will have made up 25% of industrial processes by the year 2030 [4]. In the EU, this should translate to 1 million of new work places, mostly in rural areas [5].
The objective of this article is to demonstrate how the history of developing biomass-based chemicals has come full circle, from the type of an alliance between agriculture and the chemical industry established in the previous century in sciences (chemurgy) to the current need of building the same alliance in the context of developing the biomass industry (bioeconomy).

Biorefinery – the context of circulation economy
The key installation in bioeconomy is a biorefinery, in which organic matter, through a certain succession of processes, that is a cascade of biological and chemical processes, is converted to food, feed, biochemicals, biomaterials, biofuels and bioenergy [6].Theoretically, each chemical compound present in plant biomass can be extracted and functionalized or formulated to become foodstuff, non-food products, industrial semi-products or syntones [7]. Special importance is attached to a new portfolio of organic chemicals which belong to the high-value-low-volume (HVLV) group, a group of substitutes to today's petroleum products [8].
The profitability of a biorefinery arises from a chain of many bioproducts, at the end of which energy is generated from the remaining waste. It is seen in the context of an analysis of life cycles of individual products, processes and the whole biorefinery. The pro-environmental aspect of such an approach is supported by the fact that energy required to power biorefining processes can be generated from renewable resources. This means that changes in bioeconomy must be designed system-wise, from environmentally safe production/acquisition/logistics of biological raw material through economically effective multi-product processing as well as own energy safety by using energy potential of local energy sources, to the replenishment of environmental resources of minerals, water and organic matter. Such an approach ensures restitution of the natural resources in the environment as well as low emission and minimal environmental pollution. It also represents circular economy in its broader sense.
A good example on how a problem-oriented policy may stimulate the progress in sustainability is the global policy on renewables. What is the lesson we have learned from the technological progress in biobased energy generation and why this knowledge may increase the interest in chemurgy? First of all, the new processes (or „old" processes rediscovered anew) of (bio)(thermo)chemical conversions of biomass to useful energy have been adopted in practice. Secondly, it has been shown that there is a great amount of organic wastes, by-products and co-products which have an undeveloped potential for further chemical conversions. Thirdly, it has been demonstrated that biomass-based energy products present only a marginal potential in the rationalization of biomass conversion. Taking this knowledge into account, it may be concluded that a chemical processing of agricultural raw material requires a similar policy of giving directional support for biobased chemicals together with development of raw material supply, investment security and consumer confidence associated with organic chemicals and the whole bioeconomy market.

Chemurgy – a Polish R&D perspective
In Poland, like in other EU countries, the chemical and agricultural industries function independently, in two separate and viable economic sectors. Regarding the value of production output sold, the share of the food industry in the whole domestic economy is 16% (27 bn euros) and that of the chemical industry equals 12% (35 bn euros) (Fig. 1).

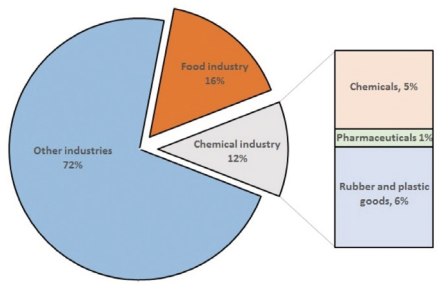

Figure 1. Structure of the contribution of the food and chemical industries to the value of production sold in Poland in 2014 [9]

The key raw materials for the Polish chemical industry are fossil fuels and fossil fuel energy is mainly used to power production processes. The dominant sectors of the chemical industry are the ones producing petrochemicals, plastics and mineral fertilizers. These are followed by the pharmacuetical and cosmetics industries, which have a much lower share in the whole chemical industry. The biomass industry in Poland is oriented primarily towards food processing and to a lesser extent towards energy generation. Production of biomaterials and biochemicals is being developed, but has not gained an important position in the country's economy. The main reason is the lack of mature technologies. However, research and development in this area are considerably advanced and various concepts of biorefineries have been proposed. Based on the authors' own research, it can be concluded that some processes designed for a lignocellulose biorefnery with a fuel profile generating 2nd (ethanol) and 3rd (algae) generation biofuels has already been implemented on the market while the concept of a sugar biorefinery relying on sugar beet processing is now being developed.
Lignocellulosic biorefinery. According to the developed model presented in Figure 2 biomass is converted to a sequence of products in a closed loop of a sustainable production system. For example, when considering cellulosic feedstock from short rotation woody crops (SRWC) and assuming that one of the processing pathways is associated with bioethanol production (Fig. 3), we can see many phases of the process beginning from the primary source of biomass, its logistics, pretreatment, hydrolysis, fermentation, distillation, and rectification, processing to other chemicals and materials, up to the final stage of post-processing waste conversion to energy and fertilizers to restitute nutrients in the environment. At each stage of biomass conversion there is a specific amount of post-processing waste, which may be treated as raw material for other pathways of conversion. All the products, processes as well as the whole biorefinery circulation are subjected to analyses of life cycles and trade-offs.

[1] Organization for Economic Co-operation and Development

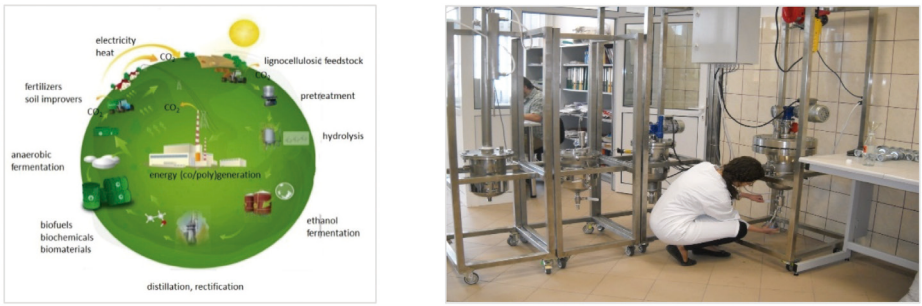

Figure 2. The concept of lignocellulosic biorefinery (on the left) and the lab installation for conversion of raw material from SRWC (willow, poplar, robinia pseudoaccacia) to ethanol. [10]

Sugar biorefinery. The development of sugar biorefinery is based on raw material of sugar beet. This feedstock is widely produced in the EU countries and owing to (i) high yielding, (ii) relatively low sensitivity to climate conditions, and (iii) a potential to be cultivated in relatively broad spectra of soil types, the area of cultivation dedicated to supplying sugar beets to sugar biorefineries may be significantly extended. In the development of a biorefinery concept, sugar beet is the only crop able to compete with sugar cane (Fig. 3). The dry matter of sugar beet contains more sugars and soluble compounds, but less insoluble compounds than found in the dry matter of sugar cane.

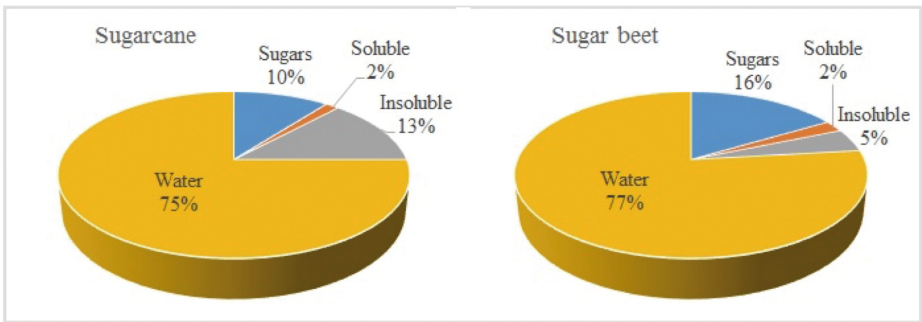

Figure 3. Comparison of the chemical composition of sugar cane and sugar beet (dry matter is divided into sugars, soluble and insoluble compounds) [11].

Regarding sustainable agricultural production, it should be mentioned that water footprint (WF) in sugar beet production is 935 m3/t, while in sugar cane cultivation it equals 1500 m3/t. Converted the production of ethanol from sugar beet, the WF is 355 L/L, compared to WF=2885 L/L calculated for ethanol produced from sugar cane [12].

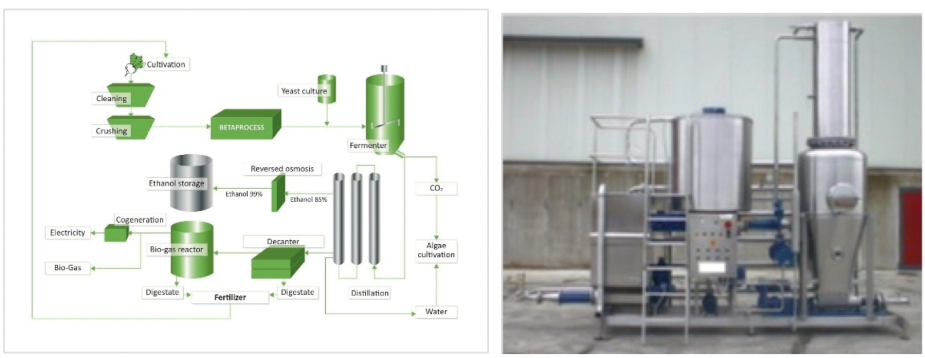

Figure 4. The concept of sugar beet direct processing (on the left) and Betaprocess® installation [13].

The sugar beet biorefinery relies on the processing of whole sugar beet plants, starting with Betaprocess® and leading to the production of primary bioproducts, such as ethanol (Fig. 4). These bioproducts can be used at further stages of chemical processing to obtain other biochemicals, such as bioplastics. Processing waste and residue, including carbon dioxide, can be used for growing algae and generating energy (methane fermentation) that can serve as a self-supply of the processes. Analogously to the lignocellulose biorefinery, it is essential to balance processing data in terms of LCA and trade-offs.

Summary

The current economic situation implicates that the following decades will be the key time for the development of market bioeconomy, for which the primary raw material will consist of biomass from agriculture, agroforestry and aquaculture. As mentioned in this paper, a situation when industrial products are made from organic raw materials is not a new one. Back in the early 20th century, we could witness the development of a new science called chemurgy, which dealt with research on the chemical processing of raw materials and waste from agricultural production with the aim of making industrial products. At that time, however, a good supply of inexpensive fossil fuels made the manufacturing of bioproducts unprofitable. The chemical industry concentrated on fossil fuels, thus inhibitng the further growth of chemurgy. It is only now, when we face unfavourable climate change and fluctuations on the fossil fuels market, that the economic processes are being re-oriented towards sustainable development, using energy from renewable resources, including biomass. A natural step in this development is an attemtp to rationalize biomass use by creating a cascade of recovering valuable chemical compounds from biomass in order to make industrial products, including energy for self-powering of all biorefinery's processes. The above considerations should stimulate a renewed interest in the chemical processing of agricultural raw products. For the development of chemurgy, it will be important to implement new production processes in biorefineries, installations analogous to petrochemical refineries. This article presents some development concpets of biorefining processes proposed in Poland, with two types of installations given as examples: a lignicellulose biorefinery and a sugar beet biorefinery, both processing feedstock originating from agriculture (SRWC and sugar beet). In both cases, the solutions correspond to circular economy, in which multi-product processing includes controlled cycling of matter and energy concordant with the model 'from cradle to cradle'.

Acknowledgements

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Systemic view of bio refineries linked to the production of food and energy

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Introduction:
Biofuel new plants in Argentina were born with special characteristics since farmers have a significate participation either as direct investors or through the cooperative system. From the technical point of view this plants are in line with new concepts linked to energy optimization and environment care. Evaluations methods employed in their study are in line with the bio refinery and bio economy concepts. Which have different definitions according to the literature. Due to the strategic importance of this industry INTA through its rural engineering institute started a series of studies centered in the products analysis, the coproducts emission and the Energy efficiencies. The fist research that included social and economic aspects as well were developed in Ag Energy Company of Viluco group in Frias Santiago del Estero 2010-2013. At the end of 2014 the firs corn based plant study ended over BIo4 de Rio Cuarto in Córdoba province and this year the plant of ACABIO located at Villa María in the same province was ended and presented in this paper.

The new studies are based in a systemic approach looking at biofuels and bio products incorporating the whole transforming biomass chain identifying interactions between products. Complementary research analyzes the logistic and infrastructure aspects shared between the different products being produced. Local and international Environmental regulations and legal aspects define in which way this bio refinery industry is developing.
There is a deep revision now days of the paradigms that use to negatively consider I generation biofuels production generated from as food coproducts from flex crops. This sources well studied present several advantages over II generation alternatives lately heavily promoted in the whole world. Summarized in the following points:

- By agricultural surface affected they produce significate human and animal food products of high quality.
- When unexpected commercial rule changes or climate disorders of agricultural plagues that alter Food production they have the flexibility to stop biofuel production and increase Food outputs from the same biomass source...
- They rely on well and mature technologies in continuous improvements with well-developed farm and industrial machinery.
- They already have established a robust logistic chain that is shared by a variety of products.

A methodology to understand the complex interactions of biomass extraction and transformation is used in order to analyze how they affect the agricultural and agro industrial Argentine sector. International protocols and methodologies were followed in order to obtain a characterization of biofuels and bio products that could be used to comply with overseas commercial requirements. Results highlight the importance of farming variability can significantly affect results magnifying the importance of long term research. The second significate aspect is related to allocation importance between the different products being produced.

Methods and materials:
Several visits seminars and working sessions were used in the companies in order to acquire the information systems involving the critical management sectors in the different transforming chain divisiones. The transforming chain were divided into biomass production, logistics industrial transformation and end product delivery. European Union norms consistency models were constructed and GHG emission estimation tools were constructed.. During the studies specific Energy and supply flows were defined. The specific calculator for each company was developed through several improvement steps improving estimations and including new aspects and procedures along time.

The European Directive was followed according to the defined procedures[1] for this type of calculations related to biofuels. Some factors were not included according the specific situation of the step and company being considered. To simplify the transforming chain procedures the following sections were taken into account:

- Agricultural production (e_{ec}): it includes all farm operations.
- Raw material transport[2] : includes all transport aspects from farms to intermediate storage facilities and final industry.
- Production of biofuels and co products (e_p): includes the industrial operation from the grain reception to the biofuel and coproduct output at the delivery facility.

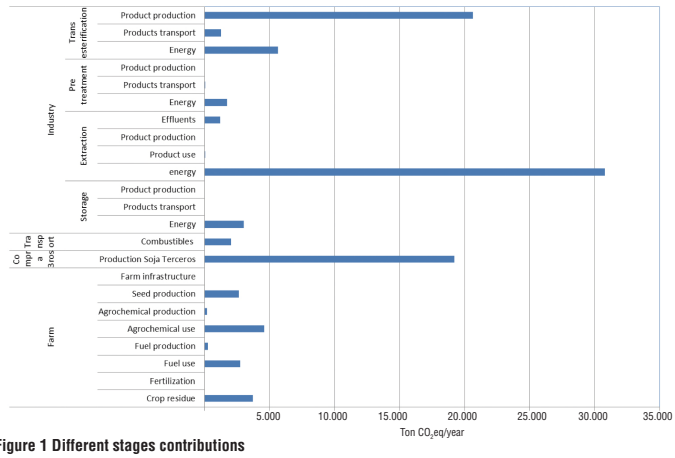

Figure 1 Different stages contributions

- Final logistics (e_{td}): includes transport to local and overseas destinations. For international delivery Rotterdam port was chosen).

For emition and other concepts and factors the guides from IPCC 2006 national inventories were followed. Since this methodologies are focused on national balance specific adaptations were made in order to make a specific study of a chain or product.

In order to quantify the annual variability a three year study was performed in one of the companies capturing climatic and field yield variations.

Results and discussion:
Results from studies over the soybean transforming chain in VILUCO S.A, indicate an overall annual emition of 88.860 Tons of CO_2eq. From this total 69% is produced at the industrial stage, 27% at the farms and 4% is generated by transporting operations With in this sectors specific studies were performed in order to highlight the significate stages were emition and Energy consumptions are concentrated. In the following figure the results from the soybean plant is presented as an example. This company produces soybean feed, expellers animal feed specialties, biodiesel and oil form the seeds complete processing. It is placed in a northern Mediterranean province Santiago del Estero with low industrial development, its position is considered critical for the successful development of the region especially in the animal production sector.
In all cases allocation factors were calculated according to the three most common methodologies used in the literature; mass balance, energy content and market price of each coproduct. Differences between them were significate in all the cases being considered in this paper.

Due to EU emition reduction requirements and limits a specific study was made in each case calculating the amount an emition reductions of the biofuels produced considering the default value defined at the directive 2009/28/CE. The different colors represent the overall contribution done by farm, industry and transport including overseas freight to Rotterdam grs.CO_2eq/Mj.

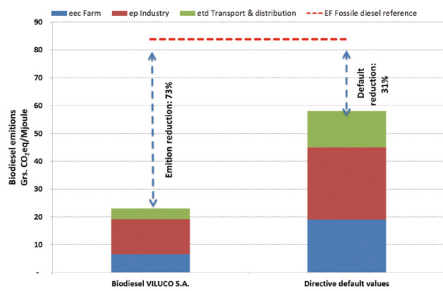

Figure 2 Final reduction achievement of VILUCO Biodiesel

The results indicate the fulfillment of gas emition reductions for all the periods since the biomass was generated at fields that were in agricultural production before January 2008[3] ,

Methodological tradability and consistency improvements were achieved by the repetition of this studies over three years this series of studies also made possible

[1]Annex V: Standard to calculate BioFuels bio liquids impact compared with fossil reference.
[2]The EU directive includes within transport all the associate emition of raw materials movements.
[3]No land use change was considered neither capture of carbon by means of better Management. No variation in field carbon stocks.

to capture climate and yield fluctuations in the region in order to study the overall impact in final results. Important drought occurred and affected the final results in a significate way indicating the importance of historic rather than unique studies to achieve better and representative results.

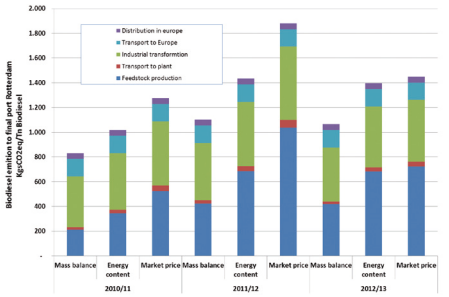

Figure 3 Inter annual variations in results according to yield changes

The two bioethanol plants gave different results according to the industrial technology and different source of coproduct being produced by them,

The results from BIO4 S.A corn plant during March 2013- February 2014 were 102.193 t CO_2eq. from this total 49% belong to the biomass production, 49% for the industrial transformation and 2% for transport and logistics at the local market

Regarding the final emition results for the principal products being generated if we wrongly apply all the emition only to the bioethanol the end result was production we arrive to a number of 43 grCO_2/MJ which is consistent with the level found in the literature. If a proper and correct allocation between products is made using the energy content procedure we arrive to a final number of 11, 4 grCO_2/MJ for the bioethanol and to 27,9 grCO_2/MJ for the DDGS The emition reduction in the first case was 49 % and 86 % in the second

The second bioethanol planta ACABIO has a capacity of 400 m3 of bioethanol per day 125.000 m3/year. The preparation of corn is done by dry milling in order to extract the starch. One of the distinctive advantages in this plant relies in the zero effluent concept. Water output contains vapor condensations and rain water.

In this plant the following end products were considered; oil, carbon dioxide, DGS and DDGS. This company is equipped with a cogeneration plant that is capable of generations all the heat and electricity employed during the different industrial stages fulfilling all the requirements. The maximum high protein animal feed production can reach 140.000 tons.
As carbon dioxide output from the plant was fixed by corn plants on the same year it is not considered as net emition. The natural gas not burned in the closed factory is considered an asset.
The total emition for 2014/15period reaches 124.150 tons of carbon dioxide which is after Energy allocation a level of 23 gmCO^2/MJ of bioethanol. In the following table energy content criteria emition are exposed.

Apropiation by energy content					
Product	**Production Tn**	**%**	**TnCO-$_2$eq**	**KgCO$_2$eq/ Tn**	**grs CO$_2$eq/ Mj**
Bioethanol	93.730	48%	58.446	624	22,58
DDGS	24.334	9%	11.372	467	
WDGS	183.665	42%	50.211	273	
Vegeta-ble Oil	973	1%	813	835	
Total	**302.703**	**100%**	**120.842**	**399**	

Table 1 Allocation according to Energy content of each product.

From the analysis of the different steps 54 % of emition come from the industrial phase, 35 % form farm feedstock production and around 13 % for transport.

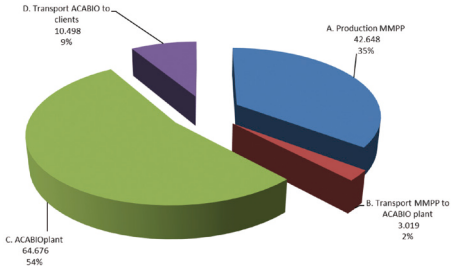

Figure 4 Emition distribution along the transforming chain

The incorporation of the carbon dioxide capture and purification plant improve the overall results of all the rest of the products. The following figure captures the reduction percentage difference taking into account the local gasoline emition factor according the third Argentinian communication 2015.

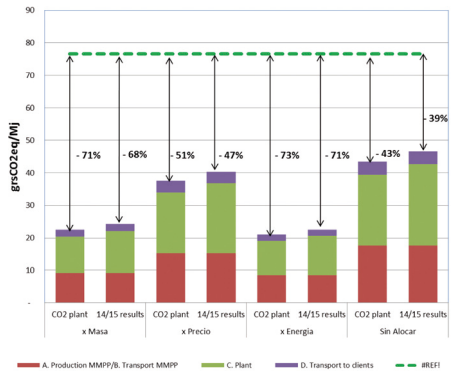

Figure 5 Effect of carbon dioxide use over the overall GHG reduction percentage

Since previous studies demonstrated that the crop yield is a very sensible factor a special study was developed in order to be able to forecast differences produced by a certain crop yield decrease or increase. The following figure shows the overall effect of a reduction of 50 % in corn yield.

Figure 6 Effect of a 50 % in mean corn yields over emition.

Conclusions

The developed experience over three different plants in Argentina demonstrated the importance of this type of studies for this type of bio refineries

- Highlight the relative importance of each step during the whole transforming chain in order to prioritize which ones significatively contribute in emitions and Energy use.
- Value the differences between years in order to measure the effect over the emition factor of the different products being produced.
- Increase awareness over the importance of correct allocation standard and criteria in order to divide GHG production and Energy use between the different products.
- Establish a methodology to make continuous improvement possible minimizing inconsistencies and increasing data trazability.
- Analyze the impact of the incorporation of new products derived from the same feedstock.

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Productivity and costs of two low-investment biomass harvesting systems applied in a situation of mixed forest of semi-natural origin

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Abstract

This study compares the productivity and costs of two low-investment biomass harvesting systems, likely to be used by owners of small and medium-sized forests in south-central Chile. The focus is harvesting the natural regeneration of *Acacia Melanoxylon* R. Br. (Australian blackwood), characterized by a high density, around 6,900 trees ha^{-1}, with an average diameter at breast height (DBH) of 5.3 to 5.6 centimeters, under the cover of a *Eucalyptus globulus* Labill (Tasmanian blue gum) plantation. Whole-tree harvesting system was used and included felling and skidding activities. A manual system, felling with chainsaws and the skidding with oxens and a semi-mechanized system with two chainsaws equipped with a felling frame and an agricultural tractor with a grapple for skidding were used. Time studies were done, productivity models were developed, and the harvesting costs were calculated. The cost of both harvesting systems was relatively similar. For an 80-meter logging distance the total costs were 20.5 USD Mg_w^{-1} in the manual system and 22.9 USD Mg_w^{-1} in the semi-mechanized system. For this last system, the use of a felling frame reduced the physical workload, increasing productivity, which allowed for the compensation of its biggest hourly cost. This study contributes by developing information about productivities and costs and by identifying the variables with the greatest influence on harvesting operations by using two low-investment systems, likely to be used by owners of small and medium-sized forests.

Introduction

To achieve the potential that a natural forest biomass offers as a source of renewable energy in south-central Chile, the development of a supply chain that meets the quality requirements and is stable and safe over time is required. This is fundamental to the implementation of energy projects in the long run (1). The biomass supply chain includes growth phases, harvesting (felling and skidding), pre-treatment (chipping and drying), and transportation (2). Harvesting and pre-treatment are critical, since these present a high technical complexity and also make up the largest share of cost (3, 4, 1). One of the biggest technical complexities in harvesting is related to the characteristics of the forest biomass used for energy purposes, many times presented in the form of thin trees or forest waste of varied volumes (5, 6). With this regard, becomes necessary to technically analyze different harvesting systems' configurations, implemented at a local level, which may be adequate for the characteristics of biomass and also the terrain conditions. Unlike high-investment harvesting systems, low-investment systems are characterized by high use of labor and low level of mechanization, designed for small-scale jobs (7, 8). In Chile, these systems adapted to local conditions have a high potential to be utilized by owners of small and medium-sized forests, in situations with mixed forest of semi-natural origin characterized by abundant natural regeneration of *Acacia spp.* within forest plantations. The general objective of this study is to provide information about productivities and costs of two low-investment systems and to identify the variables with the greatest influence during the harvesting operations of the natural regenerations of *A. melanoxylon* under the cover of a forest plantation.

Experimental

Description of the field of study

This study focuses on biomass harvesting through the utilization of two low-investment systems, applied in a mixed forest situation of semi-natural origin. A natural regeneration of *A. melanoxylon*, characterized by a high density, was harvested, with around 6,900 trees per hectare, with average diameters (DBH) of 5.3 to 5.6 centimeters, under the cover of an *E. globulus* plantation. The study was carried out in Las Palmas (39°44´50´´S - 73°08´50´´O), located 20 km north of the city of Valdivia, Los Ríos region, Chile. The climate is temperate rainforest with Mediterranean influence, the average annual precipitation totals 2,292 mm.

Description of the harvesting systems

Whole-tree harvesting method was used for the natural regeneration of *A. melanoxylon* and comprised felling and skidding activities. Two harvesting systems were utilized (Figure 1).

- Manual harvesting system: the manual harvesting system included an operator with a Sthil MS 360 chainsaw in charge of cutting all the *A. melanoxylon* trees, plus an assistant (Figure 1 A). The skidding of bunches was carried out by a team of oxen (Figure 1 B). At the time of harvest, the oxen were four years old, had an average weight of 500 kg, and had two years of log skidding training.
- Semi-mechanized harvesting system: two operators worked independently the felling. One operator was equipped with a Husqvarna 440e chainsaw (operator 1) (Figure 1 C) and the other was equipped with a Sthil MS 260 chainsaw (operator 2) (Figure 1 D). Both chainsaws were equipped with a felling frame of Finnish origin designed for felling small-sized trees (Apuri felling handle 2010). The skidding was carried out with a John Deere 6403 agricultural tractor with double traction equipped with a hydraulic grapple and conditioned for forest work (Figure 1 E).

Figure 1. Biomass harvesting systems

Results and discussion

Productive times and delays by system

In felling, the least productive times were observed in the manual system, equivalent to 63.9% of the scheduled work time (Figure 2). The main component of delay was due to rest breaks for the operators (personal delays), representing 20.8% of the scheduled work times. The operational delays represented another 14.5%. In the semi-mechanized system, the productive times for felling represented 77.5% of the scheduled work time. The personal delays for the operators to rest represented only 9.0%, the operational delays represented 9.6%, and the mechanical delays represented the remaining 3.1%, mainly due to problems with adjusting the felling frame on the chainsaws.

In skidding, the productive times of the manual system were equivalent to 46.1% of the scheduled work time (Figure 2). The 19.0% of the personal delays of the manual system were due to rest breaks for the operator or the oxen. In the semi-mechanized system, the productive times for skidding represented 72.7% of the scheduled work time. The rest breaks for the tractor operator represent 11.2% and the operational delays 15.6%.

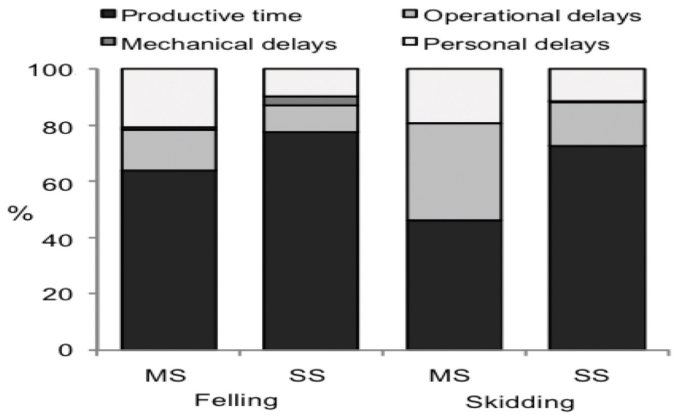

Figure 2. Percentage breakdown of the productive machine hour and delay times by harvesting system. MS: manual system; SS: semi-mechanized system.

Felling productivity and unit costs per ton

The average productivities in the felling phase of the two harvesting systems were very similar, from 1.25 and 1.34 Mg_w PMH^{-1} for the manual and semi-mechanized systems respectively. According to these productivities and considering an effective workday as 6.5 schedule machine hour (SMH, excluding preparation times and the end of the task), the felling production in the manual system reached 5.2 Mg_w $daily^{-1}$ (production of one Mgw every 75 minutes) and with the semi-mechanized system, 6.8 Mg_w $daily^{-1}$ (production of one Mgw every 63 minutes). The biomass output in the semi-mechanized sector reached 95.9 Mg_w ha^{-1}. According to this output, around 19 worked days are required for the felling of a hectare with manual system, while approximately 14 work days are needed with the semi-mechanized system.

The felling productivities decreased significantly in the face of increases in understory density and the terrain slope over 20%. According to the felling productivities per schedule machine hour (Mg_w SMH^{-1}), the felling production cost with the manual system was 10.9 USD Mg_w^{-1} and with the semi-mechanized system, it was 11.3 USD Mg_w^{-1}. These costs can decrease by up to 7.0 USD Mg_w^{-1} with the manual system and up to 8.8 USD Mg_w^{-1} with the semi-mechanized system when the delay times are equal to zero. On the other hand, in the semi-mechanized system, the felling costs increased to 13.5 USD Mg_w^{-1} in conditions of low understory density. With respect to the slope, the felling costs increased to 14.9 USD Mgh^{-1} in high-slope conditions (21-30%) and decreased to 9.1 USD Mgh^{-1} in low-slope conditions (0-10%).

Skidding productivity and unit cost per ton

With a skidding distance of 40 meters and an effective workday of 6.5 SMH, the biomass extraction reached an estimated productivity of 4.68 Mg_w $daily^{-1}$ with oxen and 27.89 Mg_w $daily^{-1}$ with the tractor. Considering the biomass output of the semi-mechanized harvesting sector (95.9 Mg_w ha-1 of stem biomass), with the oxen around 20 workdays were required for the extraction of one hectare of biomass and around 4 workdays with the tractor. Beside, for an 80-meter skidding distance, for oxens and the tractor the estimated productivities were 3.38 and 14.95 Mg_w $daily^{-1}$; therefore, around 28 and 7 workdays per hectare were required, respectively.

The hourly cost of the manual system was 13.69 USD SMH^{-1} and in the semi-mechanized system, it was 38.37 USD SMH^{-1}. With regard to skidding, the hourly cost of the tractor per scheduled hour of work was more than 5.3 times larger than the oxen hourly cost. These cost differences have the main impact of a significantly greater investment in the tractor with respect to the oxen (fixed costs) and its higher level of fossil fuels (variable costs) for its operation. The skidding cost comparison was done by simulating the productivity for the whole range of skidding distances carried out by the oxen and by the tractor (Figure 3). The skidding costs per wet biomass ton, were larger for the tractor than oxen, when delays are not considered for the entire range of distances. For example, the skidding cost with the oxen and with the tractor for a skidding distance of 40 meters was 3.2 and 4.5 USD Mg_w^{-1}, and for a distance of 90 meters was 4.7 and 9.4 USD Mg_w^{-1} respectively. Considering the delays time, for a distance of 40 meters, the skidding costs for the oxen and for the tractor were 6.9 and 6.2 USD Mg_w^{-1} and for a 90-meter distance, they were 10.2 and 12.9 USD Mg_w^{-1}respectively. In common, the skidding costs for the tractor were less than for the oxen up to an approximate distance of 50 meters. For a larger distance, the skidding costs for the tractor were higher.

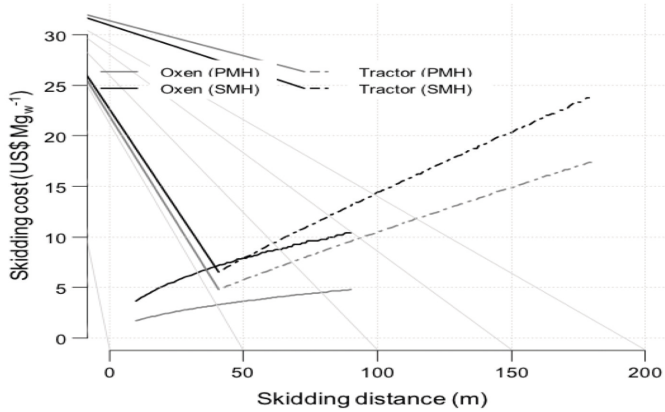

Figure 3. Skidding costs per wet ton of biomass, considering the productivities per productive machine hour (PMH) and per scheduled machine hour (SMH), according to the logging distance.

Conclusions

The biomass harvesting systems was carried out in a mixed forest situation of semi-natural origin characterized by a high density and small-sized trees under the cover of a forest plantation. Under these operating conditions, the two low-investment harvesting systems reached relatively similar costs per unit.

For an 80-meter skidding distance, equivalent to the average skidding distance for the oxen and the tractor, the total costs, including all of the felling and skidding activities, were 20.5 USD Mg_w^{-1} in the manual system and 22.9 USD Mg_w^{-1} in the semi-mechanized system.

For felling, the use of chainsaws with felling frames helped to decrease the physical workload, reducing delays times, and for that matter, increasing the productivity per scheduled work time, in this way compensating for its largest hourly cost.

The understory density and terrain slope had a highly significant impact on productivity and felling costs.

For skidding, the logging distance and the load size significantly affected productivity and costs, while the terrain slope, between a range of 0 to 20% had no significant effects on productivity.

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Assessment of supply chains for pre-treatment of forest residues in Chile

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Abstract
Forest residues are a renewable and sustainable source of energy that can be used to produce electricity and heat. A challenge hindering the intensified use of forest residues for energy production is the highly dispersed nature of the feedstock and the associated costs of collection and transport to the power plant. This study explores the potential for cost reductions through three different pre-treatment technologies: (1) pelletization, (2) torrefaction combined with pelletization and (3) fast pyrolysis. To this end, a mixed integer linear program (MILP) is formulated. The MILP represents decisions regarding the optimal locations, technologies and capacities of pre-treatment plants and the amounts of feedstock and final products to be transported between the selected locations. It minimizes the objective function of overall costs for the entire supply chain from the recovery of forest residues to the distribution of final products. The model is applied to a case study in four Chilean regions, which form the centre of the Chilean forestry industry and feature a high potential of currently unexploited forest residues that could alleviate the dependency of Chile on imported fossil fuels. The assessment concludes that it is possible to provide forest fuels at delivered costs of approximately 6 €/GJ for regular pellets, 8 €/GJ for torrefied pellets and 10 €/GJ for bio-slurry. Sensitivity analysis and the evaluation of different scenarios indicate that decentralized supply chain configurations with short feedstock transportation distances are generally preferable to more centralized concepts.

Introduction
There is an increasing interest in intensifying the use of biomass resources for the production of electricity, heat and biofuels. Biomass is generally considered a sustainable and renewable source of energy which mitigates climate change and promotes the development of rural areas. Despite these benefits, bioenergy production is frequently hindered by technical and economic challenges. Biomass feedstock is often characterized by high moisture content, low energy density and varying chemical composition. Due to these properties, raw biomass is a low-quality fuel in comparison with fossil fuels. Besides, raw materials accrue over wide regions which leads to increased collection, transportation and handling costs. The use of pre-treatment technologies has gained interest in recent years as it has the potential to overcome both the technical and logistical hurdles associated with bioenergy production. Currently, the most important pre-treatment technology is pelletization. Pellets have a higher bulk density and lower moisture content than raw biomass and show superior properties with respect to handling and combustion. In addition, thermal processes such as torrefaction and pyrolysis can be used to produce bio-based energy carriers with increased energy density and properties similar to those of coal and oil.

Problem statement
The integration of a densifying pre-treatment step into a biomass supply chain requires substantial capital investments associated with the respective technology. As is the case for many industrial processes, the processing costs of a pre-treatment plant decrease with increasing plant size. To benefit from these economies of scale, pre-treatment must take place at only few centralized plants with high capacities. To achieve a high throughput at a pre-treatment plant, long-distance transportation of loose biomass has to be accepted. In contrast, decentralized pre-treatment at several plants close to harvesting sites is associated with lower feedstock transportation costs.
It is the aim of this article to investigate this trade-off relationship between transportation and processing costs for three pre-treatment processes: (1) pelletization, (2) combined torrefaction and pelletization and (3) fast pyrolysis. First, a mixed-integer linear programming (MILP) model is presented. The model optimizes transportation and pre-treatment processes as well as plant locations and capacities in a bioenergy supply chain. Second, the model is applied to a case study in the south of Chile.

Mathematical model
The model represents a simple biomass supply chain: Feedstock of type b can be collected at harvesting sites i according to the biomass potential $u_{i,b}$. Harvested and chipped feedstock $a_{i,b}$ can be transported from sources i to destinations j (shipment x_{ij}) or to candidate locations k (shipment x_{ik}) where one of the pre-treatment processes p and the plant capacity $\omega_{p,k}$ are chosen. The pre-treatment products $x_{kj,p}$ are then transported to destinations j, where the demand v_j for bioenergy must be fulfilled.
The objective function minimizes the total supply chain cost including feedstock acquisition cost (FC), feedstock transportation cost (TC_1), pre-treatment processing cost (PC) and product distribution cost (TC_2):

(1) $$minC = FC + TC_1 + PC + TC_2$$

The set of biomass types b is limited to residues from plantations (pl) and native forests (nf). The feedstock cost of plantation residues follows directly from the acquisition cost c_{pl}^{acq} and the purchased amount $a_{i,pl}$ of residues. It is assumed that the acquisition cost of residues from native forests increases with a higher utilization of the total biomass potential. This is modelled with a non-linear supply curve consisting of different segments and cost levels. The amount of residues is represented by $\omega_{nf,l\_nf,i}$ while the cost level of the chosen segment l_{nf} is represented by c_{nf,l_nf}:

(2) $$FC = \sum_i a_{i,pl} \cdot c_{pl}^{acq} + \sum_i \sum_{l_{nf} \geq 2} c_{nf,l_{nf}} \cdot \omega_{nf,l_{nf},i}$$

Transportation costs are comprised of a fixed component c^{tf} and a variable component c^{tv}. TC_1 refers to the transportation of wood chips (wc), TC_2 to the distribution of pre-treatment products (p). Distances between sources, candidate plant locations and destinations are represented by three distance matrices d_{ik}, d_{ij} and d_{kj}:

(3) $$TC_1 = \sum_i \sum_k c_{wc}^{tf} \cdot x_{ik} + c_{wc}^{tv} \cdot d_{ik} \cdot x_{ik} + \sum_i \sum_j c_{wc}^{tf} \cdot x_{ij} + c_{wc}^{tv} \cdot d_{ij} \cdot x_{ij}$$

(4) $$TC_2 = \sum_k \sum_j c_p^{tf} \cdot x_{kj,p} + c_p^{tv} \cdot d_{kj} \cdot x_{kj,p}$$

The processing costs are subject to economies of scale and are therefore represented by non-linear cost functions. In order to obtain a linear formulation of the optimization problem, the cost functions are broken down into piecewise linear segments (g). The processing cost at each plant is then calculated with the capacity $\omega_{p,g\_p,k}$ and the intercept y_{p,g_p} and slope s_{p,g_p} of the respective segment. The binary variable $\mu_{p,g\_p,k}$ indicates whether a segment g is chosen:

(5) $$PC = \sum_p \sum_k \sum_{g_p \geq 2} s_{p,g_p} \cdot \omega_{p,g_p,k} + y_{p,g_p} \cdot \mu_{p,g_p,k}$$

The amount of wood chips transported from a harvesting site to pre-treatment plants and destinations must equal the total a_(i,b) of wood chips from plantations and native forests:

(6) $$\sum_k x_{ik} + \sum_j x_{ij} = \sum_b a_{i,b} \qquad \forall i \in I$$

The amount of wood chips procured cannot exceed the respective biomass potential:

(7) $$a_{i,b} \leq u_{i,b} \qquad \forall i \in I, \forall b \in B$$

The energy provided to a destination must equal the demand of this destination:

(8) $$\sum_i x_{ij} \cdot h_{wc} + \sum_k \sum_p x_{kj,p} \cdot h_p = v_j \qquad \forall j \in J$$

The sum of the flows from each harvesting site i to a plant k must equal the total capacity of the plant:

(9) $$\sum_i x_{ik} = \sum_p \sum_{g_p \geq 2} \omega_{p,g_p,k} \qquad \forall k \in K$$

The total flow of products from a plant k to each destination j must be in accordance with the capacity and the feedstock-to-product ratio (FPR) of the respective process p:

(10) $$\sum_j x_{kj,p} \cdot FPR_p = \sum_{g_p \geq 2} \omega_{p,g_p,k} \qquad \forall k \in I, \forall p \in P$$

Only one segment (with the upper interval limit n) of a piecewise linear cost function can be chosen for each pre-treatment plant as well as for each biomass potential of logging residues from native forests:

(11) $$n_{p,g_p-1} \cdot \mu_{p,g_p,k} \leq \omega_{p,g_p,k} \qquad g_p = 2, \dots, G_p, \forall k \in K, \forall p \in P$$

(12) $$n_{p,g_p} \cdot \mu_{p,g_p,k} \geq \omega_{p,g_p,k} \qquad g_p = 2, \dots, G_p, \forall k \in K, \forall p \in P$$

(13) $$\sum_{g_p \geq 2} \mu_{g_p,k} \leq 1 \qquad \forall k \in K, \forall p \in P$$

(14) $$n_{nf,l_{nf}-1} \cdot \mu_{nf,l_{nf},i} \cdot u_{nf,i} \leq \omega_{nf,l_{nf},i} \qquad l_{nf} = 2, \dots, L_{nf}, \forall i \in I$$

(15) $$n_{nf,l_{nf}} \cdot \mu_{nf,l_{nf},i} \cdot u_{nf,i} \geq \omega_{nf,l_{nf},i} \qquad l_{nf} = 2, \dots, L_{nf}, \forall i \in I$$

(16) $$\sum_{g_{nf} \geq 2} \mu_{g_{nf},i} \leq 1 \qquad \forall i \in I$$

The model was implemented in GAMS and solved using the CPLEX solver.

Case Study: Pre-treatment of forest residues in the south of Chile

The presented MILP model is applied to a case study in four Chilean regions (IX, X, VIII, XIV). The model requires input parameters with regard to the transportation network, the biomass resources and the pre-treatment technologies.

Transportation network

The model includes three sets of locations: sources, destinations and candidate locations for pre-treatment plants. These locations are derived from the administrative division of Chile. For each of the 128 municipalities (comunas) within the four regions, two locations — the capital and the centroid — are identified using ESRI ArcMap. The capital of each comuna is considered as a possible destination while the centroids represent the biomass resources. With the list of centroids and capitals, the distance matrices d_{ik}, d_{ij} and d_{kj} are calculated. Since the transportation costs of torrefied pellets and bio-slurry in Chile (**Table 1**) are unknown, they are derived from the transportation cost of wood products such as timber and cellulose [1].

Table 1: Specific transportation costs

	Moisture content	Bulk density	Transportation cost
Forest residues	40 %	150 kg/m³	6.58 €/t + 0.28 €/t/km
Wood chips	40 %	230 kg/m³	4.29 €/t + 0.18 €/t/km
Wood pellets	9 %	600 kg/m³	2.63 €/t + 0.11 €/t/km
Torrefied pellets	2 %	800 kg/m³	2.63 €/t + 0.11 €/t/km
Bio-slurry	20 %	1,200 kg/m³	4.81 €/t + 0.15 €/t/km

Biomass resources and feedstock acquisition costs

Two types of feedstock – residues from plantations and from native forests – are taken into consideration in this case study. Plantations and native forests differ with regard to the available amount of residues and the cost of recovery. The supply of plantation residues in each comuna is derived from the plantation area published in the Anuario Forestal [2]. The specific yield of residues is calculated as a fraction of the mean annual increment (MAI) and ranges from 1.4 t/ha/year to 2.0 t/ha/year. It is assumed that 40% of all logging residues generated in Chile are available for energetic use [3]. With this information, the total amount of forest residues from plantations in all four regions is estimated at approximately 550,000 dry tons per year. It is assumed that the forest residues are chipped directly at the harvesting site. The acquisition cost of wood chips is estimated at 8.7 €/t [4]. This cost estimate includes collection and piling, forwarding, roadside storage and chipping but excludes transportation. The bioenergy potential of residues from native forests was investigated in a project of the Universidad Austral de Chile (UACh) [5]. It was shown that the acquisition costs of residues from native forests increase with a higher utilization rate due to a lack of accessibility. To account for these additional costs, the cost function published in the UACh report is applied to the acquisition cost of plantation residues and linearized for the MILP model. The available amount of residues from native forests in each comuna is also calculated using estimates from the UACh report [5].

Pre-treatment technologies

The MILP model requires two inputs related to the pre-treatment technologies: First, feedstock-to-product ratios which are involved in the mass balance restrictions. Second, cost curves which are needed for the objective function. The feedstock-to-product ratio (FPR) is the ratio between the amount of product obtained after the last processing step and the amount of feedstock before drying. Due to the heat demand for drying, which is met by using a share of the feedstock, the FPR strongly depends on the moisture content of the biomass [6]. In order to estimate the FPRs of all three pre-treatment processes (**Figure 1**), mass and energy balances are adapted from the literature [6, 7].

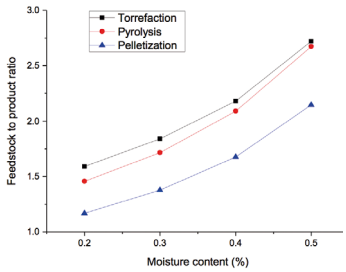

Figure 1: Feedstock-to-product ratios

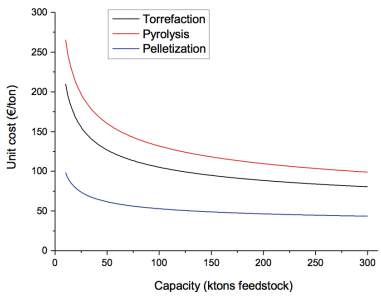

Figure 2: Pre-treatment cost functions

In order to derive the cost functions, investment estimates taken from the literature are collected and used to calculate the total production cost according to a factorial approach [8]. Calculating the total production cost over a range of capacities leads to the cost functions (**Figure 2**) which are linearized for the MILP model.

Results

The model was applied to different scenarios which differ with respect to the number of destinations and the demand for pre-treatment products. It was found that pelletization is preferable to torrefaction and pyrolysis in most scenarios. Since pelletization plants require the lowest capital investment, they are most suitable for a decentralized supply chain configuration with small capacities and low economies of scale. The tendency towards decentralized configurations can be explained by the feedstock-to-product ratios of the processes. Since approximately two tons of feedstock must be delivered to the pre-treatment plant for each ton of product delivered to a customer, feedstock transportation costs account for a high share of the total cost and cannot be outweighed by economies of scale.

The spatial distribution of biomass resources and plant locations in a scenario with shipments to all 128 comunas is shown in **Figure 3**. It can be seen that the plants (all pelletization) are located close to the biomass resources (Ø 45km) in order to keep the feedstock transportation cost low. In contrast, longer distances (Ø 85km) are accepted for product distribution thanks to the increased energy density of pre-treatment products. The supply chain model does not account for product properties which can be of advantage for subsequent applications. Gasification, for instance, benefits from a lower O/C ratio which results from torrefaction and pyrolysis, but not from pelletization. The pre-treatment processes are therefore assessed individually in different scenarios (**Table 2**). The assessment shows that feedstock transportation is particularly problematic for the pyrolysis process as the high capital investment requires large capacities and therefore long distances between harvesting sites and pyrolysis plants. The model recommends that a share of 20–30% of the feedstock is sourced from native forests. However, this translates into a cost reduction of only 2–5% in comparison to scenarios without procurement from native forests.

Finally, it should be noted that the integration of a pre-treatment step is not justified by lower product transportation costs alone. With a maximum transportation distance of 200 km in this case study, the reduced transportation cost cannot outweigh the processing cost of the pre-treatment step. Thus, if wood chips are accepted as a fuel, no pre-treatment may be the most economical option.

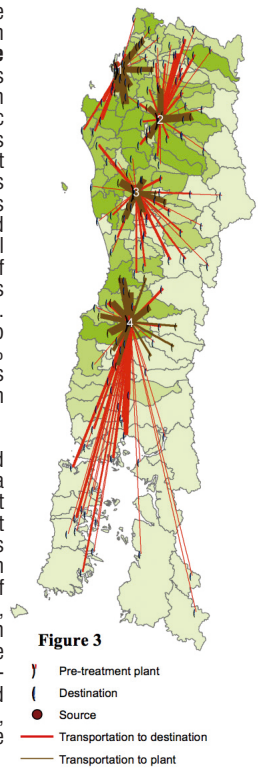

Figure 3

Table 2: Delivered cost per technology

Process		PEL	TOR	PYR
# plants		4	3	1
Ø delivered cost	€/t	95	152	171
Ø plant capacity	t/a	98	144	500
Feedstock (FC)	€/GJ	0.78	0.80	0.89
Transport (TC1)	€/GJ	1.90	2.11	4.16
Processing (PC)	€/GJ	3.16	4.11	4.47
Transport (TC2)	€/GJ	0.49	0.89	0.87
Delivered cost	€/GJ	6.33	7.92	10.38

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Kinetic model for the oxidation of hazardous compounds in an industrial effluent from forest biomass processing

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Introduction

Industrial effluents from alkaline sulfite treatment of wood contain several compounds that are recalcitrant to microbiological treatment (aromatic derivatives from wood extractives and labile lignin fractions) thus requiring a suitable treatment system [1]. Advanced Oxidation Processes (AOP) exploit the high reactivity and low selectivity of hydroxyl radicals (•OH), which attacks organic molecules during oxidation. The information regarding the kinetic study on the degradation of mixed recalcitrant compounds in industrial streams by Fenton-type oxidation is still limited; therefore, the principal objective of this work was to propose a kinetic model for the reaction.

Experimental

Industrial wastewater used

Spent liquor from an alkaline sulfite treatment of wood was used, in which wood chips are impregnated with low quantities of sodium hydroxide and sodium sulfite (pH 9 - 10). It has a pH of 7-8, deep red color, and it is mainly composed by extractives and small fragments of lignin derived from the chemical reaction.

Analytical Techniques

Solids were determined according to Tappi T629. Inorganic content was assessed by determining ashes at 525°C according to Tappi T211. COD was measured following the technique SM 5220-B (Standard Methods for the Examination of Water and Wastewater, 17th Edition). Total Organic Carbon (TOC) was used to measure the organic compounds content in liquors, using a TOC analyzer (Shimadzu, TOC-VCPN model).

Catalyst preparation and characterization

Pellets of Υ-Al_2O_3 (Alfa SASOL) were used as support. They were impregnated with an aqueous solutions of $Cu(NO_3).2.5H_2O$ (Riedel-de Haën, p.a.) as precursor. The incipient wetness impregnation method consists of mixing the support with an aqueous solution containing an appropriate amount of salt so that, after calcination, the catalyst contains the required metal content. The volume of the prepared solution is equal or slightly smaller than the pore volume of the support. The maximum load is limited by the solubility of the precursor in the solution. Solids were air dried during 24 h, and then oven dried during 24 h at 120 °C. Finally, they are calcined in air atmosphere during 4 h at 900°C.

Support and catalysts were characterized using the following techniques:

- Surface areas were calculated from nitrogen adsorption at –196 °C by using a Micromeritics FlowSorb II 2300.
- Oxide structures and cluster size of the synthesized metal oxides supported on alumina were determined by techniques of powder XRD using PANanalytical, X'Pert Pro equipped with Cu Kα radiation. The patterns were recorder over 10° <2θ < 70° range and compared to the JCPDS files to confirm phase identities. The main peaks corresponding to the gamma alumina phase are 2θ = 66.7° (100), 46.1° (80), 37.4° (60), and 39.7° (30).
- The catalysts elemental composition was determined by energy dispersive X-ray spectroscopy (EDS) using an EDAX Genesis XM4-Sys 60 equipment, with a EDAX multichannel analyzed model EDAM IV, sapphire detector Si(Li) and Be window, super ultra-thin, software EDAX Genesis version 5.11.

Fenton-Type oxidations

A PYREX glass batch reactor of 250 mL with a glass stopper equipped with a condenser, a thermocouple, and pH meter were used. To minimize external mass transport effects, experiments were carried out with a high-speed stirring. Testing was performed in contact with air, at atmospheric pressure. The reaction volume was 100 mL. The experiments were conducted using 0,5g/L of pellet catalyst and 433 ppm of initial TOC. Experimental oxidation conditions are summarized in Table 1 (where $[H_2O_2]o$ is the initial peroxide concentration).

Table 1: Conditions of the catalytic reactions.

Oxidation reaction	Temperature °C	[H2O2]o g/L
Ox 1	45	1.786
Ox 2	60	1.786
Ox 3	70	1.786
Ox 4	80	1.786
Ox 5	45	2.437
Ox 6	60	2.437
Ox 7	70	2.437
Ox 8	80	2.437

2.5 Kinetic study of Fenton-type reactions

The rate and the order of the heterogeneous catalytic oxidation reactions were obtained using the linear form of "n" order reactions. The order of the reaction was determined by fitting the equations to the experimental data and by verifying the coefficient of determination (R2).

Results and discussion

Catalysts characterization

Table 2 summarizes the characterization results of the Υ-Al_2O_3 (gamma alumina) and the CuO/Υ-Al_2O_3 catalyst. The analysis of X-ray diffraction revealed only the presence of the characteristic peaks of the Υ-Al_2O_3 phase, which means that the concentration of impregnated active phase is quite low and the formed particles are well dispersed copper oxides [2]. According to BET analysis (despite the high calcination temperatures), the catalysts show acceptable surface areas after calcination. The composition of each catalyst via spectrometry and energy dispersive X-ray (EDX) was determined as the average of 330 points from the surface to the center of the pellets, and remains almost constant through all the distance, indicating a uniform distribution of the active phase [3]. The high calcination temperature probably promotes the diffusion of copper species to inner zones of the support [4].

Table 2: Characterization results

Sample	Theoretical CuO content (%)	BET area (m^2/g)	Phase detected by XDR	Active phase quantification by EDAX
Υ-Al2O3	-	200	Υ-Al2O3	-
1.25Cu900	1.25	133	Υ-Al2O3	1.24
2.25Cu900	2.25	170	Υ-Al2O3	2.34
5Cu900	5	187	Υ-Al2O3	4.69

Kinetic study

Variations of TOC in the performed oxidations can be divided into two stages (see figure 1). In the first stage (named "seconds stage") the organic matter decreased abruptly, whereas in the second stage (named "minutes stage"), it decreased smoothly until the end of the reaction. This mechanism probably indicates that the "seconds stage" follows a higher-order kinetics, and/or other reaction mechanisms take place [5]. The "two step kinetic model" [6] which can be applied to TOC reduction, admits two sequential steps of oxidation, as follows:

$$A + H_2O_2 \xrightarrow{k_{ss}} B \qquad (1)$$

$$B + H_2O_2 \xrightarrow{k_{ms}} C \qquad (2)$$

This suggests that two parallel reactions should be responsible for TOC degradation [7]. Where unstable species easily oxidized are named as A, pollutants that are difficult to oxidize are designed as B, the desired final products (CO_2 and H_2O) are commonly referred as C, and kss and kms correspond to the "seconds stage" and the "minutes stage" kinetics constants respectively.

As seen can be seen in Figure 1, the rapid decrease in TOC concentration observed approximately within the first 20 seconds requires specialized equipment that allows the determation of the order of the reaction. The time-concentration curves from the oxidation experiments (correspond to the "minutes stage"), obtained for the reduction of total organic carbon (TOC) were fitted to potential kinetic equations. In all cases, the oxidation rates can be described by pseudo-zero order kinetics. This kinetic model is observed at catalyst surface saturation by the reactants [8], and suggested that the rate of reaction in this study does not vary with their concentration.

Despite that oxidation kinetics of compounds in the forest biomass processing effluent can be described as of pseudo-zero order, the overall rate is determined by the reaction between the substances to be degraded and the consumed hydroxyl radicals (•OH), which supposedly reach a constant concentration in the solution [9]. Although the degradation of an organic compound by Fenton's reagent is complex, several researchers, by many techniques, have offered evidence of the formation of the •OH radical as the principle active oxidant in this system [10]. The global kinetic of the reaction can be represented as follows:

$$-\frac{dC}{dt} = r = k_{OH}[\bullet OH] = k \qquad \rightarrow k_{OH} * [\bullet OH] = k \qquad \frac{d[\bullet OH]}{dt} = 0 \quad (3)$$

Where r is the reaction rate, Co is initial concentration of TOC or aromatic compounds, C is concentration at time t, k_{OH} is reaction kinetic rate constant and, k is apparent pseudo-zero order kinetic constant.

Thus, kinetic reactions and the final final kinetic expressions that are obtained from their integration can be represented as:

$$-r_{TOC_A} = -\frac{dTOC_A}{dt} = f(TOC, k_{ss}) \rightarrow -\int_{TOC_A}^{TOC_B} \frac{dTOC_A}{f(TOC)} = k_{ss}\int_0^t dt \qquad (4)$$

$$-r_{TOC_B} = -\frac{dTOC_B}{dt} = k_{ms} \rightarrow \qquad TOC_C = TOC_B - k_{ms} * time \quad (5)$$

Where, TOC_A and TOC_B are the initial total organic carbon, and k_{ss} and k_{ms} are the reaction rate constants of both reactions, respectively. This model does not discriminate the presence of non-oxidizable matter in the initial mixture (mainly acetic acid). Being the remaining TOC along the reaction due to the presence of both, the refractory pollutants and oxidizable compounds.

The kinetic equation for the "minutes stage" can be described as follows:

$$-r_{TOC_B} = \frac{k_{ms}}{R} \qquad R = \frac{[H_2O_2]_{oWork}}{[H_2O_2]_{oStochiometric}}$$

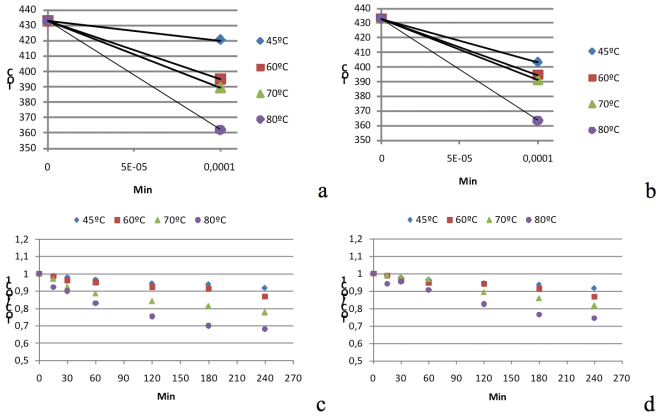


Figure 1: TOC evolution. a. "seconds stage" at stoichiometric [H_2O_2]; b. "seconds stage" at sub-stoichiometric [H_2O_2]; c. "minutes stage" at stoichiometric [H_2O_2]; d. "minutes stage" at sub-stoichiometric [H_2O_2]. TOC1 is the initial total organic carbon for the "minutes step"

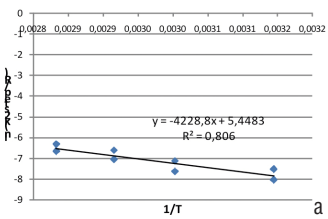
The Arrhenius expression, showing the relationship between the reaction temperature and the specific kinetic rate is expressed as follows:

$$k = A\exp\left(-\frac{E}{RT}\right)$$

Where: E is the Arrhenius activation energy for the oxidation process, k is the kinetic rate constant, A is the Arrhenius factor, R is the gas constant (8.314 J/mol K), and T is the temperature.

Arrhenius activation energy indicates the minimum energy that the reactants must have for the reaction to proceed. For these oxidation processes it resulted to be 35.158 kJmol-1 (Figure 2a).

The kinetic constants of the respective zero-order kinetics are shown in Figure 2.b.



Oxidation reaction	Kms*RH_2O_2	R^2
Ox 1	5.36E-04	0.976
Ox 2	7.98E-04	0.943
Ox 3	1.33E-03	0.983
Ox 4	1.78E-03	0.963
Ox 5	3.21E-04	0.976
Ox 6	4.83E-04	0.951
Ox 7	8.62E-04	0.964
Ox 8	1.27E-03	0.944

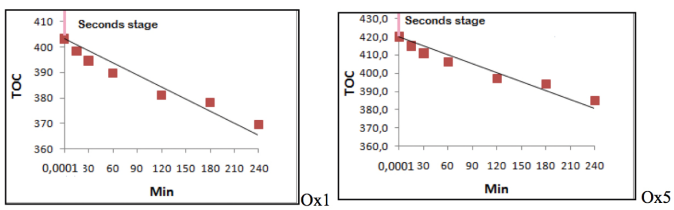
b

Figure 2: a. Arrhenius plot of zero-order kinetics constants; b. Experimental kinetic constants of the second step (minutes stage).

TOC (or TOC/TOC1) evolution along the oxidation reaction can be determined according to:

$$\frac{TOC}{TOC_1} = \left(1 - \frac{A_o \exp\left(\frac{-E}{R*T}\right)}{R_{H_2O_2}} * time\right)$$

Experimental and calculated TOC according to the model for the "minutes stage" are shown in Figure 3. The constant kap grows smoothly with the increase of [H_2O_2]o and has a steep increase with temperature in all the studied range.



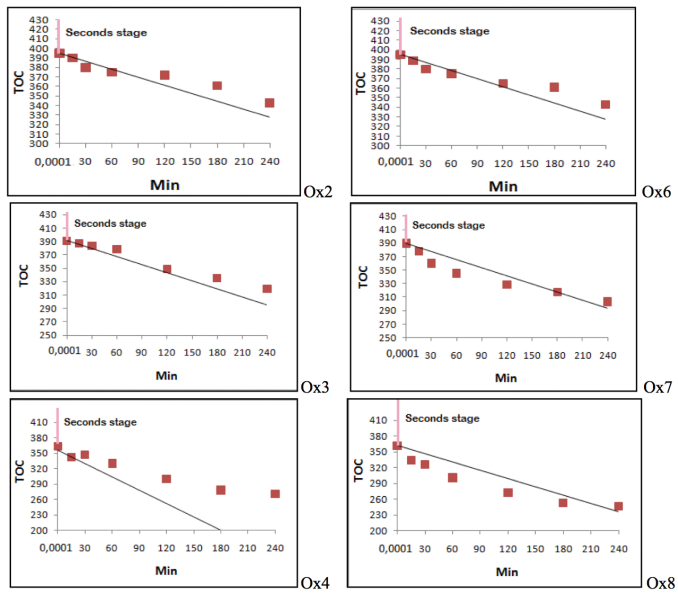


Figure 3: Experimental TOC (points) and calculated TOC (line).

Conclusions

Based on the obtained results. the used model to estimate the content of total organic carbon is applicable quite well under the conditions shown in Fig. 2.a (R> 0.80).

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Biorefineries

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Oral Presentations

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Chemical conversion

EuroBioRef: Designing next generation biorefineries

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Abstract

Project context and objectives

EuroBioRef (www.eurobioref.org) was a biorefinery project (2010-2014) dealing with the entire process of transformation of biomass, from non-edible crops production to commercial products. It involved 29 partners from 15 countries in a large scale, integrated, highly collaborative network.

Final results, intentions for use and impact

This project generated scientific innovation, technical advancements, and business opportunities. The elaboration of delocalized and virtually integrated biorefineries will enable creation of specialized jobs in rural areas, thus re-boosting local economies in the whole territory of the EU. An exploitation plan has been designed, and we assessed the number of jobs that will be created/saved in Europe (a few thousands along the VCs, including indirect jobs).

Project context and objectives

The EuroBioRef project (European Multilevel Integrated Biorefinery Design for Sustainable Biomass Processing; www.eurobioref.org) was a 4 years program coordinated by CNRS, France. It was launched on March 1st 2010, and achieved on February 28th 2014. It was supported by a 23 M€ grant from the European Union 7th Framework Program (FP7) for a total budget of 38 M€. EuroBioRef had this unique feature of dealing with the entire process of transformation of biomass, from non-edible crops production to final commercial products. It involved 29 partners (industry, SMEs, academics) from 15 different countries in a highly collaborative network, including crop production, biomass pre-treatment, fermentation and enzymatic processes, catalytic processes, thermochemical processes, assessed by a life cycle analysis and an economic evaluation of the whole development chain. With this strategy to develop next generation biorefineries, the project generated a lot of results, with an important impact on the European bioeconomy, including new energy & new chemicals production strategies.

This project has been closely followed by the EC, with an excellent appreciation: "EuroBioRef – How a radical re-design is strengthening economic viability in the bioeconomy". "For most people, the bioeconomy is the way of the future. A shift towards an economy based on renewable resources not on fossil fuels is no longer just an option, it's a necessity."

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http://goo.gl/WFfQey

Project results

Main results

Hereafter are listed the main results of the project:

- 5 lignocellulosic plants (willow, giant reed, miscanthus, switchgrass, cardoon) and 10 oil crops (castor, crambe, cuphea, lesquerella, jatropha, safflower, as well as sunflower, camelina and rapeseed for comparison) were grown;
- Large test fields were set: willow and crambe in Poland, giant reed and safflower in Greece and castor in Madagascar;
- Win-win culture rotation strategies between food and non-food crops were developed;
- A biomass supply logistics model has been developed and populated with data for 4 crops (willow, castor, safflower and giant reed);
- Efficient biotech technologies were developed to yield platform molecules from glycerol and biomass hydrolyzates, outperforming the current state of the art;

Figure 1. Potential locations for non-edible crops new cultures in Europe.

- A brand new pilot plant in Norway able to operate more than 50 kg of dry lignocellulosic materials per hour was constructed, using a new and versatile pretreatment process validated at the lab scale on miscanthus, giant reed and switchgrass. Full-scale unit is planned for 2017;
- 33 patents were filed;
- 65+ scientific papers were published;
- A 20 min video explaining the project is available on the EuroBioRef Website together with a 6 min video accompanied with a ca. 70 pages booklet both summarizing the outcomes of the project (www.eurobioref.org);
- Two books, namely *'Biorefinery: From Biomass to Chemicals and Fuels'*, Ed. by Aresta, Michele / Dibenedetto, Angela / Dumeignil, Franck, de Gruyter, ISBN: 978-3-11-026028-1 (2012), and *'BIOREFINERIES – An Introduction'*, Ed. by Aresta, Michele / Dibenedetto, Angela / Dumeignil, Franck, de Gruyter, ISBN 978-3-11-033153-0 (2015), have been published;
- Value chains corresponding to different scenarios of biorefineries integrating results and concepts developed in EuroBioRef were designed and multidimensionally assessed to realize demonstrations of the developed technologies, but also to test scenarios of industrial exploitation.
- On 11-12 February 2014, we successfully organized a two-days conference 'Tomorrow's Biorefineries in Europe' in Brussels with our sister projects, namely Biocore and Suprabio, notably to present our results and propose our technologies to stakeholders (https://colloque.inra.fr/eubiorefineryprojectsfinalconf).

Summary of the achieved work

We designed and refined 6 value chains (VCs) in which our concepts, technologies, know-how and achievements were implemented:

- Value Chain 1: Castor oil to polymers;
- Value Chain 2: Crambe/Safflower oils to polymers;
- Value Chain 3: Alcohols to fuels (ATF);
- Value Chain 4: Lignocellulosics to acrylates *(abandonned due to low technological advancement)*;
- Value Chain 5: Syngas-based products;
- Value Chain 6: Integrated productions in existing assets.

The description and main outputs of the value chains are as follows:

Value Chains 1 & 2. Both value chains are dealing with vegetable oils and are technologically the most advanced ones. The purpose of VC1 is to start from castor and produce a high value monomer with some co-products being used as fuel. VC2 starts with oleaginous crops (crambe, safflower, **Figure 1**) producing high value monomers and short fatty acids, suitable for fuel application once esterified. VC1 and VC2 have several steps in common. Both these VCs have the possibility to start from castor, crambe and safflower. Further, a route was proposed to castor oil (VC1; **Figure 2**), and combines it with the chemistry of VC2 to deliver monomers even more interesting than those initially planed in VC2. Thus, as aforementioned, due to similarities and common outputs, these VCs were merged.

Figure 2. Castor processing unit.

Value Chain 3 & 5: Fuels and syngas-derived products. These VCs relate to the production of "ATF" used for aviation fuels (VC3) and to the conversion of black liquor to syngas-derived products (VC5) including alcohols (**Figure 3**). Then, VC3 is closely related with VC5 as both share the same route of syngas production via gasification and its consecutive conversion to alcohols. However, VC3 also considers another way of production of heavy alcohols/branched paraffins via advanced chemical routes to be blended as components of aviation gasoline and jet fuel. Some road fuels candidates were also successfuly tested (**Figure 4**).

Figure 3. Higher alcohols.

Figure 4. Test engine.

Value Chain 4: Biobased acrylates. This Value chain deals with conversion of lignocellulosic crops to hydrolysates, fermentation to 3-hydroxypropionic acid, then dehydration to acrylic acid, and in parallel fermentation of sugar hydrolysates and glycerol to n-butanol. Due to lack of technological maturity / economical viability, it was decided to drop the demonstration of this VC and to redistribute some useful competencies in the other VCs.

Value Chain 6: Integration of EuroBioRef technologies in existing assets. VC6 offers a framework to consider EuroBioRef chemistries and technologies as additions to existing, preferably European plants. Several such "co-location" scenarios have been proposed as modifications of VCs 1 to 4, VC5 being a co-location model by itself. On the other hand, 11 co-location models have been identified for EuroBioRef conversions, which were not studied in any of the other VCs. The work was re-focused on the most promising VCs. With the addition of the 2 products coming from VC4, VC6 demonstrated in which cases it makes sense to add a biobased production in an existing asset (plant) and capitalize on skilled personnel, available infrastructure, and plant integration. In this case, the Integrated Biorefinery is looking at the integration of a biobased product in a fossil (or bio) existing asset.

Expected final results, intentions for use and impact
Business results were obtained on:

- Demonstration of the economic and technical performance of biobased products including bio-aviation fuels and chemicals;
- Demonstration of the increase in economical performance due to use of second-generation feedstock by using the whole plant in a zero waste concept;
- Demonstration of the sustainable value chain of non-food crops cultivated in synergy with food-crops, through rotation strategies that will benefit to both food and non-food crops yields;
- Definition of final products specifications and tests of new products to be able to propose them directly to customers.

Scientific innovation is focused on:

- Methods for conceptual process design widely applied in the chemical sector towards bio-/ chemical applications;
- Heterogeneous, homogeneous and enzymatic catalytic systems including fermentation and optimization of the formulations taking into account the purity of the feedstock;
- New low energy separation techniques and adaptation to biomass-derived products, which will enable lowering of the overall cost;
- New reactor technologies for minimizing production of by-products while enabling substantial energy savings;
- Co-products reutilization technologies in order to further increase attractivity of the process;
- Integrated reaction/separation technologies for optimized process design;
- Development of new purification technologies of fermentation broth using green solvents, which will further improve the overall sustainability extent.

Substantial advancements were made on:

- Crop rotations optimization for Northern/ Southern Europe and Africa, selection of appropriate sustainable biomass feedstock for diverse EU environments;
- Rationalization of the chain elaborated to yield each product and global integration/ optimization of the whole process;
- Quality control of a variety of feedstock for a variety of end-products to set high level standards;
- Demonstration at the lab/bench scale of sub-units and demonstration at the pilot scale of integrated chains for significant products;
- Integration of several reaction and separation steps for high selectivity and conversion, energy and Capital (CAPEX) reduction.

Sustainability assessment and performances:

- Specific logistic methodology for cultures in Northern and Southern Europe;
- LCA methodology for evaluation of environmental performances;
- Economic modelling for assessment of economic viability;
- Sustainable assessment of the whole chain for economics.

Socio-economic impact and societal implications of the project:

- Creation of specialized jobs in rural areas; The investigated value chains could contribute to about 100 to 200 direct jobs, and up to 3600 jobs when taking into account the indirect jobs corresponding to each implementation;
- Developing business/side businesses in local economies.

Preparation of the Exploitation Plan of the project (Figure 5)
EuroBioRef prepared its exploitation plan taking into account sales from each partner in 2017 and at mature market, and self-assessing a probability of success. The work plan was accordingly adjusted in order to increase the chances to reach the market and to cross the "Valley of Death".

Partners

1. CNRS, Centre National de la Recherche Scientifique (UMR8181, UMR5256, UMR6509) France
2. ARKEMA FRANCE SA /CECA, France – jean-luc.dubois@arkema.com
3. BORREGAARD Industries. Ltd., Norway
4. NOVOZYMES A/S, Denmark
5. Partner 5 left the project without contributing and was replaced by partners 29 and 30 below
6. CRES, Center for Renewable Energy Sources, Greece
7. HALDOR TOPSØE A/S, Denmark
8. CERTH, Centre for Research & Technology Hellas, Greece
9. PDC, Process Design Center BV, Germany
10. QUANTIS, Switzerland
11. EUBIA, European Biomass Industry Association, Belgium
12. DTI, Danish Technological Institute, Centre for Renewable Energy and Transport, Denmark
13. Technische Universität Dortmund, Germany
14. MERCK KGaA, Germany
15. FEUP Faculdade de Engenharia da Universidade do Porto, Portugal
16. RWTH Aachen, Germany – retired from the project on 31/08/2011
17. CIRCC, University of Bari, Italy
18. WSK "PZL-Rzeszow" S.A, Poland
19. OBRPR, Ośrodek Badawczo-Rozwojowy Przemysłu Rafineryjnego Spółka Akcyjna, Poland
20. SINTEF Materials and Chemistry, Norway
21. SOABE, Société Agricole de Befandriana-Sud & Partners Sarl, Madagascar
22. UMICORE, AG & Co KG, Germany
23. Nykomb Synergetics AB, Sweden
24. Alma Consulting Group SAS, France
25. Ruse Chemicals AD, Bulgaria – demerger from Orgachim AD, Bulgaria from 1st January 2014
26. Imperial College of Science, United Kingdom
27. Novance, France
28. University of Warmia and Mazury in Olsztyn, Poland
29. Technische Universität Hamburg – Hamburg, Germany – entered the project from M24
30. BKW Biokraftwerke Fürstenwalde GmbH, Germany – entered the project from M24

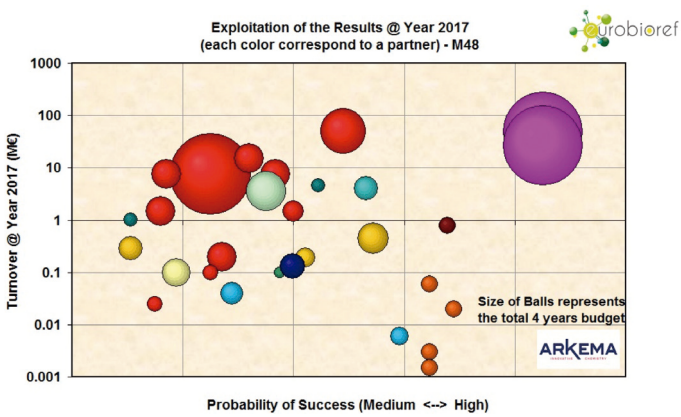

Figure 5. Exploitation of the results @ Year 2017 (each color corresponds to a partner) – Results at M48, End of the Project.

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2G Bioethanol Biorefinery using sugarcane lignocellulosic biomass residues

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Abstract

This paper intends to evaluate an advanced bioethanol biorefinery using sugarcane bagasse and trash using computational tools for process simulation. Based on the use of SuperPro Designer tool and input data, mostly generated within the EU ProEthanol2G project funded by EC, detailed information for mass and energy balances has been generated for assessing the feasibility of advanced 2G biofuel plants. A bioethanol yield of 249 L/dry ton biomass was obtained together with 2.1 ton/h biogas and a surplus of electricity of 14.6 MWh susceptible to be supplied to the grid.

Introduction

Nowadays, sugarcane is mainly used for producing sugar, bioethanol and electricity. However, first generation bioethanol is generally criticised due to the (indirect and direct) land use change effects that allows a competition between food and energy. In order to reduce negative impacts such as additional greenhouse gas emissions, integrative systems referred to as advanced (second-generation) biorefineries are being developed worldwide. These biorefineries technologically convert whole feedstock, including solid residues and wastewater streams for minimizing energy consumption with the goal to reach near-zero waste. In Brazil, sugarcane bagasse, occurs as a by-product of sugar cane processing in sugar and/or ethanol plants. The combined use of bagasse and trash from sugarcane fields in ethanol production is supposed to increase the ethanol yield per ha, to provide more renewable energy, to reduce greenhouse gas emissions as well as other beneficial environmental impacts.

Experimental

A concept for a sugarcane biorefinery aiming at a profitable process for conversion of sugarcane bagasse and other lignocellulosic biomass residues (sugarcane trash) into bioethanol was developed. Second-generation bioethanol production from lignocellulosic biomass can be divided into five steps: 1) Feedstock handling operation units 2) Pretreatment and solid-liquid separation 3) Hydrolysis and fermentation 4) Distillation and dehydration 5) Combined heat and power generation. Other steps may also be included such as yeast inoculum, enzyme production and biogas burning from the wastewater treatment plant.

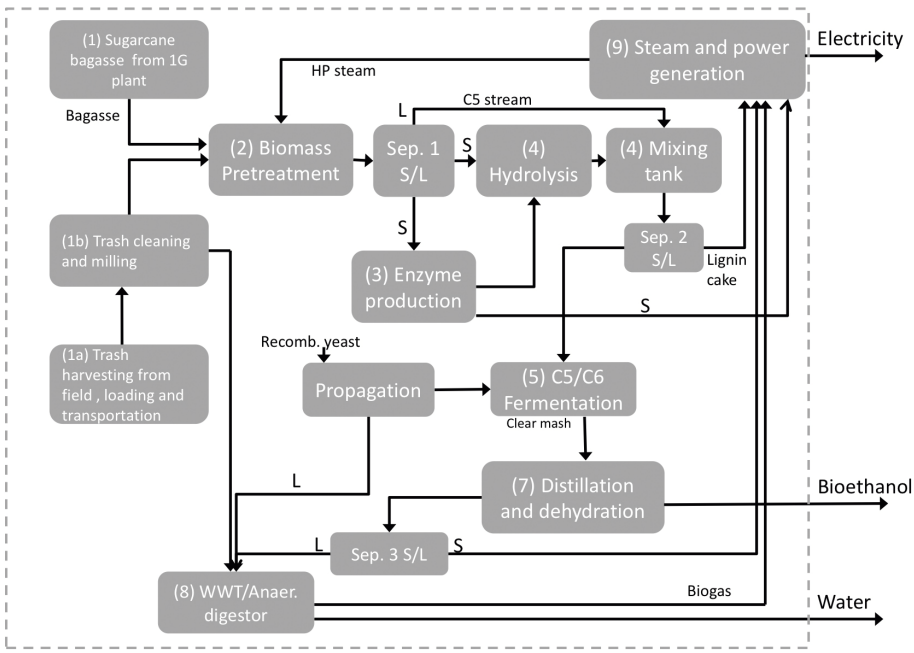
The material and energy balances were modelled with *SuperPro Designer* v.8.5 software using global information provided by Inbicon A/S, literature data and complemented with information generated within the EU ProEthanol2G project.

The 2G bioethanol biorefinery was dimensioned to process 70 ton sugarcane bagasse (dry mass) and 40 ton trash (dry mass) per hour. This amount of trash is based on the assumption that 50% of the total available straw is left behind in the field.

The biomass pretreatment step simultaneously processes both sugarcane bagasse and sugarcane trash. A hydrothermal pretreatment is carried out at 190°C with a residence time of 12 minutes (Petersen et al. 2009). The pretreated material, with 70-75% moisture content, is submitted to an S/L separation step. At this stage, the pretreated solids are mainly composed of cellulignin with approx. 50-60% moisture, and the liquid fraction is a hemicellulose hydrolysate (C5 liquor) with cellulose and lignin solubilization less than 5%.

The enzymatic hydrolysis step is performed at 20% wt solids (Larsen et al. 2012) at 15 FPU/g DM and 50°C during 48 h in an horizontal tank reactor (Dasari et al. 2009). The C5 liquid fraction coming from pre-treatment is added at a later stage of the hydrolysis step. The model also considers on-site enzyme production, co-fermentation of C5 and C6 sugars, yeast inoculum production, distillation and dehydration unit, wastewater treatment and a CHP unit (Figure 1).

Figure 1. Advanced bioethanol production flowsheet.



Results and discussion

The input parameters for process simulation were mostly based on experimental data obtained from EU ProEthanol2G project as well as some specific data provided by Inbicon A/S Demo plant, Kalundborg, Denmark (Table 1).

The data from Table 1 was used to develop a detailed process model including all unit operations, which then allowed attaining complete mass and energy balances of the all process.

With all these balances available it was possible to evaluate the performance of the biofuel factory in relation to biomass yield, electric and steam consumption. Downstream processing used 60% of the total energy consumption of the plant (steam and electricity) and Enzymatic Hydrolysis and Fermentation were the biggest consumers of electricity (36% each). Any improvement in these sections will increase the performance of the plant.

Enzyme production section required, as stated in Table 1, 8.25% of the pre-treated solids and resulted in the consumption of 19% of total electricity and 6% of total energy.

The obtained bioethanol yield, 196.1 kg/dry ton biomass (Table 2), corresponding to 248.5 L/dry ton biomass is 25% higher than that obtained by the Inbicon A/S cellulosic (C6) ethanol plant from wheat straw (Larsen et al. 2012). The use of the C5 fraction in the present model contributes to the increase of ethanol yield, even if this process considers on-site enzyme production, using part of the cellulose for this process.

Table 1. Advanced bioethanol plant input data for process modelling

2G Plant Processing Capacity:	
Sugarcane straw (ton DM/h)	34
Bagasse (ton DM/h)	70
Pre-treatment:	
Total solids (% DM)	40
Temperature (°C)	190
Duration (min)	12
Hemicellulose solubilisation (%)	70
Cellulose solubilisation (%)	2
Enzyme Production:	
Pre-treatment solids used (%)	8.25
Yield (kg enzyme/kg glucose)	0.24
Enzyme titter at harvest (FPU/ml)	25
Enzymatic Hydrolysis:	
Total solids (% DM)	20
Enzyme loading (FPU/g cellulose)	10
Temperature (°C)	50
Duration (h)	48
Cellulose hydrolysis (%)	80
Hemicellulose hydrolysis (%)	70
Yeast propagation:	
Yield (kg biomass/kg sugar)	0.44
Temperature (°C)	30
Duration (h)	30
Fermentation:	
Temperature (°C)	35
Duration (h)	12
Yeast inoculum (g DM/L)	7.5
Glucose consumption (%)	100
Xylose consumption (%)	80

Table 2. Advanced bioethanol plant output data

Methane production (ton/h)	2.08
Low pressure steam consumption (ton/h)	104.17
High pressure steam consumption (ton/h)	17.40
Power consumption (MW.h)	29.24
Power to the grid (MW.h)	14.63
Bioethanol production (ton/h)	19.08
Bioethanol yield (kg/dry ton biomass)	196.10

Conclusions

This paper reports the improvement of advanced ethanol yield to almost 249 L ethanol per ton of dry biomass (sugarcane bagasse and trash) compared with previous published data reported on advanced ethanol Demo units in Europe of 198 L/ton (Larsen et al. 2012). Moreover, 2G ethanol production from sugarcane bagasse and trash generates an electric surplus that can be sold to the grid thereby lowering 2G ethanol final costs. The biomethane generated from wastewater streams is not very high but contributes for a substantial reduction of residues generated in the process leading to a near-zero wastewater streams in the whole plant.

Acknowledgments

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Where are we with green Biorefineries?

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European Union sets the goal of development of more sustainable and balanced growth of the EU by the reduction of wastes and residues as well as by wise exploitation of these renewable feedstocks towards new products produced in the flexible and reduced environmental footprint processes. On the other hand, looking at the definition of biorefinery, which *is an industrial facility (or network of facilities) that cover an extensive range of combined technologies aiming to full sustainable transformation of biomass into their building blocks with the concomitant production of biofuels, energy, chemicals and materials, preferably of added-value,*[1] it can be clearly stated that the Green Chemistry principles are widely echoed in the Biorefinery concept. Especially important is the use of renewable resources, use of safer solvents and auxiliaries. Among safer solvents are e.g. ionic liquids (ILs) and supercritical fluids (SCF).

A fluid is termed supercritical when it is above the critical temperature and pressure and below the pressure needed for condensation. Supercritical carbon dioxide ($scCO_2$) fulfils these requirements and is broadly employed in the extraction[2] and only recently has been used in renewable biomass processing.[3-5] Water is also one of the fluids considered as potentially interesting in high-pressure biomass treatment.[6] ILs are substances completely composed of ions and are liquid at or close to room temperature. ILs are characterised by near-zero vapour pressure,[7] thermal stability[8] and widely tuneable properties as regards polarity, hydrophobicity and miscibility with other solvents through appropriate modification of the cation and the anion.[9] Also the data concerning the ILs toxicity and biodegradability are every time broader available,[10] hence these aspects become every time a less limiting factor in the IL use. Although ILs have been associated to be expensive solvents, the development of bulky low price ILs capable efficiently pre-treat biomass were recently reported.[11] This development opens a new window for processing of lignocellulosic biomass, where the employment of ILs could be a feasible alternative to conventional processes.[12]

Due to the aforementioned benefits coming from the use of green solvents in the biomass (either wastes or residues) processing, it is extremely important to develop appropriate methods of biomass processing in the delivery of value added products. However, the exploitation of both SCF and ILs in the biomass conversion was rather dedicated to either biomass dissolution or fractionation in case of ILs or to downstream processing in case of SCF. Hence, there is still a tremendous gap in ILs and SCF application in biomass processing to deliver value-added products. The major gaps are registered in the fields of (i) Biomass pre-treatment and extraction, (ii) Bioprocessing in ILs and SCF, (iii) Chemical conversion towards value-added products and (iv) **Downstream processing.**

Biomass pre-treatment and extraction

Pre-treatment with ILs and SCF offers advantages superior to conventional methods allowing to: (i) alter physicochemical properties of the biomass macromolecular components, such as reducing the lignin content and cellulose crystallinity; (ii) extract a specific macromolecular component, such as isolation of lignin and cellulose and (iii) perform different fractionation approaches after biomass dissolution.

Biomass pre-treatment using ILs normally results in total or partial dissolution of biomass and alteration of its original properties. The resulting regenerated biomass contains polysaccharides as main content. The regenerated material can be later selectively separated to obtain cellulose and hemicellulose fractions, while IL is recovered from liquid stream with prior to lignin precipitation. During this process, a decrease of cellulose crystallinity is observed that guides to enhancement of cellulose saccharification yield.[13]

Supercritical fluids, mostly CO_2 are generally used as the extraction media however these have recently started to be used as well in biomass pre-treatment.[4,14] This is mostly due to the fact that at high-pressures CO_2 easily penetrates into small pores of recalcitrant biomass structure, resulting in structural changes and enhancing the glucan and xylan accessibility for enzymatic hydrolysis. In the case of CO_2 explosion, the quick release of CO_2 facilitates the disruption of cellulose structure, decreasing a degree of crystallinity and enhancing the permeability of cellulose as well as increasing the accessibility of enzymes to an extended surface area.15 In addition, the removal of acetyl groups from hemicellulose reduces the steric hindrance of hydrolytic enzymes greatly enhancing polysaccharide digestibility[16] and allows to produce liquid stream rich in pentose sugars ready for further biological or chemical conversion.

Bioprocessing in ILs and SCF

Supercritical CO_2 is a promising green solvent for many bioprocesses. Among them are normally enzyme-catalysed chemical reactions as for example, fermentation (e.g. by yeast) is ineffective due to the denaturation effect of high pressure. The finding of enhanced enzyme stability and activity in alternative solvents, e.g. with lipases,[17] suggested a wide range of high-pressure CO_2 applications as selective biocatalysts for synthesis and/or hydrolysis reactions. Recently, the number of studies involving enzymatic catalysis under $scCO_2$ conditions has increased, since it has been demonstrated that enzymes are stable and active in high-pressure CO_2 and that CO_2 favours transport properties, accelerating mass-transfer in enzymatic reactions.[18] At the same time, high-pressure CO2 was also successfully used as a medium for the recovery of products and reactants.[19] The most important factor in achieving high enzymatic hydrolysis rates is the stability of the enzymes. This on turn depends on pressure and temperature exerted, pH, number of depressurization steps, water content, carbonic acid formation, solid content and enzyme specie, as well as the substrates and types of end products, due to the inhibition of enzymatic processes, by blocking the active sites of the enzyme.[20] However, enzyme activity is more difficult to maintain than for other typical solvents, mostly caused by lowering of pH at higher CO_2 pressures. The CO_2 pressure itself, even at the range of 200 bar, does not have effect on enzyme denaturation but the pH generated by this exerted pressure may inactivate the enzyme. To maintain the enzyme activity, a specific water activity is also strongly required as it affects diffusion and thus influences both reaction equilibrium and enzyme structure by non-covalent binding or disruption of hydrogen bonds.[21] Because of the relatively low activity of crude enzymes in $scCO_2$, many attempts were made to stabilise the enzyme by modifying the form in which they are used. Enzymes in several forms (powder, reversed micelles, etc.) or immobilised in a carrier such as a sol-gel matrix or a resin can be used in the presence of SCF without significant losses of their properties. Under CO_2 at supercritical conditions, the activity of the immobilised enzyme increased more than 4 times the activity versus the crude enzyme at the same conditions.[22] Additionally, the yields of glucose in $scCO_2$ can be higher than at atmospheric pressure.

In case of processes with ILs, one of the major problems is the toxicity of ILs against the microorganisms. A broad range of studies were performed in this field.[23] In general, an increase in alkyl chain length increased the toxicity towards rods, cocci and fungi.[24] Enzymatic biocatalysis in the presence of ILs has been successfully reported for a number of reactions including esterification, transesterification, alcoholysis, hydrolysis, perhydrolysis, aminolysis, ammonolysis and polymerisations.[25] A wide variety of enzymes have also been employed such as lipases, cellulases and oxidoreductases in a wide range of ILs and have been shown to give comparable or higher catalytic activities in ILs than conventional solvents.[26] However, there is still a strong need to understand how an IL affects enzyme stability, selectivity and activity before a suitable IL can be selected for a particular enzymatic reaction.

Chemical conversion towards value-added products

Several hundreds of literature reports were published that presented ILs employed as catalysts and/or reaction media. The most comprehensive reviews in this field are those published by Wasserscheid, Welton and their co-workers.[27,28] However looking for particular examples of the use of ILs in the conversion of biomass towards platform chemicals, the works on these subjects are still rather limited. One of the most extensively studied examples are furans namely 5-hydroxymethylfurfural (HMF) [29] and furfural and even so the works are rather limited to dehydration of monosaccharides rather them real biomass feedstock. In many studies, high conversion of sugars with limited HMF yield due to the formation of by-products, such as levulinic acid and humins was observed. It is inevitable for the conversion of cellulose or oligosaccharides from inedible bioresources to HMF to encounter the complexity of the structure and the existence of the other biomass components, such as lignin and protein. Normally, the process further includes dissolution of lignocellulose biomass and cellulose depolymerization to produce glucose for the synthesis of HMF. Hydrolysis of disaccharides and polysaccharides is catalysed by a base while dehydration of the resulting sugar monomers is favoured the acidic conditions. Thus, the selection of solvent and catalyst are the key challenges for high efficient synthesis of HMF. Dehydration of xylose leads to the formation of furfural. The generation of furfural from xylose goes through a complex mechanism. In aqueous media, xylose can either undergo retroaldol fragmentation into acids, aldehydes and ketones, or converted into intermediates whose chemical nature is controversial. Most studies on furan production from carbohydrates (including mono-, oligo- or poly- saccharides) in ILs (frequently, imidazolium salts) have been performed in the presence of catalysts. In particular, chromium

halide catalysts enabled the furfural synthesis from xylose under mild conditions in ILs, through the isomerization of xylose into xylulose, and the further dehydration of the latter into furfural. Recently, acidic ionic liquids (AILs) have been successfully used to carry out a number of organic transformations,[30] owing to their ability to perform as reaction media and catalysts at once. Due to their catalytic activity, AILs offer the advantage of avoiding the problems of the catalysed systems related to pollution, separation, and recycling.[31]

High pressure processes, chiefly hydrothermal treatments are studied for the production of hemicellulose-derived sugars either in oligomeric or monomeric form. Thus produced monosaccharides can be converted into a wide variety of products such as biofuels (either cellulosic ethanol or other furanic fuels), chemicals and biomaterials. Degradation products are also formed during hydrolysis and they may inhibit the fermentative processes. The presence of CO_2 lowers pH of the medium helping to biomass hydrolysis, and have been investigated as an alternative technology for classical methods of biomass hydrolysis.[32]

The solubility of CO_2 in H_2O determines the pH of the medium and unlike acid-hydrolysis pre-treatment, the acidity of medium produced by the high-pressure CO^2 process does not represent an environmental problem, as after depressurization, the pH become solely dependent on the other compounds in solution. Therefore, the pH of the solution mixture can be controlled by a degree of dissolution of CO^2 in H_2O which, on the other hand, is dependent on temperature and pressure. Analogously to IL-based processes the dehydration of biomass-derived cellulose and starch to produce furans was studied in many systems including supercritical H_2O.[33] H_2O under supercritical conditions is the most often used SCF to produce 5-HMF. However, high-pressure H_2O technologies require temperatures in the range of 100-400°C so that, on an industrial scale, a potential reduction of dehydration temperature means energy savings. The way to achieve this is the use of a CO_2/H_2O mixture. Other type of catalytic reaction studied in the presence of SCF is hydrogenation. The solubility of gases (e.g. H_2) in H_2O or other typical organic solvents is very low. $ScCO_2$ is an alternative solvent that is feasible for carrying out these reactions with either homogeneous or heterogeneous catalysts. The CO_2 under supercritical conditions is still a liquid and is completely miscible with H_2. At the same time, $scCO_2$ having liquid-like density, is a good solvent for non-polar organics. Thus, taking these dual properties of CO_2 under supercritical conditions, it acts as a carrier for gas to the liquid phase, minimising the mass transfer limitations. [34]

Downstream processing

Downstream processing constitutes a vast portion of the overall cost of the process. Thus, study of this part of the process is extremely important. Comparing the downstream processing with SCF and ILs it can be stated that extraction and purification using SCF technology is probably the most developed field of use green solvents in the biomass biorefinery. This is because of the need of the cellulosic ethanol extraction by employing $scCO_2$.[16] Even so, to apply the benefits of selective solubility of ILs and SCF in the downstream processes of biomass-derived products there is a strong need of the basic research such as phase equilibria of multicomponent and multiphase systems with those fluids and value-added products. The careful analysis of the current achievements and potential of the listed issues it is clear that there is still a strong need for development of all aspects of biorefinery concept with ILs and SCF. To help to identify opportunities, threads, weaknesses and strength of the use of ILs and SCF in biomass processing the SWOT analysis was performed and is depicted in figure 1.

The SWOT analysis confirms the wide range of opportunities and strengths for the use of both types of solvents (ILs and SCF) in the biomass processing, which on the other hand, is still strongly hindered by series of external threats which must be minimised to accomplish the objectives of Green Biorefineries.

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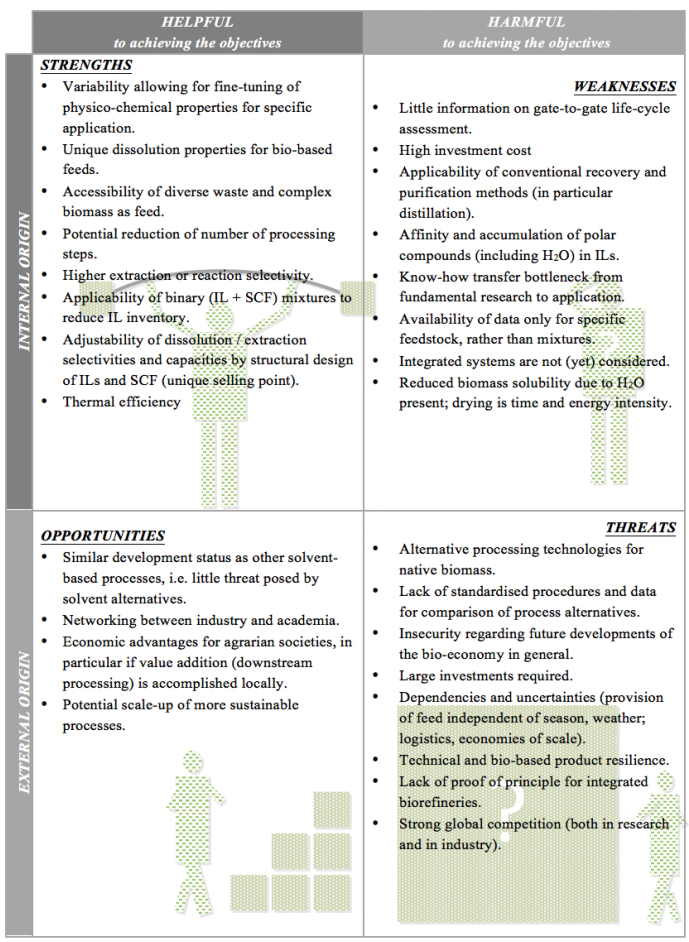

Figure 1. SWOT analysis of ILs and SCF engagement in the biomass processing

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Conversion of inulin-containing and lignocellulosic biomass to the platform chemical 5-hydroxymethylfurfural in water

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Abstract
Hydroxymethylfurfural (HMF) is an attractive platform chemical and can be synthesized from hexoses available in biomass. Two possible raw materials are inulin-containing biomass, which can be converted with 30-38 mol% to HMF, as well as lignocellulosic biomass. For the latter a suitable pretreatment is necessary. Therefore, steam explosion for a first pretreatment and a subsequent diluted acid hydrolysis as second step for the production of glucose-rich solutions are applied. The isomerization of glucose to fructose is catalyzed by heterogeneous catalyst like hydrotalcite.

Introduction
In view of a bio-economy in which fossil carbon sources are replaced by biomass, chemical building blocks play a key role. The goal is to produce so-called platform chemicals from biomass, for which a large number of secondary compounds and products are available. Very important among the group of platform chemicals are the furfurals, which were named under the top 10 value-added bio-based chemicals by the US Department of Energy [1]. One representative is 5-hydroxymethylfurfural (HMF) which is an attractive platform compound for various chemical transformations because of its two different functional groups. Unfortunately, an economic industrial production process for HMF for large scale applications does still not exist. The main reasons are the low reactivity of glucose and the relatively high costs of fructose, which are potential feedstocks. However, the company AVA Biochem started in 2014 the production of HMF with a plant capacity of 20 tons per year with purity up to 99.9 %, using a fructose-rich raw material [2].
In general all biomass that contain hexoses or their oligomers/polymers can be used as feedstock for the HMF production. One possible raw material is inulin-containing biomass, for example jerusalem artichoke or chicory. The cultivation of the latter provides the opportunity to combine the chicory salad production with root utilisation (rich in inulin) for production of platform chemicals.
Another possible raw material for HMF is lignocellulosic biomass which is cheap, largely available and is not in competition with food production. However, the use of lignocellulose provides additional chemical challenges, since the lignin-carbohydrate matrix prevents cellulose from fast hydrolysis to glucose. For this reason a suitable pretreatment step has to be developed. The second issue for the HMF production from lignocellulose is the isomerization of obtained glucose-rich solution to fructose. A schematic reaction pathway from largely available biomass to HMF is shown in Figure 1.

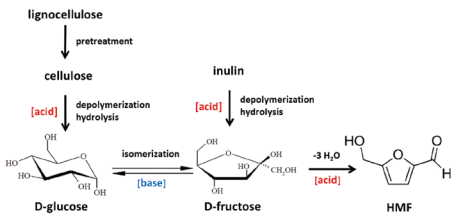

Figure 1 – Schematic reaction pathway from biomass to HMF.

Experimental
Materials
Two different inulins are used in this study: inulin-type fructan Orafti® P95 (DP = 2-8) from BENEO and inulin-type fructan from Sigma Aldrich (DP = 36). Dried beech wood chips are provided thankfully by Joh. Sinnerbrink (Verl, GER). This chip sized hardwood lignocellulosic biomass has a size of ca. 30×15×1-2 mm and is used without further comminution. A Na-X zeolite (Na86[(AlO2)86(SiO2)106]·264 H2O) named „Molecular sieves, 13X“ was obtained from ALFA AESAR and calcinated for 6 h at 500 °C before use. A synthetic hydrotalcite was obtained in powder size from Sigma Aldrich and calcinated for 6 h at 450 °C. All other chemicals were purchased from Sigma Aldrich with p.a. quality.

Conversion of biomass
Inulin conversion experiments are conducted in self-made micro-batch autoclaves as triplicates. About 1 g of inulin is added to 10 ml of 0.1 mol/l diluted acid (sulfuric, nitric or phosphoric acid). The autoclaves are heated to 160 °C in an oven and cooled down after 3 min of residence time.
Pretreatment of beech wood chips is performed in a self-made steam-explosion gun. About 100 g of beech wood are placed in a 1l stirred reactor and heated up by introducing of superheated steam. After a certain residence time an outlet valve is quickly opened and thereby the biomass is rapidly discharged in a collecting tank. The intensity of the pretreatment is determined with the severity parameter S0 introduced by Overend and Chornet [3]. The adapted formula is shown in equation 1.

$$(1) \quad S_0 = logR_0 = log\left(\int_0^t exp\left(\frac{T_r[°C]-100\,°C}{14.75}\right)dt\right)$$

Acid hydrolysis of steam-exploded biomass is done in a semi-continuous mini-plant. About 15 g of dried biomass are placed in a fixed bed reactor. The reactor is purged with 15 ml/min water and heated to 180 °C. When the desired temperature is reached in the biomass fixed bed, 0.05 mol/l sulfuric acid is introduced into the reactor with a flow of 15 ml/min. The hydrolysis product liquid is collected in intervals and analysed afterwards.
Isomerization experiments with heterogeneous catalysts are performed under atmospheric conditions in glass flasks at least in duplicates.

Analytics
The analysis of sugars in beech wood hydrolysis liquids is done by GC after derivatization of sugars into corresponding alditolacetates, according to an adapted method of Sawardeker [4]. The detailed procedure is described elsewhere [5]. The analysis of fructose and glucose from isomerization experiments is done via photometry after enzymatic conversion in a test-kit from r-biopharm. HMF was analyzed via HPLC with a UV-detector at 290 nm. A Lichrosper 100 RP-18 column at 20 °C is used with water/acetonitrile eluent (9:1 v/v) at a flow rate of 1.4 ml/min.

Results and discussion
Conversion of inulin to HMF
Inulin can be easily hydrolysed into its fructose monomers by an acid catalyst, which will also promote the dehydration step to HMF. Three different mineral acids are used as a homogeneous catalyst for inulin hydrolysis and the dehydration of sugar monomers to HMF. In figure 2 the HMF yields from different carbohydrates are shown, which were obtained with batch reactors at 160 °C for 3 min. The inulin with small chain length of DP = 2-8 as well as the long-chain inulin with DP = 36 show similar HMF yields which are also comparable with the results using the sugar monomer fructose. This indicates that the hydrolysis of inulin to monomers proceeds fast without large by-product formation. It can also be estimated from figure 2 that the type of the acid is not influencing the HMF yield heavily, however slightly smaller yields were obtained with phosphoric acid.

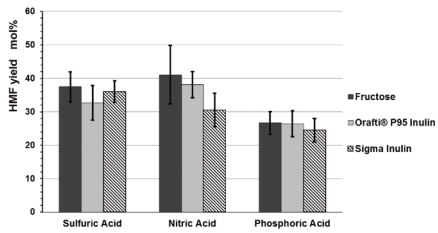

Figure 2 – HMF yield of fructose, Orafti® P95 inulin (DP = 2-8) and Sigma Aldrich inulin (DP = 36) at 160 °C for 3 min in diluted mineral acid (cA = 0.1 mol/l).

Conversion of lignocellulose to HMF - pretreatment
The investigated concept comprises the combination of steam explosion for a first pretreatment in order to break up the lignin-carbohydrate matrix of lignocellulose as well as a subsequent diluted acid hydrolysis for the production of glucose-rich solutions. Beech wood chips are treated in steam-explosion with three different severity parameters. Then the steam exploded wood material is hydrolyzed in a flow-through reactor with 0.05 mol/l sulfuric acid at 180 °C. The concentration profiles of the main carbohydrate building blocks of hardwood, xylose (from hemicellulose) and glucose (mainly from cellulose), are shown in figure 3. As higher the severity parameter S0, the less xylose can be obtained from the pretreated biomass. It is general observed that during steam explosion a part of the hemicellulose hydrolyses to low molecular products as well as repolymerize to form so called pseudo-lignin. These reactions reduce the xylose yields in subsequent acid hydrolysis.

The concentration profiles of glucose (see figure 3) show no positive influence of steam explosion pretreatment to glucose formation. Although the macrostructure of the wood material is largely altered

and broken up (examined by SEM pictures), the hydrolysis of cellulose to glucose is not accelerated.

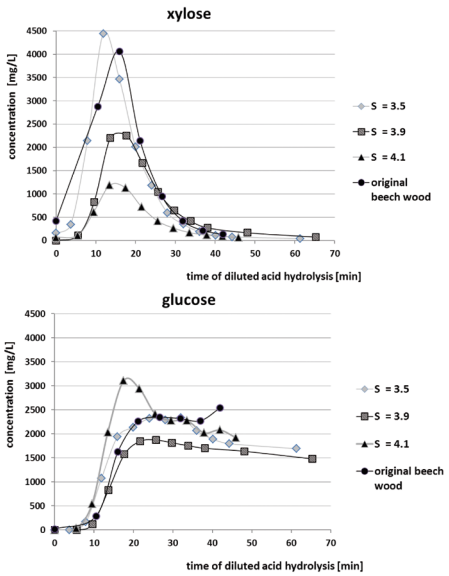

Figure 3 – Concentration profiles of xylose (above) and glucose (below) in the liquid product during 0.05 mol/l sulfuric acid hydrolysis at 180 °C of steam-exploded (S0 = 3.5 - 4.1) or untreated beech wood.

Conversion of lignocellulose to HMF - isomerization

The second issue for the HMF production from lignocellulose is the isomerization of the obtained glucose-rich solution to fructose. It is widely believed that this key reaction is base-catalyzed. This leads to a challenge in the process chain, because the biomass hydrolysis and the dehydration of fructose are acid-catalyzed, while the intermediate glucose-fructose isomerization reaction requires basic conditions. We investigate different heterogeneous isomerization catalysts and among them hydrotalcite and Na-X zeolite appeared to be the most promising. Figure 4 shows the fructose yields of isomerization experiments. Results from literature [6,7] could be roughly reproduced with glucose model solutions in water. When pH is reduced to typical values from acid hydrolysis the hydrotalcite showed still moderate selectivity (38 % glucose conversion, 24 % fructose yield, see figure 4). However for the Na-X zeolite, the glucose conversion drops under acidic conditions. If product liquid from wood hydrolysis is used as source of glucose, the conversion rise using hydrotalcite. It is expected that by-products from acid hydrolysis cause side reactions during isomerization step.

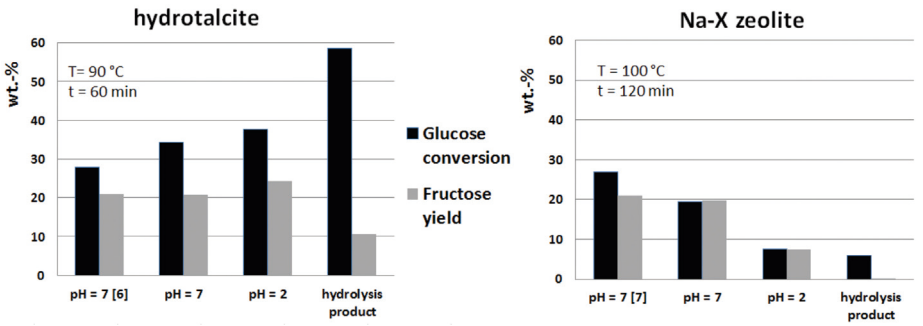

Figure 4 – Isomerization of glucose to fructose with hydrotalcite and Na-X zeolite. As educts glucose model solution in water (pH = 7), glucose solution in sulfuric acid (pH = 2) or sample from diluted sulfuric acid hydrolysis from beech wood chips are used.

Conclusion

The yields of HMF from different inulins reach almost the same level than the yields of HMF from fructose, indicating that the hydrolysis of inulin to sugar monomers proceeds fast without larger byproduct formation.

Although steam explosion opened the structure of lignocellulosic biomass, it has no positive effect on the hydrolysis of cellulose to glucose. When steam exploded biomass is used, the xylose yields during diluted acid hydrolysis of hardwoods are significantly reduced.

The isomerization of glucose to fructose is a key reaction in the process chain from lignocellulosic biomass to HMF. This reaction can be catalyzed by hydrotalcite even in acidic hydrolysis liquids.

Although hot compressed water processes achieve moderate HMF yields, they offer important benefits. Water is cheap, environmental friendly and a good solvent for sugar monomers and HMF. The use of water as reaction medium provides the opportunity for direct use of wet biomass [8].

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Lipid extraction from *Chlorella vulgaris* using electromagnetic field

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Abstract

In order to comply with criteria of green chemistry, and with the intention to mitigate the use of solvents for oil extraction from fresh microalgae *Chlorella vulgaris*, the aim of this study was to developed an efficient, economic and environmental sustainable process with the use of magnetic fields (direct current) and ultrasound bath by using surfactant (Sodium dodecyl sulfate SDS) as extraction agent, with a 20% of concentration for all the samples. Magnetic fields treatment reached a maximum oil recovery (extraction yield) of 39% (0.02 g lipid/g dried microalgal biomass) at the highest moisture (97%) and magnetic flux (316 mT). For ultrasound stimulation, the maximum oil recovery was 26% (0.014 g lipid/g dried microalgal biomass), where additionally to temperature, the power supplied (200W) to the samples had a significant effect. However, the best results for extraction yield were obtained at the lowest biomass moisture (85%).

Keywords: Magnetic field, ultrasound, microalgae, lipid extraction, SDS.

Introduction

The third generation biofuels specifically derived from microalgae are considered to be technically viable alternative energy resource [1] with a higher photon conversion efficiency (approximately 3-8% against 0.5% for terrestrial plants) which represents higher biomass yields per hectare [2], it use marginal areas unsuitable for agricultural purposes (e.g. desert and seashore lands) and thereby does not compete with arable land for food production, its production is not seasonal, can be harvested batch-wise nearly all year round, cultures can be induced to produce a high concentration of feedstock (oil, starch, biomass) and they produce high value bioproducts as proteins, polysaccharides, pigments, biopolymers, animal feed and fertilizers [2].

However, lipid extraction performed by conventional extraction methods to obtain these valuable products, involve the use of organic solvents which are highly-toxic, environmentally unfriendly, expensive and can deteriorate lipid structure [3;4]

An aqueous extraction process (AEP), which uses water as the extraction medium, is one potential vegetable oil extraction method [5], which results easy to adapt for microalgae oil extraction. One way to enhance AEP is to incorporate a surfactant, resulting in an operation known as aqueous surfactant-assisted extraction or "ASE" and the use of magnetic field or ultrasound. A surfactant is an amphiphilic compound comprised of polar and non-polar parts, thus enabling the surfactant to reduce interfacial tension (IFT) of the system. By definition, IFT is the repulsive energy between two different species of molecules [6], which is the minimum energy required to disperse one phase into another, increasing the surface area between the two phases. Moreover, reduced IFT aids disruption of the oil trapped inside the microalgae matrix and the droplets are then readily transported to the aqueous phase [7]

[5] report canola oil extraction using aqueous surfactant-assited extraction (ASE) process, obtained 80% of total oil extraction efficiency using sodium dodecyl sulfate (SDS). [8], scaled this process up to extract 150 g of ground canola and peanut using an extended surfactant in the extraction medium; however, scale-up with a commercially-available surfactant has not been reported [5]

The electromagnetic fields are a physic field produced by electrically charged objects [9], which have been used successfully in many fields such as chemical engineering, biomedical engineering and agriculture [10]. The knowledge in the electromagnetic fields effect, determines the extraction process efficiency which consists in lipid viscosity reduction by increasing temperature, which favors the contact with water present in the biomass, inducing regrouping water molecules, to facilitate their sedimentation [11]

Water treatment with magnetic fields, modifies the properties of the medium as conductivity, pH and surface tension [12], which depend of field intensity, exposure time [13], the substances present in the system [14] and pH [15].

Ultrasound-assisted extraction (UAE) is now recognized as efficient extraction techniques, used to enhance extraction yields since fifties [16]; more recently the ultrasound improves the extraction of oils from plant materials [17], mainly through the phenomenon of cavitation. The mechanical effect of UAE promotes the release of soluble compounds from the plant body by disrupting cell walls, enhancing mass transfer and facilitating solvent access to the cell content. This effect is much stronger at low frequencies (18-49 kHz) [17]

Actually, there are few studies about microalgae lipid extraction using ultrasound and magnetic fields, therefore, showing the unconventional technological possibilities for this purpose. Therefore the use of alternatives technologies makes the extraction lipid be efficient, economical and environmentally sustainable processes which are important for the production of biodiesel.

Experimental

Chlorella vulgaris was supplied by Ensenada Center for Scientific Research and Higher Education, CICESE, Mexico. The cultivation system was and horizontal tubular photobioreactor with a capacity of up to 700 liters. The culture media was made with fertilizers (nitrogen and phosphorous source), acid pH. It was used as a sunlight source sunlight and temperature of about 30-35 °C.

Microalgal pre-treatment

Prior to pretreatment, microalga paste were evaluated for moisture content by infrared moisture balance (Ohaus MB 45). The moisture content was approximately 80 wt % (wet basis).

The biomass was homogenized with a stirrer Heidolph RZR 2120® at 400 rpm for one hour. Subsequently, the samples were subjected to a cell disruption process in autoclave at 105°C for 5 minutes.

One the samples reached room temperature, it was added SDS at 20% and were mixed for 30 minutes at 1100 rpm on a Fisher Scientific ® (Isotemp) hot plate/stirrer model.

Ultrasonic assisted extraction in batch of aqueous medium

Ultrasound-assisted extraction was carried out using an ultrasonic device operating at low frequencies (25 kHz) with a 200 W ultrasonic bath (TI-H15 Elma GmbH & Co). 4 g of solids biomass was used at each experiment.

Magnetic assisted extraction in batch of aqueous medium

For the extraction process using magnetic field, a power source Statron typ 3203 GLEICHSPANNUNGSREGLERH, was connected to an electromagnet CENCO® 79637-77 (Central Scientific Company), to induce magnetic field at laboratory scale. The magnetic flux density was determined with PHYWE® teslameter.

4 g of solids biomass was used at each experiment.

Post-treatment

The slurry was centrifuged 10 minutes at 21000g (14000 rpm, Hettich EBA 21).

After centrifugation, two fractions were obtained: liquid fraction (oil and emulsion (lipids-SDS)) and solid fraction (microalgal biomass). The liquid fraction was demulsified by n-hexane:2-propanol (3:2 v/v) at 1:1 (supernatant:solvent) at room temperature.

The samples were allowed to stand in a separatory funnel for 30 minutes to separate the two immiscible phases. The bottom aqueous extraction medium was decanted; the upper organic phase (oil plus n-hexane) was desolventized at 70°C overnight until constant weight (Figure. 1) as described by [5]

The extracted lipid yield (EY), were defined as the total lipid (TL) for microalgae using a modified procedure described in [18] and extracted lipids using electromagnetic field or ultrasound (EL) (g lipid extracted/g total lipids) (Eq. 1) [5]

$$\text{Extracted lipid yield (\%)} = EL/TL*100 \quad (1)$$

The oil extraction efficiency was calculated based on the weight of extracted oil (EL) and total of solids (TS) used for the extraction process (Eq. 2)

$$\text{Oil extraction efficiency (\%)} = EL/TS*100 \quad (2)$$

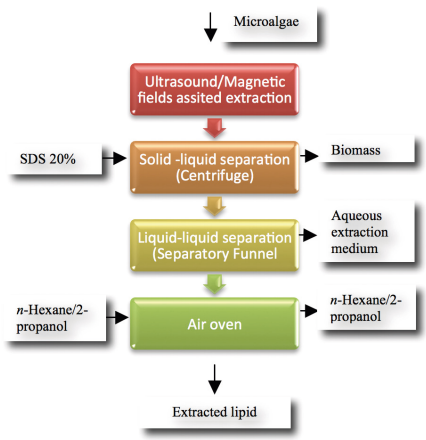

Figure. 1. Diagram of lipid extraction process.

Experimental design and statistical analysis

In order to obtain the best lipids extraction conditions using ultrasound and magnetic fields, three-factor and three-level Box-Behnken design under response surface methodology was made using extraction temperature, biomass moisture, magnetic flux (for treatments with magnetic fields) and power (for treatments with ultrasound) as factors in the efficiency of oil extraction from Chlorella vulgaris (Table 1), to determine de effect of each factor and, the most important point, their interactions.

Table 1
Level and Variables involved in both treatments

Magnetic field treatment			
Level	X_1 Temperature(°C)	X_2 Moisture (%)	X_3 Magnetic flux density (mT)
-1	30	85	55
0	45	91	185
+1	60	97	316

Ultrasound treatment			
Level	x_1 Temperature(°C)	x_2 Moisture (%)	x_3 Power (W)
-1	30	85	20
0	45	91	110
+1	60	97	200

Statistical analysis was performed with an ANOVA using Statgraphics Centurion XV (Statistical Graphics Corporation, Rockville, MD, USA) to determine de effect of each factor and, the most important point, their interactions on the extracted lipid yield and oil extraction efficiency. The adequacy of the models was determined using model analysis and coefficient of determination (R^2).

Results and discussion

Lipid extraction

In a heterogeneous system (Surfactant solution and microalgae), the surfactant monomers are adsorbed onto the particles surfaces. This reduce de IFT among the solid and two liquid phases (lipids and water) leading to detachment of oil into the surfactant solution [19,20]
[5] report good canola oil extractions efficiency with 0.02 M SDS. However, an increase in SDS concentration decreased oil extraction, because of reduced recovery of the detached lipids which was dissolved with the surfactant. In this investigation the use of SDS at 20%, represents 0.014 M SDS, which was selected for subsequent experiments.

Fitting the model

The highest Extracted lipid yield and Oil extraction efficiency obtained for magnetic field treatment was 39.64% and 0.0203 g lipid/g dried microalgal biomass, respectively and for ultrasound treatment was 26.52% and 0.015 g lipid/g dried microalgal biomass, respectively. Thus, the magnetic fields have a better effect in microalgae lipid extraction.
The Table 2, presents the statistical significance of each coefficient through p-value by an analysis of variance (ANOVA) for the Extracted lipid yield (%) response variable for both treatments.

Table 2
ANOVA for experimental results

Source	Sum of squares	Mean Square	F-ratio	*p*-Value
Magnetic field treatment (R^2=89.52%)				
A	1,0658	1,0658	10,74	0,022
B	0,1953	0,1953	1,97	0,219
C	0,4186	0,4186	4,22	0,095
AA	0,4609	0,4609	4,64	0,084
AB	0,0056	0,0056	0,06	0,821
AC	0,6006	0,6006	6,05	0,057
BB	0,00074	0,00074	0,01	0,934
BC	0,16	0,16	1,61	0,260
CC	0,2231	0,2231	2,25	0,194
Error total	0,4963	0,0992		
Total (corr.)	3,5929			
Ultrasound treatment (R2=93.65%)				
A	0,5442	0,5443	12,82	0,016
B	0,9832	0,9832	23,16	0,005
C	0,0667	0,0667	1,57	0,265
AA	0,0501	0,0501	1,18	0,327
AB	0,3256	0,3255	7,67	0,039
AC	0,2794	0,2794	6,58	0,050
BB	0,4708	0,4708	11,09	0,021
BC	0,3567	0,3566	8,40	0,034
CC	0,4814	0,4814	11,34	0,019
Error total	0,2123	0,0424		
Total (corr.)	3,348			

A:Temperature, B:Magnetic Flux (Magnetic field treatment)/Power (Ultrasound treatment), C:Moisture

These data showed that Temperature term (A) was significant ($p < 0.05$) for the lipid extraction used in both treatments. Power (B) and interaction between temperature-power and power-moisture for lipid extraction using extraction with ultrasound. These significant terms have a remarkable impact on the response, playing a dominant role in lipid extraction yield. Results observed by the p-Value significance can also be pointed out on a Pareto chart of standardized effects present on Figure 2.
These results mean that a higher temperature, a higher amount of lipids will recover, because decreases the viscosity of lipids, allowing that lipids present inside the microalgae have better interaction with external environment.
On the other hand, in ultrasound treatment, power had a significant effect, therefore extraction yield increases as the power does. This is because power, increases the internal motion of particles, allowing a better interaction between the solid phase (microalgae) and liquid phase (water-oil-SDS). However, ultrasonication provided lower oil extraction, because a high shear force could enhance the emulsion formation, reducing free oil extraction efficiency [5]
The overall results show an innovative and effective method that modify the properties of treated water such as conductivity, pH and surface tension [12] the water and the lipids characteristics present in the system, allowing the extraction. Similar results were obtained by [21], whom reported, the total lipid yield of pure hexane system=0,015 g lipid/g dried microalgal biomass, and 0.088 g lipid/ g dried microalgal biomass using sonication with a chloroform-methanol (1/1/ v/v) mixture for Botryococcus sp.

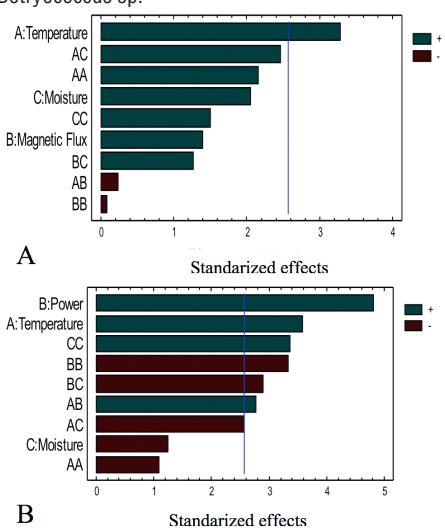

Figure 2. Standardized Pareto chart. A: Temperature, B: Magnetic Flux/power, C: Moisture for A. Magnetic field treatment and B. Ultrasound treatment.

Conclusions

aqueous surfactant-assited extraction (ASE), with the use of magnetic field and ultrasound, it's a clean process easily adapted for microalgae lipid extraction, which can be use at ambient temperature using non-toxic and non-inflammable solvents. High treatment temperature significantly impacted on lipid extraction for both treatments.
The only drawback is that the yield is still below of the conventional method. However, the use of magnetic field, ultrasound with SDS, corroborates that the method allows an extraction with good yield, less pollution and significant economic and safety benefits.

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Research as tool to create value-addedforest- based bioeconomy

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Abstract

Wood is an abundant source of biomass globally. Traditionally forest bioeconomy, albeit the term has only recently evoked, has been based on pulp and paper as well as solid wood industry. The by-products formed during these processes have been mainly burned for energy. Current research seeks to develop future forest bioeconomy concepts, in which wood biomass can be efficiently fractionated to its components, i.e. cellulose, hemicellulose, lignin and extractives. As a result the biorefinery can simultaneously produce bulk, high volume and high value, low volume products. The products can be textile fibres, nanocellulose, hemicellulose polymers, lignin chemicals and even chemicals for medical purposes.

An economically sustainable multiproduct biorefinery requires development of efficient biomass fractionation methods as well as novel end-use concepts. Current research activities are focusing e.g. on using ionic liquids (Kilpeläinen et al 2007) or deep eutectic solvents (DES) (Kroon et al 2013) as tools to fractionate biomass. Also other methods such as Pressurized hot water extraction (PHWE) (Kilpeläinen et al 2014) or alkaline oxidation treatment (AlkOx) are being explored (Kallioinen et al 2013).

Different value-added applications using cellulose in its polymeric or fibrous forms are being investigated by different groups. Technologies for production of nanofibrillated cellulose have been developed with concomitant development of various end-uses for nanocellulose. Replacing cotton with wood-derived fibres also offers a lot of possibilities. Man-made cellulosic textile fibres have been developed using ionic liquids in the IonCell F process (Sixta 2014). Totally new types of textile fibres have been developed by extrusion-based processes from nanocellulose (Håkansson et al 2014) or from pulp fibres (Salmela et al 2013). Hemicellulose and lignin can also be valorized to value added chemicals by e.g. chemical functionalization. Attempts to create hemicellulose- or lignin based barrier materials have been reported by several groups (e.g. Hult et al 2013).

Sustainable forest based bioeconomy requires research also in the primary production in different levels. Understanding of the genetics behind biosynthesis of wood components offers possibilities to screen varieties with e.g. high extractives production (Partanen et al. 2011) or varieties with more easily degradable lignin (Fagerstedt et al. 2015).

In this paper the current research topics and their potential impact to forest bioeoconomy are discussed.

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Development of selective fractionation methods for the integrated upgrade of corn cobs

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Abstract
In this work diverse fractionation strategies based on corn cob delignification were developed. An organosolv process using ethanol:water mixtures was optimized and compared with alkaline delignification (using NaOH solutions). Both methods allowed a significant delignification (79% and 94% delignification yield, respectively) with organosolv also enabling the streamlined production of a liquid phase containing an appreciable concentration (14.6 g/L) of (arabino)xylo-oligosaccharides (XOS). The above processes were also used in a combined strategy consisting of a hydrothermal processing (autohydrolysis) for the selective hydrolysis of hemicellulose followed by delignification. The first allowed to produce XOS-rich hydrolysates (67.3 g/100 g initial xylan) and a cellulose and lignin-rich solid. The further delignification processes, alkaline or organosolv, led to global delignification yields of 76% and 93%, respectively. In all cases the glucan-enriched solid residues obtained, presented high enzymatic saccharification yields (83-90%). The fractionation strategies proposed, as well as the results obtained are very promising enabling the integrated upgrading of this material.

1 Introduction
Corn residues are the most abundant agricultural residues worldwide and their chemical composition and low-cost turn them into very attractive biorefineries' feedstocks. Corn cobs are especially interesting as currently they do not have any significant use. Unfortunately, their upgrade is not straightforward, and several significant hindrances that limit their industrial use still exist. One of these bottlenecks is the development of efficient and selective fractionation processes (1) that would enable to separate their main components (hemicellulose, cellulose and lignin). Diverse physical, chemical, physicochemical and/or biological processes have been developed (2) targeting either the removal and (partial) hydrolysis of hemicellulose or lignin removal. Amongst the promising biomass pretreatment options available, hydrothermal processes such as autohydrolysis and organosolv treatments, or their combination, may lead to a selective fractionation of the major macromolecules. In this work, diverse pre-treatment strategies were used in order to produce added-value marketable products, namely xylo-oligosaccharides and lignin derivatives and easily upgradable cellulose.

2 Experimental
2.1 Raw material
Corn cobs were kindly provided by AgroMais (Golegã, Portugal). The material was dried at room temperature, shredded and then ground using a knife mill to pass 6 mm. Milled corn cobs were homogenised in a single lot and stored in plastic containers.

2.2 Organosolv treatments
Organosolv treatments were carried out in a 2 L stainless steel reactor (Parr Instruments Company, USA). Biomass was mixed with an ethanol solution 50% (w/w) to reach a liquid-to-solid ratio (LSR) of 7 (g/g) and left to react at the desired temperature for 2 h. After reaction completion, the reactor was rapidly cooled down, the liquid and solid phases were separated by pressing, and the solid phase was washed (ethanol solution) and dried.

2.3 Alkaline treatment
Alkaline treatments were carried out in 500 mL Schott flasks at 120ºC in an autoclave (30-120 min). Sodium hydroxide solutions at 1%, 1.5% or 2% (w/v) were added to biomass in order to reach a LSR of 7 (g/g). After cooling, the samples were vacuum filtered. The liquid phase was collected and the pH was adjusted to approximately 5 before analysis. The solids were washed with distilled water, dried and used for further composition analysis.

2.4 Hydrothermal treatment
Autohydrolysis treatments were carried out at 195ºC (non-isothermal operation) as described above for organosolv, using only water instead of the ethanol solution. After cooling and pressing, the liquid phase was filtered. The solid phase was washed and dried and used for subsequent delignification studies.

2.5 Analytical Methods
2.5.1 Chemical characterisation of solid samples
Raw material and processed solids were ground (< 0.5 mm) and the moisture, ash, extractives and protein content as well as macromolecular composition (after quantitative acid hydrolysis) were quantified as described elsewhere (3).

2.5.2 Chemical characterisation of the liquors
Monomeric sugars, organic acids and furan derivatives were analysed by HPLC (3). Oligosaccharides concentrations were calculated from the increase in sugar monomers, after acid post-hydrolysis and analysed by HPLC under the same conditions. Total phenolic compounds were determined by the Folin-Ciocalteu method (4) and expressed as mg GAE mL-1 (Gallic acid equivalents).

2.5.3 Enzymatic hydrolysis
The enzymatic digestibility of the untreated, and selected treated samples was evaluated based on the NREL protocol (5) using Celluclast® 1.5L and Novozyme 188® enzymes. Samples were tested, at least, in duplicate carrying out proper blank assays. The hydrolysis was carried out for 72 h (50ºC, 250 rpm). After this period, enzymes were inactivated in a water bath (90ºC, 5 min), filtered and analysed by HPLC.

3 Results and discussion
3.1 Raw material characterisation
Corn cobs presents a high content of cellulose (35.7%, estimated from glucan content), and hemicellulose (33.3 %, estimated from xylan, arabinan and acetyl groups) that together account for close to 70% of the feedstock. The cellulose content is higher than the reported in (6) but lower than the reported in (7). Lignin and protein contents are in the range usually reported (8-10). Ash content is very low, which is an advantage, as compared with straws (e.g. (11, 12). The extractives content is similar to the value reported for corn straw (3).

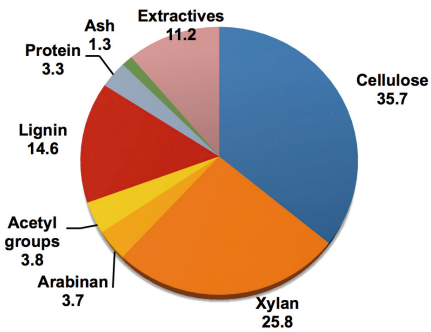

Figure 1 - Chemical composi-tion of corn cobs (% of dry weight).

3.2 One-step processes towards the delignification of corn cobs
Table 1 shows the behaviour of the three main macromolecular components of corn cobs, cellulose, hemicellulose and acid insoluble lignin after the organosolv (O.) or alkaline treatments (A.).

As expected, lignin is the main fraction affected by these treatments. For organosolv, the highest removal of lignin (79%) was attained at 190ºC. Under these conditions, lignin content in the remaining solid was 6.1%. This delignification yield was higher than that reported for other materials such as *Miscanthus* and *Buddleja davidii* (13, 14). Along delignification the concentration of phenolic compounds in the liquid stream increased with increased severity, reaching 2.25 g/100 g initial lignin for the severest condition. However, for the highest delignification yield, the recovery of phenolics was only 0.83 g/100 g initial lignin (5.72 g/L). This probably occurred because the method used for quantification of phenolic compounds mainly detects low molecular weight phenolic, whose concentration increased for more severe conditions. For alkaline treatment, delignification yield increased with the increase of NaOH concentration, as expected. Using 2% NaOH the delignification yield obtained was above 90% for all reaction times tested. In contrast, for 1% NaOH the yield was just above 50%. In general, most of the data reported in the literature only achieved these delignification yields for higher NaOH concentration (15-17). The maximum delignification yield (94%) was obtained for the treatment with 2% NaOH (1 h). However, under milder conditions (1.5% NaOH, 0.5 h) an important delignification yield (88.2%) was also obtained, leading to solids with a residual content of lignin (2.16%). Comparing with organosolv assays, alkaline treatment carried out at lower temperature (120ºC vs 190ºC), displayed better delignification results. The hemicellulosic fraction was also affected by these treatments, although this was more significant for organosolv than for alkaline treatments. Organosolv presented a sharp decrease in (arabino) xylan (Xn) content, together with the increased production of sugars, mainly in the oligomeric form ((arabino)xylo-oligosaccharides, XOS), xylose and arabinose, as well as furfural. The highest XOS yield was obtained in the same operation conditions of the highest delignification, and it corresponded to a production of 41 g XOS/100 g initial Xn. Conversely, the liquors obtained from alkaline treatment showed residual monomeric sugar content. In this case, the material balances for xylan also suggest a limited degradation of hemicellulose into XOS. Lactic acid and glycerol also appear as degradation products, whereas neither HMF nor furfural, were detected. For both organosolv and alkaline treatment, glucan was only slightly solubilized (max. 11%) together with some production of gluco-oligosaccharides (GlcOS), that reached a maximum (3.7 g/100 g initial glucan) for organosolv. Glucose yield never surpassed 1 g/100 g initial glucan and minor formation of HMF was found. This behaviour led to a higher glucan content in the solid fractions, which ranged from 40.3 to 81.2% (organosolv) and 45 to 51% (alkaline).

3.3 Two-step process towards the fractionation of hemicelluloses and lignin
Since hydrothermal processing (autohydrolysis) have shown to be an adequate method for the selective fractionation and recovery of hemicelluloses, strategies based on two-step processes (autohydrolysis followed by organosolv or alkaline treatment) were also studied. Previous optimization of autohydrolysis conditions (data not shown) enabled to hydrolyse Xn producing XOS as the main products (max. 67.3g/100 g initial Xn) whereas glucan and lignin were retained in the solid phase to yield solids with the following composition: 53.0% glucan, 13.8% (arabino)xylan, 18.8% lignin and 14.4% others (by difference).

Table 1 – Yield of lignin, (arabino)xylan and glucan derivatives for different assays

Assay		g/100 g initial KL		g/100 g initial Xn				g/100 g initial Gn			
		KL	Phe-nolics	Xn	XOS	X+A	Fur	Gn	GlcOS	Glc	HMF
O.	150ºC,2h	58.8	0.26	97.3	8.0	2.2	0.1	97.7	2.0	0.9	0.0
	160ºC,2h	53.3	0.30	98.7	8.6	1.8	0.1	97.2	1.5	0.5	0.1
	170ºC,2h	40.6	0.50	63.0	38.7	2.5	1.2	93.5	2.4	0.4	0.2
	180ºC,2h	31.3	0.52	56.8	40.4	2.0	0.8	97.9	3.7	0.1	0.2
	190ºC,2h	21.2	0.83	32.4	41.2	2.8	5.1	99.6	3.0	0.2	0.4
	200ºC,2h	22.9	1.23	14.8	14.5	3.4	10.6	102.5	2.4	0.4	0.9
	210ºC,2h	30.3	2.25	5.3	4.6	0.9	10.9	89.5	1.6	0.2	1.6
A.	1%,0.5h	35.0	n.a.	95.4	n.a.	2.8	0.0	97.6	n.a.	0.3	0.0
	1.5%,0.5h	11.8	n.a.	85.5	n.a.	0.6	0.0	88.9	n.a.	0.6	0.0
	2%.0.5h	9.9	n.a.	82.5	n.a.	0.2	0.0	91.8	n.a.	0.0	0.0
	1%,1h	32.0	n.a.	98.1	n.a.	0.0	0.0	101.8	n.a.	0.0	0.0
	1.5%,1h	10.4	n.a.	85.5	n.a.	0.7	0.0	91.0	n.a.	0.5	0.0
	2%,1h	5.6	n.a.	81.7	n.a.	1.2	0.0	93.8	n.a.	0.4	0.0
	1%,2h	44.3	n.a.	96.9	n.a.	0.3	0.0	106.1	n.a.	0.0	0.0
	1.5%,2h	12.8	n.a.	85.9	n.a.	5.6	0.0	91.1	n.a.	0.3	0.0
	2%,2h	8.5	n.a.	77.7	n.a.	1.8	0.0	88.8	n.a.	0.4	0.0
H.O.	160ºC	52.8	0.77	67.0	30.1	1.2	0.4	101.0	0.5	0.1	0.0
	170ºC	48.0	1.44	56.8	31.2	1.6	1.2	99.5	0.4	0.1	0.0
	180ºC	42.3	1.21	44.4	36.7	4.0	2.7	96.4	0.5	0.1	0.1
	190ºC	34.7	1.05	23.4	35.0	9.0	3.8	94.1	0.7	0.1	0.3
	200ºC	40.8	1.38	24.3	13.1	9.9	11.6	94.7	1.2	0.2	0.6
H.A.	1%,0.5h	35.3	n.a.	71.1	n.a.	0.0	0.0	102.5	n.a.	0.0	0.0
	1%,1h	32.0	n.a.	48.9	n.a.	0.0	0.0	107.6	n.a.	0.0	0.0
	2%,1h	10.4	n.a.	42.4	n.a.	0.2	0.0	92.8	n.a.	0.0	0.0
	1%,2h	34.0	n.a.	67.7	n.a.	0.1	0.0	106.3	n.a.	0.0	0.0

O. - organosolv; A. - alkaline; H.O. - autohydrolysis+organosolv; H.A. - autohydrolysis+alkaline; KL – Klason Lignin; Xn - (arabino)xylan; XOS - (arabino)xylo-oligosaccharides; X + A – Xylose + Arabinose; Fur - furfural; Gn – glucan; GlcOS - gluco-oligosaccharides; HMF - 5-hydroxymetilfurfural; n.d.- not available

3.3.1 Autohydrolysis followed by organosolv

In this two-stage process (Table 1, H.O.) the maximum delignification of pre-treated solid reached 65.4% (190ºC). Temperature increase favours delignification yield, although it was possible to attain 47.2% at the lowest temperature (160ºC). However, considering the global delignification yield, it can increase up to 75.6% (190ºC). A comparable lignin removal was already reported for Eucalyptus globulus after similar sequential treatments (18). Although a major part of hemicellulose fraction was already removed by autohydrolysis the delignification step reduced xylan content even further (to 24% of initial in the pre-treated solid) releasing some monomeric pentoses (max. 9.9 g/100g initial Xn) and a quite remarkable concentration of XOS (13.1 g/100g Xn). The glucan fraction still remains virtually unaffected for all assays, a favourable trait as compared to other reports (19). The optimal condition for the second step of the combined treatment is in the temperature range of 180-190ºC, which produces a solid phase containing 73% glucan, 10% xylan and just 10% lignin.

3.3.2 Autohydrolysis followed by alkaline treatment

In the strategy encompassing autohydrolysis followed by alkaline treatment (Table 1, H.A.), most of the glucan and a considerable fraction of xylan were recovered in the solid phase and as expected, lignin was the main component affected. Solids presented a glucan content ranging from 70 to 77%, xylan around 15% and minor lignin content (min. 2.8%). The delignification yield reached 90% (2% NaOH, 1 h). Comparing the direct alkaline treatment with this two-step process, it was noted that in general the delignification yield is higher for the later except for the maximum attained (94.4% vs 92.7% for the two-step process at the same conditions). These yields are higher than the reported for a two-step treatment of Miscanthus using aqueous ammonia followed by NaOH and oxidants (20). Similarly to the one step alkaline process, no major sugar monomers were detected in the liquid phase, although a significant amount of formic acid was produced, together with some glycerol and lactic acid (data not shown).

3.4 Enzymatic hydrolysis

Figure 2 shows the enzymatic digestibility of both raw and treated corn cobs. As expected, the raw material presented a low enzymatic digestibility (38.6%), and all treatments improved it, especially the organosolv treatment (210ºC) that increased the enzymatic digestibility up to 96.9%, in what seems to be dependent on treatment severity.

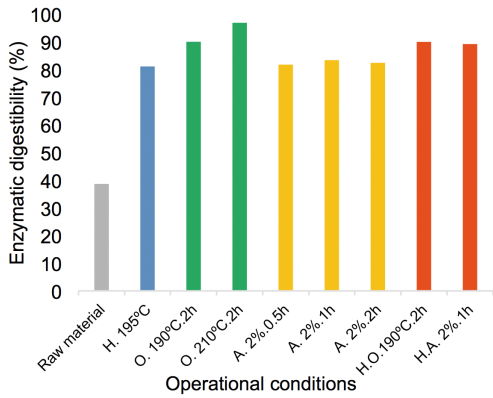

Figure 2 - Enzymatic digestibility of raw corn cobs and of selected solids resulting from several assays:
H. - autohydrolysis; O. - organosolv;
A. - alkaline treatment;
H.O. - autohydrolysis + organosolv;
H.A. - autohydrolysis + alkaline treatment

Conversely, no changes on the enzymatic digestibility were observed for alkaline treatments with increased severity. Combined treatments increase digestibility further as compared to the autohydrolysis treatment, but they do not surpass the effect of the direct organosolv treatment. These results can be explained by the amount of (residual) xylan and lignin present in the samples.

4 Conclusions

The proposed set of strategies showed that direct organosolv treatments enabled a significant delignification and hemicellulose removal, yielding a liquid phase rich in added-value phenolic compounds and XOS that can be easily purified. The resulting solid phase was clearly enriched in glucan. Alkaline treatments were also very efficient in lignin removal, producing a cellulose and hemicellulose -rich solid. The two-step processes enabled to obtain a high yield of XOS in the first step and a solid fraction enriched in lignin and glucan. This later fraction was efficiently delignified, either by organosolv or alkaline processes to produce lignin derivatives, with high yield and solids more enriched in glucan. Promising saccharification yields of the solids were also obtained, aiming at a complete valorisation of the fractions and integration in a biorefinery framework.

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Using magnetic resonance imaging to monitor process flows of multiphase systems

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Abstract

We describe the use of magnetic resonance imaging (MRI) to study the flow of fiber systems. With the advent of permanent magnet-based MRI systems, it is increasingly becoming accessible for use in real industrial processes. We focus on MRI to study the flow of cellulosic suspensions that are used in the manufacture of paper and ethanol. Such suspensions are opaque, exhibit complex rheology, and, in the case of ethanol production, are undergoing chemical reactions. We study the flow of cellulose suspensions in a pipe connected to a batch reactor. We will show how these processes can be optimized. Once enzymes are added liquefaction begins and the yield stress decreases with time. These measurements benefit the operability and design of these multiphase reactors.

Introduction

Magnetic resonance imaging (MRI) is a non-invasive technique that has enabled imaging of a wide variety of systems that are of interest medically, biologically and industrially. Generally, it is known for imaging of static systems, such as brain scans. A less common application of MRI is in the study of flow as well as other transport processes (1-18). For example, it is possible to measure the motion of a particular constituent as a result of diffusive processes. Likewise, MRI can be used to study flowing systems. In particular, MRI is capable of determining the velocity of a flowing material. For this application, the typical system is a slurry with a suspending fluid and a solid particulate phase. The MRI measurement is actually based on proton imaging so that the velocity of the liquid phase is measured. This aspect of MRI makes it especially amenable to measurements that involve complex industrial systems. For solid phases with high aspect ratios, such as fiber suspensions, a high solids loading is 20% by weight fiber. In such a case, the liquid comprises 80% of the system and provides a liquid substrate that produces an adequate MRI signal for measurements. In fact, with low aspect, spherical particle suspensions where the concentration can be quite large, about 50%, MRI has been successfully used to image velocities and concentration distributions of the solid phase.

Other measurement techniques generally impose limitations on the type of system being studied. Laser Doppler Velocimetry or Particle Image Velocimetry both require transparent systems. These can either be dilute or systems for which the particulate and the liquid phases have matched indices of refraction. Ultrasonic Pulsed Doppler Velocimetry works with opaque systems but multiple scattering effects can produce errors in the measurements. For the study of particulate systems effective use of MRI does not require that the system be transparent. Further, like the other techniques, MRI is non-invasive. It is, uniquely, can provide information about the individual chemical constituents in a system. This combination has lead our group to apply MRI to study diffusion, absorption, rheology, fluid mechanics and suspension mechanics (1-18).

Experimental

Over the course of our work, there has been a significant evolution in the basic MRI unit. In our earliest work, the MRI unit used a superconducting magnet, installed in a shared use faculty and far from our laboratories (1-10). Our work required that our flow system was constructed so that it could be assembled and used over a 24 hour period and then removed from the MRI unit as it was prepared for the next user. For a short period of time our work used an electromagnet-based system. Although this was housed in our laboratory, it was limited. Over the last decade, permanent magnet-based MRI units have become available (15-18). These systems easily fit in a typical fluid mechanics laboratory. All MRI measurements were obtained using a spin echo sequence. In our case, we used a 1 Tesla permanent magnet (Aspect Imaging, Hevel Modi'in Industrial Area, Shoham, Israel) with 30 G/cm peak gradient strength. The radio frequency coil was a solenoid with 3 turns, encasing a cylindrical volume 38 mm in diameter and 36 mm long.

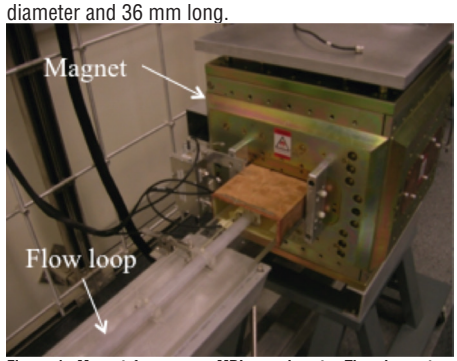

Figure 1. Magnet for process MRI experiments. The pipe enters the magnet as shown in the photo and passes through the coil that applies the magnetic field gradients, which provide the spatial resolution.

Figure 1 shows MRI system that we have used for these studies. The "Flow Loop" in Figure 1 points to the pipe that goes through the magnet. The entire flow system consists of a tank, a mixer used to agitate the slurry, a pump and a pipe that goes though the magnet which has magnetic gradient coils. The pressure drop is also measured. The combination of the MRI determined velocity and the pressure drop allows the radially resolved viscosity to be determined. Because the measurement is made in situ, the technique can be used for time resolved rheological measurements which allows the liquefaction process to be followed.

The materials consisted of a delignified cellulosic fiber, commercially called Solka Floc (Solka-Floc, International Fiber Corp., Tonawanda, NY) and pretreated corn stover, **Table 1.**

Table 1. Characteristics of the Fibers.

Fibers	Length (mm)	Width (μm)	Aspect Ratio
Solka-Floc 200EZ	0.207	26.4	7.8
Solka-Floc C100	0.349	31.7	11
Pretreated Corn Stover[1]	0.001 - 2		

Results and Discussion

The basis for the argument that liquefaction can be continuously monitored is that MRI can be used to continuously monitor the yield stress or other rheological properties during liquefaction. As described above, two measurements are made: velocity profile and pressure drop. Using the conservation of linear momentum for steady flow in a pipe without reference to any specific constitutive relation, the shear stress, τ, at a radial position, r, is $\tau(r)= (\Delta P/2L)r$, where $\Delta P= P_{upstream}-P_{downstream}$ is the pressure drop over the length L.

Using the velocity profile, $v(r)$, it is possible to calculate the radially resolved shear rate using $\dot{\gamma} = |dv/dr|$. The viscosity, , can be calculated at any point in the pipe using $\eta(r) = \tau(r)/ \dot{\gamma} (r)$. By finding the shear rate that corresponds to each radial position, it is possible to obtain the usual representation of the viscosity as a function of shear rate.

For fiber suspensions, typical velocity profiles show a region near the center of the pipe where the velocity is practically flat, such as that shown in **Figure 2**. Suspensions showing such behavior are considered to have a yield stress, τ_y. The value of τ_y y is found by determining the radius at the edge of the flat region, Rplug, typically where the velocity fall below 0.99 of the maximum velocity, and calculating the shear stress at that point.

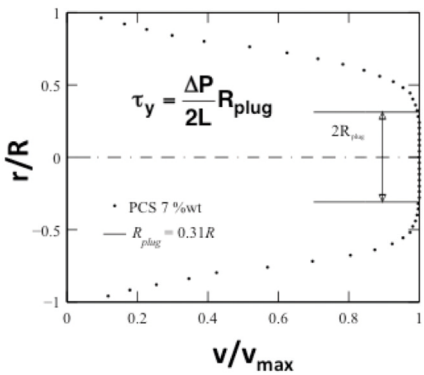

Figure 2. Velocity profile of a pretreated corn stover suspension.

Data for the yield stress of all of the suspensions show that the MRI based system provides data that have fewer errors and are more reproducible than those obtained using traditional rheometers (16). There are two principal reasons for this. First. typical rheometers are parallel plate, cone and plate or, for yield stress measurement cup and vane. In all cases, the nominal fiber dimension is comparable

[1] Pretreated corn stover samples are extremely heterogeneous. Values were obtained only for the length and a drawn from the literature (19)

to the characteristic separation between the two surfaces that create the shear flow. This gives rise to geometrical effects in the measurements which are increasingly severe as higher concentrations of fibers are used. Even if capillary viscometers are used, they are seldom several centimeters in diameter, such as that used here, and, hence also subject boundaries that confine the system so that it is not representative of processing conditions. Second, in order to make a measurement, samples must be removed from the process stream and loaded in the rheometer. For many reasons, the sample may undergo changes and the final measurement may not be representative of the system in the process stream.

MRI overcomes both of these limitations. The measured property is the in situ property. Further, the dimensions of the viscometer ensure that the length of the fiber is smaller than the diameter of the pipe. Of course, wall slippage may be present in all viscometers. It is well known that fiber suspensions may form a fiber depleted water layer near a solid boundary. At low velocities in pipe flow, for example, the water layer acts a lubricating layer and the fibrous plug slips along the wall. Such a phenomenon can be observed with MRI measurements but must be indirectly inferred with other viscometers.

MRI measurements definitively show that liquefaction can be determined on a time scale that allows process control strategies to be implemented. In particular, by monitoring the yield stress in during semi-batch operations, it is possible to determine optimal times to introduce additional fibers into the reactor. This, in turn, permits high volume loadings of solids. Rather than trying to operate hydrolysis processes with high volume loadings of what are essentially solid like materials, the procedures identified using MRI allows similar loadings with clear advantages. First, pumping requirements are reduced. The demand to handle systems that might only be pumped with an extruder goes away. Less costly equipment may be used and the energy demand of the equipment will be less. Second, any limitations due to mass transfer will be significantly reduced. Mixing enzymes is less likely to result in shear damage and the mixing will be more uniform.

Conclusions

MRI provides a unique tool to analyze liquefaction of cellulosic suspensions. This leads to the ability to optimize processes in terms of capital and operating costs.

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Solid state fermentation of chemically untreated sugarcane bagasse for fungal production of single cell oil as biodiesel feedstock

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Abstract

Fungal lipids - single cell oils (SCOs) intended for biodiesel have to be produced at low cost and it may be achievable by the use of renewable carbon sources employing solid state fermentation (SSF). The present study evaluates the one-step fungal conversion of lignocellulosic biomass into biodiesel feedstock without added enzymes or chemicals, in a consolidated bioprocessing approach. It was brought about by a single microbial strain, Aspergillus terreus IBB M1 - a tropical oleaginous lignocellulolytic isolate of mangrove fungus. The sugarcane bagasse waste generated in a local sugar mill after cane milling and juice extraction was used as the SSF substrate without any thermochemical pretreatment. The moistening agent contained mineral salts formulated by considering the marine habitat of the fungus. The fungal strain was able to utilize the untreated bagasse for lipid accumulation as revealed from SCO production in wide mouth flasks yielding 27.8 mg/g of the substrate. The fatty acid profiling of SCO indicated the abundance of oleic acid as the sole monounsaturated fatty acid at 11.8 % level. The major polyunsaturated fatty acids were found to be linoleic (8.8 %) and gamma linolenic acid (7.45 %). A higher content of oleic acid along with the absence of polyunsaturated fatty acids possessing ≥4 double bonds in SCO is expected to impart good biodiesel fuel quality from *A. terreus* IBB M1. It has the potential to be an alternative source for both petro-diesel and plant oil derived biodiesel because SSF configuration has considerable advantages in process operation. Furthermore, the absence of pretreating the agro waste can make the entire process green and cost effective rendering it amenable for further development.

Introduction

The cost effective production of SCO as biodiesel feedstock could be achieved by using agro-industrial residues and a cheaper cultivation process of solid state fermentation (SSF) [1, 2, 3]. In SSF the solid matrix could be the source of carbon and other nutrients, or an inert growth support with impregnated nutrients. A number of factors give SSF an extra advantage over the submerged process e.g. the lower cultivation costs and energy requirement, higher product yields, less wastewater generation, reduced transport costs, potential environmental benefits by utilizing solid agro-industrial wastes [4]. Microbial lipids produced in SSF cultures hold the potential to become tomorrow's source of biodiesel by means of improvement of lipid yield in the SSF process [5].

The mangrove fungi are known to contribute to the intense carbon processing of this ecosystem and oleaginous isolates would be ideal candidates for lipid production in a 'consolidated bioprocessing', a new approach with potential for the direct bioconversion of lignocellulosics into biofuel of interest without added enzymes or chemicals [6].The present study was conducted with an objective of preliminary evaluation of the consolidated bioprocessing approach for the direct bioconversion of raw untreated sugarcane bagasee into lipids as biodiesel feedstock. Substrate was without any thermochemical pretreatment. The SSF process was brought about by a single microbial strain, Aspergillus terreus IBB M1 - a mangrove isolate of an oleaginous fungus capable of both utilizing the raw, insoluble lignocellulose and accumulating lipids [7, 8]. The lab scale solid state cultivation of fungus was carried out in wide mouth flasks. SCO was extracted from fermented biomass and evaluated on the basis of fatty acid profiling for biodiesel.

Experimental

Sugarcane bagasse (SCB), an agro-residue remaining after milling cane and extracting the juice was collected as dried material from a local sugar factory. Samples were treated with water to remove soluble sugars, dried and milled to 1mm particle size. A. terreus IBB M1 was grown at 30°C on malt extract agar plates for 6-7 days. Spore inoculum was prepared in sterile Tween-80 solution (0.1%, w/v) from the culture. SSF experiments for evaluation of bagasse as substrate were conducted in 250 mL wide mouth Erlenmeyer flasks each containing 5 g SCB and 20 mL of moistening agent composed of (in g/L) 0.5 NH_4Cl, 1.5 yeast extract, 15 NaCl, 5 Na_2HPO_4, 7 KH_2PO_4, 1.5 $MgSO_4.7H_2O$, 0.1 $CaCl_2.2H_2O$, 0.08 $FeCl_3.6H_2O$, 0.01 $ZnSO_4.7H_2O$ with 0.1 mg/L each of $CuSO_4.5H_2O$, Co $(NO_3)_2.6H_2O$, $MnSO_4.5H_2O$. The flasks were steam sterilized, cooled and inoculated with 1mL of spore suspension per g of substrate. The incubation was at 30°C for 8 days under static conditions.

The solid state fermented mass was harvested from the flasks and dried to constant weight in a hot air oven. Extraction and gravimetric estimation of total lipids was carried out after cryo-pulverization of biomass followed by solvent extraction with chloroform: methanol (2:1, v/v) [7, 9]. The SCO yield was reported in mg per gram of substrate. The fatty acid composition was analyzed by standard gas chromatography based method [10]. Identification of peaks was performed by comparison with authentic fatty acid methyl ester (FAME) standard mixture (AOAC 996.06 Standard; Restek Corp., USA) and quantifications done on the basis of their specific peak areas or using internal FAME standards.

Results and discussion

The fungus was able to grow and utilize untreated sugarcane bagasse for lipid accumulation resulting in SCO production of 27.8 mg/g of substrate after 8 days cultivation. This suggests the potential suitability of A. terreus IBB M1 in the consolidated bioprocessing of sugarcane bagasse for production of lipids by SSF. Most of the earlier studies have employed different strategies e.g. thermo-chemical pretreatment of substrate, externally added enzymes and additives such as other substrates. It has to be noted that in the present study, sugar cane bagasse was used without any chemical or enzymatic pretreatment. Besides, no additional substrate has been used and therefore, the yield obtained is significant. In a study by Peng and Chen (2007), different endophytic fungal isolates produced SCO ranging from 19 to 42 mg/g initial dry substrate after 10 days culture on the solid state medium composed of steam-exploded wheat straw supplemented with wheat bran [11]. In another report, Microsphaeropsis sp. produced 42 mg/g dry substrate composed of steam-exploded wheat straw and wheat bran without added cellulase in 10 days [2]. Lin et al. (2010) investigated direct microbial conversion of wheat straw into lipids by a cellulolytic oleaginous fungus, A.oryzae A-4 in solid state fermentation. The strain produced 28.5 mg/g lipids in 6 days using wheat straw which was further improved by employing fractional factorial experimental design and additive substrates, wheat bran and bagasse [12]. Fatty acid analysis revealed that SCO contained 11.51 % oleic acid (C18:1) as the major monounsaturated fatty acid Linoleic (C18:2n6c, 8.8 %) and gamma linolenic acids (C18:3n6, 7.45 %) were the main polyunsaturated fatty acids.

Biodiesel with high monounsaturated fatty acid content [e.g. oleic acid (C18:1)] has better characteristics with respect to ignition quality, nitrogen oxide emissions, fuel stability and flow properties. The percentage of unsaturated fatty esters having ≥ 4 double bonds affects oxidative stability and increases viscosity thereby affecting the quality of biofuel. [13]. Our studies showed the presence of desirable monounsaturated fatty acids along with absence of undesirable highly polyunsaturated fatty acids which overall suggest the potential suitability of the produced SCO as biodiesel feedstock.

Conclusion

In conclusion, the direct conversion of chemically untreated sugarcane bagasse into SCO was demonstrated via SSF using an oleaginous mangrove fungal strain A. terreus IBB M1. The SCO was found to be suitable as biodiesel fuel feedstock on the basis of fatty acid profile. The studied solid state cultivation process is cost-effective and environment-friendly which can be further tuned by design of experiments methodology.

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Improvement of the lignocellulose hydrolysis by use of auxiliary enzymes

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Abstract
Bioethanol production basically requires three stages: pre-treatment, hydrolysis and fermentation. In this process, efficient hydrolysis or saccharification of lignocellulosic residues depends on the concerted action of cellulases and auxiliary proteins. In this work we have isolated fungal proteins having auxiliary activity in order to be able of designing balanced and efficient mixes of cellulase and auxiliary enzymes. Auxiliary proteins from rot fungus *Gloeophyllum trabeum* were identified by proteomic analyses of fungal proteins that showed cellulolytic enhancement activity on wheat straw. Among other hydrolytic and oxidative enzymes, a polysaccharide mono oxygenase (PMO) and a xylanase were identified. Corresponding genes were isolated and sequenced. GtPMO1 and GtXYL1 were produced in the yeast *Pichia pastoris* and purified. Different combinations of these enzymes with Celluclast 1.5L were assessed in reactions with wheat straw as substrate, for production of reducing sugars. In these experiments recombinant swollenin (TrSWO) from *Trichoderma reesei* was also included. Results indicate that GtPMO, GtXYL1 and TrSWO effectively improve the activity of conventional cellulase in 30-50%, depending on the concentration of enzyme used.

Introduction
Lignocellulosic residues are the most abundant renewable resources in the world and their utilization provides an opportunity to advance toward and maintain the sustainable development in the earth. The complex structure and recalcitrance of the lignocellulose convert the manufacture of bio-products derived from lignocellulosic residues in a technical challenge to overcome. Pretreatment methods are applied to loosen up the fibers is a first step in the process, followed by the breaking down of the polymer constituents, cellulose and hemicellulose, into monomer sugars available for microbial fermentation. Despite of cellulases were described over 50 years ago, developments are still necessary in order to improve the enzymes efficiency and to decrease the impact of this stage in the whole process's economy.

Besides cellulase, auxiliary proteins should be included in the reactors in order to ensure the complete conversion of the insoluble polymer (1-4). This group includes hemicellulases, expansin-like proteins, ligninases and oxidases. Auxiliary enzymes accomplish their role by one the three mechanisms: 1) by contributing to the loosening of the plant cell wall structure; 2) by degrading non-glycosidic cell wall components, such as lignin, or 3) by degradation of non-cellulosic polysaccharides, such as pectin or xylan. By removing these cell wall components, the cellulose becomes more accessible to the cellulolytic enzymes.
Swollenins belong to the family of the plant expansins, a protein family that can loosen the plant cell wall structure without hydrolyzing any of its components (6). Swollenin from *T. reesei* (SWOI) was discovered in 2002 and its effect on cellulose deconstruction was proved (7). Swollenins from other fungi have been identified having similar effect (8, 9).

In a second group of auxiliary proteins are the hemicellulases. Hydrolysis of hemicellulose is essential for accessing the cellulose during the degradation. Given the heterogeneous composition of the hemicellulose composition in lignocellulose, different hemicellulases must be considered as auxiliary protein, depending on the origin of the biomass. In particular (10). These differences imply that enzymes added to the cellulolytic mixtures must necessarily consider the nature of the substrate to be hydrolyzed, and emphasize the relevance of the design step in the production of substrate-specific cellulolytic mixtures.

Polysaccharide monooxygenases (PMOs, former GH61s) contribute to the cellulose deconstruction by mechanisms completely different to those exhibited by glycoside hydrolases. Studies have revealed that PMOs are metal ion dependent monooxygenases (11-13). Although at first the structure analysis led to some confusion, it was finally determined that the metal ion needed for the enzyme's activation was a copper (II) (14-16), which is bound by a set of three highly conserved residues: two histidines and one tyrosine. This structure has been shown to be critical for the enzyme activity (12, 14). The new finding of GH61 been a copper-dependent Polysaccharide Monooxygenase led finally to the reclassification of this family on the Carbohydrate-Active Enzymes Database (CAZy) to an Auxiliary Activity family number 9 (AA9) (17).

Brown Rot Fungi decompose wood cell wall by an oxidative mechanism, in which hydroxyl radicals selectively depolymerize cellulose (18). The cellulolytic enzymatic system in *G. trabeum* has been investigated for characterization of the individual enzymes. A processive endoglucanase and three GH10 xylanases have been cloned and characterized (19-21). The *G. trabeum* genome possesses four PMO genes (22). Jung et al (2015) reported the characterization of a recombinant *G. trabeum* PMO (23), demonstrating the synergistic effect when working in combination with an endoglucanase and a xylanase from the same fungus.
In this work, we have further advanced in the characterization of the ability of three auxiliary proteins, GtPMOI, TrSWOI and GtXyl10g to improve the effect of a commercial preparation of cellulase, Celluclast 1.5L, in the hydrolysis of wheat straw. Optimized combinations of these enzymatic complexes can contribute to reduce cellulase loading in the cellulose conversion processes.

Experimental
Fungus culture and strain maintaining
The fungus *Gloeophyllum trabeum* was isolated from a Chilean native forest by the Laboratory of Biodegradation and Wood Preservation of the Faculty of Forest Sciences of the University of Chile. The fungus was cultured at 28 °C in liquid medium containing 1% milled wheat straw as carbon source.

Protein identification in *Gloeophyllum trabeum* cultures
Proteins in supernatant of *G. trabeum* cultured in medium with wheat straw as only carbon source were concentrated by ammonium sulfate precipitation. This extract was fractionated in an Akta Purifier FPLC (GE Heathcare) system by size exclusion chromatography (SEC). Each individual fraction was used for hydrolysis of alkali-pretreated wheat straw. The hydrolysis reactions were conducted in 50 mM sodium acetate pH 5, using 50 mg mL-1 of fungal cellulase or Celluclast (Novozyme), equivalent to 5 PFU g-1 substrate, and 2.5 g L-1 Tween 20. Hydrolysis was conducted at 50°C for 48 h. Reducing sugars were measured by the DNS method (24).

Proteomic analysis using mass spectrometry
After SEC separation, proteins showing synergistic behavior with Celluclast 1.5L in wheat straw hydrolysis were combined in two pools. Pool 1 was concentrated by ultrafiltration, lyophilized, suspended in buffer Tris-HCl 20 mM pH 7 and subjected to proteolytic digestion and liquid chromatography (LC)/MS/MS). Proteins in Pool 2 were resolved by SDS-PAGE and proteins bands were excised from the gel and analyzed by in-gel digestion and LCMS/MS. Peptide mass list was compared with a list of theoretical peptide mass using the bioinformatic tool Mascot v2.4 (www.matrixscience.com) in combination with NCBInr database v20130315 (23778171 sequences; 8170669966 residues) and filtered using a fungal taxonomic filter (NCBInr fungi, 1752624 sequences). Significance of the data was confirmed by use of the score generated by the search algorithm MOWSE (Mascot).

***G. trabeum* cDNA synthesis, gene cloning and recombinant expression**
Total RNA was extracted from mycelia grown on solid medium as described (25). The RNA extraction was carried out in a mortar and pestle using Ambion® TRIzol® Reagent following the manufacturer's indication. cDNA synthesis was prepared using Invitrogen reverse transcriptase Superscript III following the manufacturer's indications.
Degenerated primers were designed from conserved regions present on fungal genomes, available at NCBI and EMBL-EBI databases. Starting from the partial ORFs cloned, the complete sequences were identified in the genome available in TIGR (26), which were used for specific primers design. PCR were performed using Phusion High-Fidelity DNA polymerase (New Englands Biolabs), according to the provider's guidelines. The PCR product was cloned into pGEM-T Easy vector, transformed in E. coli Top10, and sequenced by Macrogen (Korea). The mature sequences encoding for GtPMO1, GtXylI and TrSWOI were introduced into the *P. pastoris* expression vector pPIC9K using standard cloning protocols. Recombinant expression of the *G. trabeum* xylanase and PMO was carried out using the Multicopy Pichia Expression Kit (Life Technologies) following the supplier recommendations. GtPMO1 was purified by Ion Metal Affinity Chromatography; GtXyl1 and TrSWOI were purified by anionic exchange chromatography in Q Sepharose. Fractions containing the proteins, as judged by SDS PAGE, were combined, concentrated and desalted.

Wheat straw hydrolysis by Celluclast 1.5 and the auxiliary proteins
GtXylI, GtPMO and TrSWO were combined with Celluclast 1.5L (Novozymes) in hydrolysis reactions containing 1% (w/v) pretreated wheat straw. The hydrolysis reactions were conducted in 50 mM sodium acetate pH 5, using 1 mg Celluclast per g wheat straw, 2.5 g L^{-1} Tween 20 and 0.006 mg mL^{-1} β-glucosidase, at 50°C with orbital agitation by 48 h. Reducing sugars were measured with DNS. All the results were analyzed by ANOVA in order to ensure the statistical significance of the differences with $p<0.05$.

Results and Discussion
Fractioning of extracellular G. trabeum proteins and wheat straw hydrolysis
The fungus was cultured in wheat straw as only carbon source by seven days. Concentrated extracellular proteins were fractioned by filtration chromatography. Each eluted fraction was tested for hydrolytic activity on CMC, Birchwood xylan and pretreated wheat straw, confirming production of cellulases and xylanases (Fig. 1) as reported previously (). When proteins in the fractions are combined with cellulases of a commercial preparation (Celluclast™), proteins in fractions 23-26 (Pool 1) and 30-33 (Pool 2) exhibited important synergism with the commercial cellulases. In order to identify the proteins present in those fractions, proteins in Pool 1 and Pool 2 were submitted to proteolytic cleavage and the resulting peptides were identified by liquid chromatography and mass spectrometry. Results are summarized in Table 1. The most abundant proteins present in these fractions resulted to be two a GH10

xylanase, an alcohol oxidase, and two polysaccharide monooxygenases. In order to explore the effect of combining these enzymes in vitro, sequences encoding for GtLPMO1 and XylI were isolated and cloned into the *Pichia pastoris* KM71 expression system.

Recombinant expression

Genes encoding for GtPMO and Xyl I were isolated from the cDNA of *G. trabeum* and cloned into the *P. pastoris*. Blast analysis of these sequences reveals 100% coverage and a 99% identity with the already submitted sequences. The fragment encoding for the mature PMO protein was fused to the *S. cerevisiae* α peptide in vector pPIC9K downstream the signal sequence of S. cerevisiae α-peptide. The recombinant plasmids were used to transform P. pastoris KM71. The proteins were purified from the culture medium (Fig. 2) and used to hydrolyze wheat straw in combination with recombinant *T. reesei swollenin*.

Lignocellulolytic activity and synergism with Celluclast

The auxiliary activity of GtXyl1, TrSwoI and GtLPMO1 was evaluated by study the effect of each protein on the wheat straw hydrolysis by Celluclast 1.5L. Results are shown in Table 2. Consisting with results previously reported, TrSWOI and GtPMO1 showed negligible activity on wheat straw. When mixed with Celluclast 1.5 L reducing sugars production resulted in a variable increment, depending on the auxiliary protein concentration used. The better effects were reached with 50 µg/mL TrSWOI, and 5 ug/mL GtXylI, being about 50% higher in presence of the auxiliary protein compared to the Celluclast 1.5L alone. GtLPMO1 increased the release of reducing sugars in 47 % at the highest tested concentration.

The importance of xylanases in lignocellulose degradation has been very well recognized since long time ago, due to the requirements for recovering the pentoses for a more rentable process. However, in the last five years the role of the xylanases in the deconstruction of the hemicellulosic matrix for improvement of the cellulose accessibility has remarked the accessory character of these enzymes. In 2014 the recombinant expression of a thermostable xylanase from *G. trabeum* was reported (21). That enzyme and the GtXylI described in this work share significant amino acid sequence similitude, so is probable that GtXylI and Xyl10g are the same protein. However, the specific activity of our xylanase on birch xylan is 14.600 U/mg of protein, much higher than the 180 U/mg of protein reported by Kim (21). It will be interesting to identify the small sequence features responsible of this functional difference.

Conclusions

The strategy utilized conducted effectively to the identification of auxiliary enzymes for degradation of wheat straw. Experimental results have shown the auxiliary role of GtLPMO1, GtXyl1 and TrSWOI on the hydrolytic activity of wheat straw by a commercial cellulase, implying that combination of the three proteins could result significant improvement of the efficiency of the cellulose conversion processes.

Acknowledgements

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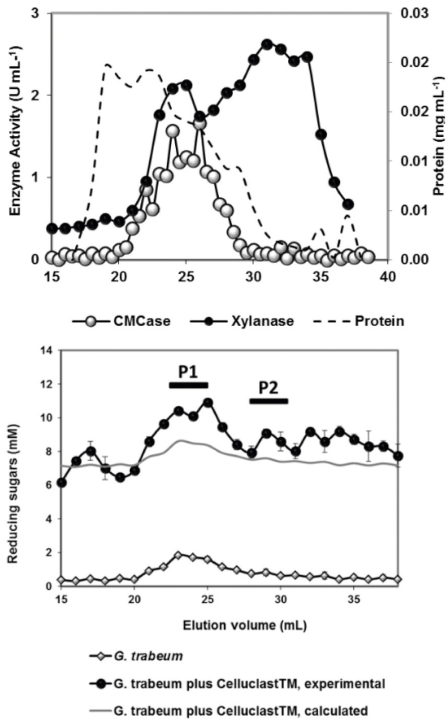

Fig.1. Fractioning of extracelular proteins from *G. trabeum* cultured in wheat straw by Size Exclusion Chromatography. A) Activity on carboxymethyl cellulose, birch wood xylan of the eluted proteins. Protein concentration was measured by the method of Bradford. B) Hydrolytic activity on alkali-pretreated wheat straw. The hydrolytic activity was analyzed in the fractions individually or in combination with Celluclast 1.5L. After 16 h at 50°C reducing sugars were measured by the DNS method.

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Table 1 Main proteins identified in fraction of *G. trabeum* extracellular proteins exhibiting synergy with Celluclast™.

Activity (NCBI access code)	CAZY Family[b]	Score Global[c]	emPAI[d]	EC number	Reference
Endoxylanase (gi|521722511)	GH10	458	1.12	3.2.1.8	Sydenham et al. 2014
Alcohol oxidase (gi|521719815)	AA3	918	0.95	1.1.3.13	This work
Endoxylanase (gi|521722509)	GH10	452	0.53	3.2.1.8	Sydenham et al. 2014
Lytic monooxygenase (gi|339219008)	AA9	142	0.17	1.-.-.-	Jung et al 2015
Lytic monooxygenase (gi|238583365)	AA9	87	0.14	1.-.-.-	This work
Endoglucanase (gi|296803329)	GH5	140	0.1	3.2.1.4	Cohen et al. 2005

Symbols: a: Access code NCBI; b: CAZY classification (www.cazy.org); c: Score is calculated as -10*Log(P), where P is the probability of the coincidence being a random event (scores > 48 indicate identity or extent homology, p<0.05); d: emPAI: Exponentially Modified Protein Abundance Index (Ishihama et al. 2005).

Table 2. Effect of auxiliary proteins GtXyl!, GtPMO1 and TrSWOI in the wheat straw hydrolysis by Celluclast 1.5L.

Protein	Concentration (µg/mL)	Reducing Sugars (mg/mL)		Increment (%)
		Auxiliary protein alone	Auxiliary protein plus Celluclast	
Celluclast 1.5L	10		6.6	0
GtXylI				
	0.5	0.3	8.8	33
	5.0	0.1	9.7	47
	50	0.5	7.0	6
GtPMO1				
	0.1	ND	8.9	36
	0.3	ND	9.7	47
TrSWOI				
	10	0.4	8.1	23
	50	0.5	9.9	50

Values are the average of experiments in triplicate. Standard deviation was between 10 and 15 %. Differences between both conditions (Celluclast and Celluclast plus TrSWOI, GtXylI or GtPMO1) are statistically significant (P<0,05). ND. Not detected.

The influence of sono-assisted alkaline pretreatment of sugarcane bagasse in enzymatic hydrolysis for cellulosic ethanol production

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Abstract
The sono-assisted alkaline pretreatment of sugarcane bagasse was optimized using a central composite design in which the glucose recovery after enzymatic hydrolysis was used as the response function. Cellic® CTec3 (Novozymes) and Thermossac® Dry (Lallemand) were used for enzymatic hydrolysis and fermentation, respectively. The best performance was achieved at 70 °C with 140 W of sonication power using 0.125 g of NaOH g^{-1} of dry biomass, yielding a total glucose recovery of 95.8 wt% in relation to the total glucan content of the original material. The fermentation efficiency of the best substrate hydrolysate was 91.4 %, whereas lower values of 87.1 and 80.0 % were obtained for steam-exploded and sono-assisted alkali-washed steam-exploded cane bagasse, respectively. In addition, when the glucan recovery of each pretreatment method was taken into account, the total C6 cellulosic ethanol production from the sono-assisted alkali-washed cane bagasse was 20.4 and 40.6 % higher than that of the other two pretreatment technologies, respectively.

KEYWORDS: Sugarcane bagasse, ultrasound, alkaline extraction, steam explosion, enzymatic hydrolysis, cellulosic ethanol.

1. Introduction

The sugarcane bagasse, as any other type of lignocellulose, is primarily composed of cellulose, hemicelluloses and lignin (Szczerbowsk et al., 2014; van der Pol et al., 2015). Hence, due to the close association that exists among these plant cell wall components, a pretreatment method is required for its efficient use as a raw material for ethanol production, which includes the need for high accessibilities to enzymatic hydrolysis and good fermentation yields. This is so because the enzymatic hydrolysis of cellulose and other plant cell wall polysaccharides is restricted by several factors including the substrate recalcitrance, which involves properties such as available surface area, pore volume, cellulose crystallinity, lignin content, and fiber coating by non-cellulosic components. These and other parameters define the enzymes' accessibility to the substrate and their ability to release fermentable sugars from plant polysaccharides (Himmel et al., 2007).
Different pretreatment methods have been proposed so far to deconstruct the plant cell wall, remove and/or modify both lignin and hemicellulose components, and enhance the accessibility of glucans to enzymatic hydrolysis, this without compromising the fermentation yield by releasing inhibitory compounds in the reaction system (Alvira et al., 2010; Martín et al., 2008; Rocha et al., 2012a). In this regard, the pretreatment step has a strong influence in the overall process costs by determining the extent of sugar recovery, fermentation toxicity, enzymatic hydrolysis rates, enzyme loadings, and other process variables (Kumar et al., 2009).
Alkaline pretreatments usually involve delignification using dilute sodium hydroxide under conditions milder than those of other pretreatment processes. This type of pretreatment consists of applying a basic solution to solubilize lignin-carbohydrate complexes by the breakdown of alkaline labile aryl-ether bonds (e.g., α-O-4 and β-O-4) and by acid-base reactions involving both aliphatic (mostly due to carbohydrates) and aromatic hydroxyl groups (exclusively due to the polyphenolic structure of lignin) to form low molar mass water-soluble phenolates. In general, this extraction procedure decreases both lignin and hemicellulose contents, modifies the substrate structural organization (including cellulose crystallinity), and induces biomass swelling, thereby improving the substrate susceptibility to enzymatic hydrolysis (Balat et al., 2008). In addition, the alkaline pretreatment produces less inhibitory compounds to enzymes and fermenting microorganisms compared to pretreatments based on acid hydrolysis (Carvalheiro et al. 2008).
Besides the alkaline extraction, steam explosion is one of the most widely employed physicochemical pretreatment methods for the fractionation of lignocellulosic materials such as cane bagasse and this has been demonstrated in both laboratory and pilot scale (Rocha et al., 2012b). Depending on the pretreatment severity, this method is able to remove the hemicellulose component almost completely and to impart chemical modifications to the lignin component so as to produce cellulosic materials that are highly accessible to enzymatic hydrolysis (Balat et al., 2008). However, due to the acid environment in which steam explosion is performed, furan compounds and organic acids are released in the pretreatment water solubles and these tend to inhibit the optimal performance of both enzymes and fermenting microorganisms (Carvalheiro et al., 2008).
In this work, the sono-assisted alkaline pretreatment of sugarcane bagasse was optimized using a central composite design in which the glucose recovery after enzymatic hydrolysis was used as the response function. Afterwards, enzymatic hydrolysates derived from the best alkali-treated substrate were fermented by an industrial strain of *Saccharomyces cerevisiae* and the resulting ethanol yields were compared with those derived from other pretreatment technologies such as steam explosion with and without post-delignification with dilute sodium hydroxide.

2.Material and methods

2.1 Material

Sugarcane bagasse was obtained from the São Martinho Mill (Pradópolis, SP, Brazil). The cellulase complex Cellic® CTec3 was provided by Novozymes Latin América (Araucária, PR, Brazil). The industrial strain of *S. cerevisiae* (Thermossac® Dry) was obtained from Lallemand (Milwaukee, WI, USA).

2.2 Methods

2.2.1 Pretreatments
Steam explosion of sugarcane bagasse was performed with 1 kg of cane bagasse (50 wt% moisture content) at 195 °C for 7.5 min in a 10-L steam reactor. The resulting steam-exploded material (SEB) was washed twice with water for 30 min at room temperature under a 5 wt% total solids. A fraction of the water-washed SEB was separated and characterized as described below. The water-washed SEB was also submitted to an alkaline extraction. The conditions used for this purpose were established by the optimization procedure described below. The resulting delignified substrate was referred to as sono-assisted alkali-washed steam-exploded bagasse (SA-AWSEB).
The alkaline extraction were performed with milled native cane bagasse (1 mm particle size in average) in 500 mL Erlenmeyer flasks containing 5 wt% total solids, using an agitation of 200 rpm for 30 min in an Ultronique Q5.9/37A ultrasonic bath. The sono-assisted alkaline delignification of cane bagasse was pre-optimized using a central composite design with two replicates at the center point for a total of 16 experiments (Table 1). The variables tested were temperature (°C), NaOH concentration (g g^{-1} total solids) and sonication power (W). After delignification, the resulting substrates were filtered and washed with a fresh NaOH solution at the same concentration used before, followed by several water washing steps until a neutral pH was reached.

2.2.2 Biomass chemical composition
The materials were subjected to extraction in a Soxhlet apparatus for the determination of total extractives following the NREL/TP-510-42619 method (Sluiter et al., 2008a). The moisture and ash contents were generated according to the NREL/TP-510-42621 (Sluiter et al., 2008b) and NREL/TP-510-42622 (Sluiter et al., 2008c) methods, respectively. The chemical compositions, before and after pretreatment, were determined according to the NREL/TP-510-42618 (Sluiter et al., 2012) and NREL/TP-510-42617 (Hyman et al., 2008) methods, generating the acid insoluble lignin (determined gravimetrically) and the acid soluble lignin (determined by UV spectroscopy), respectively. Carbohydrate composition was determined in the resulting acid hydrolysate by High Performance Liquid Chromatography (HPLC), using an Aminex HPX-87H (Bio-Rad) chromatographic column. The quantification was performed by external calibration using a series of standard solutions for each component of interest (cellobiose, glucose, xylose, arabinose, formic acid, acetic acid, furfural and hydroxymethylfurfural, the latter two used for controlling the dehydration of pentoses and hexoses, respectively).

2.2.3 Enzymatic hydrolyses
Enzymatic hydrolyses of pretreated substrates were carried out in 250 mL Erlenmeyer flasks with a final volume of 50 mL using 4 wt% total solids at 50 °C and 150 rpm for 96 h. The enzyme loading was 33 mg of Cellic CTec3 g^{-1} of substrate (dry basis), which expresses the mass of the as-received commercial enzyme preparation (wet basis) in relation to the substrate total solids. The reaction mixture was prepared in a 50 mmol L^{-1} acetate buffer, pH 5.2. Aliquots were collected at 3, 6, 12, 24, 48, 72 and 96 h and immediately analyzed by HPLC under the conditions described above. Glucose was monitored in substrate hydrolysates to express the total release of water soluble sugars. Hydrolysis yields were calculated by expressing the amount of glucose in relation to the theoretical amount of glucose of the starting material. The substrate hydrolysates were then filtered through a Gooch crucible of medium porosity prior to fermentation.

2.2.4 Fermentation
The fermentation was performed in acetate buffer 50 mmol L^{-1} pH 4.8 with 1.0 g L^{-1} of cells (from inocullum), 6.7 g L^{-1} of YNB (Difco Yeast Nitrogen Base) and enough substrate hydrolyzate to reach a final glucose concentration of approximately 50 g L^{-1}. Assays were performed in an orbital shaking incubator at 35 °C and 150 rpm for 24 h. Aliquots were collected at 3, 6, 12 and 24 h and analyzed for glucose and ethanol by HPLC using the same procedure mentioned above.

3. Results and discussion

Cane bagasse contained 40.1 ± 0.7 % of glucan, 23.7 ± 0.1 % of hemicellulose and 23.07 ± 0.7 % of total lignin. Also, this material contained 5.1 ± 0.2 % of ash and 5.2 ± 0.2 % of total extractives, with 3.5 ± 0.1 % and 1.7 ± 0.1 % of these being obtained in water and ethanol 95 %, respectively.
The delignification values are shown in **Table 1** and the highest delignification of 66.48 % was achieved for sample AWP10 (0.1875 g g^{-1} NaOH; 80.23 °C; 105 W), which had a total lignin content of 10.26 %. Such delignification efficiency was 38 % higher than that obtained for the sample that was treated with the highest NaOH concentration (AWB12 – 0.2926 g g^{-1} NaOH; 55 °C; 105 W), suggesting that the temperature was the most significant variable in the extraction process. Indeed, lower NaOH concentrations led to lower delignification levels for experiments that were performed at the same temperature and sonication power (e.g., see AWB11 and AWB12). Therefore, under a sonication power of 105 W, the use of higher

temperatures allowed the use of lower NaOH concentrations, and 0.1875 g g^{-1} (or 0.94 % NaOH at 5 wt% total solids) was already enough to achieve the highest delignification level. The glucan recoveries based on chemical composition after pretreatment were higher than 90.6 % throughout the entire experimental design.

Table 1. Alkali pretreatment conditions and their results of delignification (%) after the pretreatment and enzymatic hydrolysis (GlcEq - %) after 96h of reaction.

Experiment	[NaOH] (g g^{-1} dry substrate)	Temperature (ºC)	Sonication Power (W)	Delignification (%)	GlcEq (%)
AWB1 [a]	0.125 (-1)	40 (-1)	70 (-1)	18.4	16.6±0.7
AWB2	0.125 (-1)	40 (-1)	140 (+1)	14.1	11.0±0.8
AWB3	0.25 (+1)	40 (-1)	70 (-1)	11.6	27.1±1.5
AWB4	0.25 (+1)	40 (-1)	140 (+1)	16.0	48.9±1.2
AWB5	0.125 (-1)	70 (+1)	70 (-1)	37.1	77.4±0.1
AWB6	0.125 (-1)	70 (+1)	140 (+1)	48.9	96.9±0.5
AWB7	0.25 (+1)	70 (+1)	70 (-1)	49.7	81.6±0.4
AWB8	0.25 (+1)	70 (+1)	140 (+1)	52.3	91.9±0.1
AWB9	0.1875 (0)	29.77(-1.68)	105 (0)	21.1	29.4±0.9
AWB10	0.1875 (0)	80.23 (+1.68)	105 (0)	66.5	77.2±0.5
AWB11	0.0824 (-1.68)	55 (0)	105 (0)	29.1	32.6±3.0
AWB12	0.2926 (+1.68)	55 (0)	105 (0)	48.2	78.9±5.8
AWB13	0.1875 (0)	55 (0)	46.14 (-1.68)	39.4	59.2±2.6
AWB14	0.1875 (0)	55 (0)	163 (+1.68)	38.2	60.2±1.2
AWB15 [a,b]	0.1875 (0)	55 (0)	105 (0)	27.2	66.8±1.5
AWB16 [a,b]	0.1875 (0)	55 (0)	105 (0)	28.0	69.8±2.1

[a] **SA-AWB, sono-assisted alkali-washed bagasse;**
[b] **Center point of the experimental design, which was performed in duplicate;**

The enzymatic hydrolysis of SA-AWB revealed that, when temperatures lower or equal to 40 °C were used for pretreatment, yields ranging from 11.0 to 48.9 % were observed even after 96 h of incubation with 33 mg of Cellic CTec3 g^{-1} of total solids (**Table 1**). A relatively low hydrolysis yield was also observed for sample SA-AWB11. Hence, the use of intermediate temperatures of 55 °C was not enough to overcome the lack of reagent that in this case corresponded to the lowest NaOH loading applied in the entire experimental design (0.0824 g g^{-1} NaOH). On the other hand, at temperatures above or equal to 70 °C, the hydrolysis yields were between 77.2 and 96.9 % in 96 h. The best hydrolysis performance was achieved with sample SA-AWB6 (0.125 g g^{-1} NaOH; 70 °C; 140 W). Sample SA-AWB12 also reached a high conversion by enzymatic hydrolysis (78.9 %) and this was attributed to the use of a NaOH concentration of 0.2926 g g-1 at an intermediate reaction temperature.

The application of high sonication powers also resulted in high conversions by enzymatic hydrolysis, compared to the use of lower sonication powers. When samples SA-AWB3, SA-AWB5, and SA-AWB7 (obtained at 70 W) were compared with samples SA-AWB4, SA-AWB6, and SA-AWB8 (obtained at 140 W), for which the conditions of temperature and alkali concentration were the same, there was an increase in the substrate hydrolysis yields by 80.3, 21.2 and 12.6 %. By contrast, although performed with a high level of sonication power (163 W), SA-AWB14 showed a hydrolysis profile similar to SA-AWB13, which was exposed to a sonication power of only 46 W.

One of the main objectives of this work was to compare the sono-assisted alkali pretreatment of cane bagasse with steam explosion as well as with steam explosion followed by sono-assisted alkali washing. In this case, the sono-assisted alkaline delignification of SEB was carried out under the best condition among those described in Table 1 (SA-AWB6). Comparison was carried out on the basis of process yields including C6 fermentation to fuel ethanol using *S. cerevisiae.*

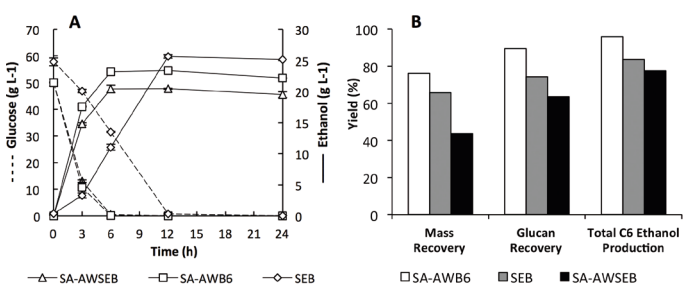

Figure 1: (A) Fermentation profile of their corresponding substrate hydrolysates; (B) Comparison of pretreatment processes in relation to the mass recovery of substrate total solids, glucose recovery after enzymatic hydrolysis and the total cellulosic ethanol production from C6.

The SEB reached lower enzymatic hydrolysis yields when compared to the other two pretreated substrates, even though its hydrolysis was carried out with a higher enzyme-to-substrate ratio. This was so because the enzyme loading was based on total solids and SEB had a considerably lower glucan content compared to SA-AWSEB. By contrast, SA-AWSEB reached 96.0 % of hydrolysis in 96 h, indicating that its glucan content was the most readily available compared to the other two pretreated substrates. To extend the comparative analysis of the different pretreatment processes, fermentation trials were performed with their corresponding enzymatic hydrolysates using an initial glucose concentration of around 50 g L^{-1} (**Figure 1A**). These trials were carried out for 24 h but the glucose consumption leveled off after 12 h, suggesting that the process could have been interrupted at this time without any loss in process efficiency. After 12 h, ethanol concentrations of 25.6, 23.4, and 20.4 g L^{-1} were obtained from SA-AWB6, SEB, and SA-AWSEB hydrolysates for fermentation efficiencies of 91.4, 87.0, and 79.9 %, respectively. SA-AWB6 gave rise to the best fermentation performance, with final conversion yields ($Y_{P/S}$) of 46.6 % that were immediately followed by SEB (44.4 %) and SA-AWSEB (40.8 %). Also, the SEB hydrolysate resulted in the highest ethanol productivity but this was only because its initial glucose concentration was about 7 g L^{-1} higher than that of the other two hydrolysates. On the other hand, compared to SEB and SA-AWSEB, the SA-AWB6 substrate produced hydrolysates that were less inhibitory to the yeast metabolism.

Figure 1B provides the most important numbers for the comparative analysis of the three pretreatment strategies involved in this study. For this, three parameters were combined to give the ultimate efficiency of the entire cellulosic ethanol production process: the mass recovery of water-insoluble fibers after pretreatment, the glucan recovery obtained after enzymatic hydrolysis in relation to the substrate glucan content, and the efficiency with which ethanol was produced from the substrate hydrolysates. Hence, the resulting figures reveal the total C6 ethanol production yield obtained in each case in relation to the theoretical amount of ethanol that could be produced from the native bagasse glucan content. In general, the sono-assisted alkali washing of native bagasse out performed both steam explosion and steam explosion followed by alkali washing primarily because little glucan was lost as a result of pretreatment. Besides, the resulting SA-AWB substrates were highly susceptible to enzymatic hydrolysis and fermentation. The ultimate efficiencies derived from these three pretreatment processes were 89.3, 74.2, and 63.5 %, respectively. Hence, the extent of delignification of cane bagasse with the sono-assisted extraction process (sample SA-AWB6) was lower than that for SA-AWSEB but enough to generate a better susceptibility to enzymatic hydrolysis and fermentation. This is a clear indication that steam explosion was not a fundamental step towards the successful saccharification of cane bagasse glucans.

4. Conclusion

The sono-assisted alkaline pretreatment of sugarcane bagasse was optimized for the release of fermentable sugars (mostly glucose) by enzymatic hydrolysis using low enzyme loadings of Cellic CTec3 (Novozymes). Enzyme hydrolysates derived from the best sono-assisted alkali-washed substrate were readily fermented by an industrial strain of *S. cerevisiae* (Thermossac Dry), yielding fermentation efficiencies higher than those obtained from steam-exploded and alkali-washed steam-exploded cane bagasse. Considering the capital cost involved in steam explosion, the sono-assisted alkali washing of cane bagasse emerges as a simpler, more efficient and more sustainable method for the production of cellulosic ethanol from cane bagasse, particularly if the fermentation of the C5 stream coming from its high hemicellulose content is also taken into account.

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Comparison between microwave and conduction-convection heating for autohydrolysis processing in the production of high added-value compounds and substrates for biofuel under the biorefinery concept

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Abstract

The pretreatments are generally performed by conventional heating, however the development of new technologies such as microwave heating brings a new interest in the biorefineries of second generation. The objective of this study was the comparison between two pretreatments: conduction-convection and microwave heating under different conditions of time and temperature in order to produce high added-value compounds as oligomers from hemicelulose and pretreated solids for bioethanol production using corn residues as raw material. The autohydrolysis processing was optimized for both processes (conventional and microwave heating) regarding the production of oligomers from hemicellulose. The optimal conditions were 168 °C/34 min for conventional heating and 175 °C/38 min for microwave heating. Under these conditions, pretreated solids were obtained with high cellulose content: 51 and 56 g/ 100 g of raw material for conventional and microwave heating, respectively. The oligomers production under optimal conditions were 16.96 and 18.98 g /100 g of raw material for conventional and microwave heating, respectively. As a conclusion it is observed that both of the pretreatments are effective to be considered in the concept of biorefinery.

1. Introduction

Corn represents one of the main feedstocks in Mexico, between 2012 and 2013 the production of corn raised 22.2 % compared to 2011, according to the information provided by the Food and Agriculture Organization of the United Nations [1]. Agroindustrial residues obtained from feedstock production are called lignocellulosic biomass and composed mainly by cellulose 30-50 %, hemicellulose 20-40 %, lignin 15-25 % and ashes 3-10 %. Thus, this residue represents an important source for the biorefinery processing for the production of high-added value compounds and sugar rich substrates for bioethanol production [2]. Autohydrolysis pretreatment is a chemical-free process that only involves water and lignocellulosic biomass. This pretreatment is a fast, economical and eco-friendly operation. In this pretreatment, the hemicellulose gets depolymerized in oligomers, as xilo-oligosaccharides (XOS), and monomers. XOS can be extracted from the liquid phase and the solid phase can be used as substrate for enzymatic hydrolysis, after this step, bioethanol can be produced [3]. Batch reactors are used as a conventional heating system to perform the autohydrolysis pretreatment and conventional heating is generally used whereas new technologies are in development bringing a new interest in the biorefineries of second generation [2]. One of the new alternatives is microwave heating system where the energy interacts with the polar molecules forming hot nucleus that warm up in a quickly and efficient manner allowing the reactions to take place in less time with better yields and more selectivity [4]. The objective of this study was the comparison between two pretreatments: conduction-convection and microwave heating under different conditions of time and temperature in order to produce high added-value compounds as oligomers from hemicelulose and pretreated solids for bioethanol production.

2. Experimental

2.1. Raw material

Corn residues were chopped using a cutter mill to a particle size of 0.5 – 2 mm. Samples were milled to pass a 0.5 mm screen for the physicochemical characterization where ashes, moisture and extractives were determinate. The chemical composition of the raw material was determined according to the methodology reported by Ruiz et al. [3].

2.2. Conventional and microwave heating system

A particle size of > 2 mm was used in both pretreatments and only was used water as catalyst. Both processes were performed under isothermal heating regimen for different residence time.

2.2.1. Conventional heating

The conventional heating process was performed in a stainless steel 316 pressurized reactor with a volume work of 750 mL adapted with PID control and speed agitation, heat resistance, manometer for internal pressure (Figure 1). For the treatments a ratio of 1:10 w/v (biomass: water) was used with different residence time (10, 30, 50 min) and temperature (150, 165, 180 °C).

Figure 1. Conventional heating reactor

2.2.2. Microwave heating

The microwave heating process was performed in a microwave CEM Mars 6 with eight reactors CEM Mars Xpress PFA each one adapted with a Teflon jacket. Different conditions of time (10, 30, 50 min), temperature (150, 165, 180 °C) and a ratio of 1:15 w/v (biomass: water) were evaluated.

2.3. Solid and liquid phase characterization after autohydrolysis pretreatment

At the end of pretreatment, the liquid and solid phases were separated by filtration and the solid residues were washed with distilled water. The liquid was analyzed by HPLC with a MetaCarb 67H (300×7.8 mm, Varian, USA) column. The samples were analyzed for glucose, xylose arabinose, 5-hydroxymethylfurfural (HMF), furfural and acetic acid. A second aliquot of liquors (20 ml) was subjected to quantitative post-hydrolysis (with 4% H2SO4 at 121°C during 60 min) before HPLC analysis for the determination of oligomers from hemicellulose. The pretreated solids were analyzed as described above (see Section 2.1) [3]. The three best oligosaccharides yields were selected for enzymatic hydrolysis.

2.4. Enzymatic hydrolysis of pretreated solids

The enzymatic hydrolysis of pretreated solids was performed in a in 150 mL Erlenmeyer flasks at 50 °C by duplicate, using cellulase (Cellic CTec 2) with a loading of 15 FPU/g of glucan, in 50 mM citrate buffer (pH 4.8) with 2% (w/v) sodium azide to inhibit microbial contamination and a final cellulose concentration of 5% (w/v). The necessary amount of deionized water was calculated and added to make the total volume of 50 mL. Agitation was carried out using a magnetic stirrer (150 rpm) and samples were taken at 4 h intervals for the first 12 h and at 24 h intervals until a total time of 96 h. The samples were kept in boiling water for 5 min to inactive enzymatic activity, and then centrifuged to remove insoluble substrate; the supernatant was filtered membrane filter analyzed for soluble sugars in HPLC as described below.

3. Results and discussion

The results of raw material characterization are shown in Table 1, glucan and hemicellulose with 32.08 % and 25.52 %, respectively. This helps to predict the amount expected of oligosaccharides and glucose production in the enzymatic hydrolysis.

In Table 2 the results of both pretreatments are shown for the best three results of oligosaccharides production in both cases. For the microwave heating pretreatment it is observed that the production of oligosaccharides improved compared to the conventional heating process. Both cases present an optimal point. In the microwave heating the optimal point was at 170°C for 38 min in a ratio w/v 1:15. On the other hand, for the conventional heating process the optimal point was at 168°C for 34 min. It is observed that the optimal point of the microwave presented a yield of oligosaccharides of 18.80 g/L compared with the 16.96 g/L obtained with the conventional heating. In previous works, Buruiana et al. [5] reported the oligosaccharides yield of 12 g/L using autohydrolysis processing. The pH in the treatments oscillates between 4.03 and 4.49, which

indicates the presence of hydronium ions and acetic acid that contribute to the autohydrolysis process, and improves the solubilization of the hemicellulose in the liquid fraction. For solid fraction, cellulose increased in both cases for autohydrolysis pretreatment.

Table 1. Raw material composition

Compound	[%]
Glucan	32.08
Hemicellulose	25.52
Xylan	20.22
Arabinan	2.21
Acetic Acid	3.08
Klason Lignin	17.20
Extractives	5.20
Ashes	4.68
Moisture	8.7

Cellulose hydrolysis of microwave and conduction-convection pretreated solids is shown in Figure 2. The glucose production for microwave and conduction convection pretreated solids was 19.9 and 18.44 g/L, respectively at 72 h. The microwave pretreated solids are more accessible to hydrolysis than conduction-convection pretreated solids. The data indicated that the enzymatic hydrolysis was improved by autohydrolysis in both cases, with similar values of hydrolysis have been reported by Ruiz et al. [6] using the same pretreatment.

Table 2. Characterization of liquid and solid fraction

Heating Pretreatment	Liquid Fraction		Solid Fraction (% dry weight)		
	Oligosaccharides [g/L]	pH	Cellulose [%]	Lignin [%]	Solid Yield [%]
Microwave					
165°C, 30min	17.75	4.43	51.09	21.66	73.39
165°C, 50min	18.35	4.49	55.67	22.44	66.42
Optimal Point					
170°C, 38 min	18.80	4.03	50.05	34.49	49.43
Conventional					
165°C, 30 min	16.85	4.25	43.55	29.68	55.43
165°C, 50 min	16.81	4.27	37.55	27.37	63.51
180°C, 30 min	12.82	4.17	51.39	32.80	53.80
Optimal Point					
168°C, 34 min	16.96	4.35	44.69	30.84	53.17

4. Conclusions

In this work two forms of hydrothermal treatment for the production of oligosaccharides and substrate for enzymatic hydrolysis were compared. For microwave pretreatment, the production of oligosaccharides from hemicellulose and glucose production shown a light advantage compared with the conventional heating pretreatment. The microwave and conduction convection heating can be used in the biorefinery concept

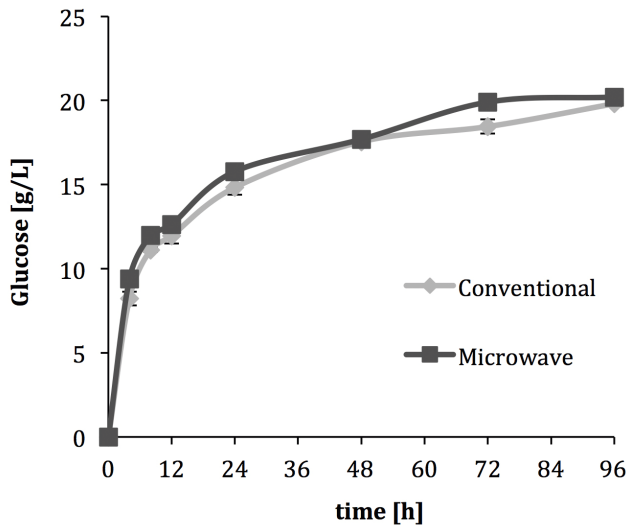

Figure 2. Kinetics of enzymatic hydrolysis

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Different strategies for lignocellulose sugars conversion into ethanol from phosphoric acid steam exploded olive tree pruning

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1. Introduction

Biomass materials, such as lignocellulosic agricultural residues, constitute a cheap and readily available resource for the production of biofuels, as well as new value-added biocompounds and biomaterials in the context of a "biorefinery" approach.

The olive sector is not only an important part of the agricultural sector in the Mediterranean countries, but its cultivation has spread globally in the last years because healthy benefits attributed to olive oil consumption. Currently, olive trees are cultivated in more than forty countries, and the total dedicated surface worldwide is about 10.4 million ha [1]. In mature trees, pruning is mainly required to renew the fruiting surface of the tree and achieve high yields. It has been estimated that pruning olive produces is the range of 2.7-3.9 t/ha [2]. The large volumes of residual olive tree pruning (OTP) biomass generated, together with the great environmental damage caused by its uncontrolled burning, has suggested the possibility of exploiting this biomass resource but, till date, no economical alternatives have been developed [3]. The use of OTP as feedstock for the biofuels industry, could provide an economic complement for the olive producers. OTP biomass can be considered a suitable raw material for the production of ethanol due to its high content of potentially fermentable carbohydrates. However, biotechnological valorization of OTP) presents significant challenges due to its heterogeneity. A typical pruning lot includes 50% of thin branches, 25% of wood and 25% of leaves with high amount of soluble compounds (extractives) and ash. Cell wall content in OTP biomass varies significantly with percentage of wood and branches but, like other biomasses of lignocellulosic composition, is a complex mixture of cellulose, hemicelluloses and lignin [4].

Pretreatment is a key step in the process to transform lignocellulosic biomass into fermentable sugars. Different pretreatments have been applied to OTP, e.g., liquid hot water [5], steam explosion [6]), dilute acid [7, 8], inorganic salts [9] and organosol pretreatments [10]. Acid catalyzed steam explosion pretreatment is probably the most commonly applied method, in which the hemicellulose fraction is solubilized and the digestibility of cellulose to enzymatic attack is increased. Harsh conditions in the pretreatment lead to a partial sugar and lignin decomposition and, therefore, to the generation of toxic compounds that affect the fermentation step [11]. Sulfuric acid is the most commonly used acid. However, the use of other acid catalysts, such as phosphoric acid, presents some advantages due to lower inhibitors formation; lower corrosiveness and the possibility of using the distillation residues as fertilizer are some of them [12)].

The hydrolysis of lignocellulosic carbohydrates renders D-glucose from cellulose and a mixture of hexoses (D-glucose, D-mannose, D-galactose) and pentoses (D-xylose, L-arabinose) from hemicelluloses.Therefore, to increase the economic competitiveness of cellulosic ethanol, the use of fermenting microorganisms capable of using the whole range of sugars available in the hydrolysate is vital. Saccharomyces cerevisiae is the preferred microorganism used to produce industrial ethanol because its excellent ability to ferment glucose and its high tolerance to ethanol and inhibitors presented in lignocellulosic hydrolysates [13]. However, it is not capable of fermenting xylose, presents in significant amounts in biomass hydrolysates. In the last decade, successful application of metabolic engineering strategies have converted industrial S. cerevisiae strains into xylose-fermenting yeast via the introduction of a xylose reductase and a xylitol dehydrogenase from Scheffersomyces stipitis [14]. This strategy has produced yeast strains capable of utilizing the pentose sugars (especially xylose and arabinose) as sole carbon sources, but these recombinant microorganisms are more sensitive to inhibitors, have low tolerance to ethanol and major difficulties still remain for xylose fermentation in the presence of glucose.

In this work, different strategies for ethanol production from water extracted OTP pretreated by phosphoric acid catalyzed steam explosion were evaluated. A recombinant yeast strain (Saccharomyces cerevisiae F12) was used with the objective to transform all sugars contained in the pretreated material (mainly glucose and xylose) into ethanol.

2. Material and methods

2.1. Materials

OTP was locally collected after olive harvesting, air-dried to an equilibrium moisture content of about 7%, and milled to a particle size smaller than 4 mm with a laboratory hammer mill (Retsch, SM 100).

S. cerevisiae F12 strain, kindly provided by Professor. Olsson from Chalmers University (Sweden), was used in SSF and prehydrolyzates fermentation. Active cultures for inoculation were obtained in 100 mL Erlenmeyer flasks with 50 mL of growth medium containing (in g/L):, yeast extract (10), peptone (10), and xylose (20) After 16 h on a rotatory shaker at 35 ºC and 150 rpm, the preculture was centrifuged at 10,000 g for 10 min. Supernatant was discarded and cells were washed with saline solution and then diluted to obtain an inoculum level of 1 g/L.

2.2. Methods

OTP at 10% (w/v) was subjected to aqueous extraction at 120 °C for 60 min and filtered. Material obtained after extraction (EOTP) was dried at 40 ºC and pretreated in a steam explosion unit equipped with 2 L reactor. The reactor was filled with 300 g (dry basis) of EOTP impregnated with 500 mL of phosphoric acid solution 1% (w/w) and pretreated at 195 ºC and 10 min. Pretreatment conditions were selected as optima for sugars recovery in a previous work [15]. After the explosion, the pretreated slurry was recovered in a cyclone, cooled to about 40 ºC and then filtered for solid and liquid recovery. The water insoluble fraction (WIF-EOTP) was washout thoroughly with water and characterized in terms of the main components (glucans, hemicellulose, lignin, and ash) according to NREL analytical methods for biomass [16]. The liquid fraction or prehydrolyzate (LF-EOTP) was analyzed for sugars and degradation compounds by HPLC system (Waters, Milford, MA) equipped with a refractive index detector (model 2414). A CARBOSep CHO-782 (Transgenomic, Inc., Omaha, NE) was used for monomeric sugars (glucose, xylose, galactose, arabinose, and mannose) quantification. Ultrapure water was used as a mobile phase at a flow rate of 0.6 mL/min and a temperature of 70 °C. Furfural, hydroxymethylfurfural (HMF), and phenols (vanillin and syringaldehyde) concentrations were analyzed in a Hewlett-Packard 1100 HPLC system (Palo Alto, CA) equipped with 1040A Photodiode-Array detector (Agilent, Waldbrown, Germany) while acetic and formic acid concertation were analyzed in the same HPLC system equipped with a RI detector. An ICSep ICE-COREGEL 87H3 column maintained at 65 ºC with a mobile phase (89% 5 mM H_2SO_4 and 11% acetonitrile) at flow of 0.7 mL/min were used for furans quantification and with a mobile phase (5 mM H_2SO_4) at a flow rate of 0.6 mL/ml for aliphatic acids quantification.

The prehydrolyzate was subjected to a detoxification step by passing the LF-EOTP through a glass filter holder, containing (0.3 g/mL prehydrolyzate) ion-exchange resin (Microionex MB 200, Rohm Haas, Copenhagen, Denmark). Then, pH was adjusted to 5.5 with sulfuric acid (2.5 N). Detoxified liquid fraction (DLF-PEOTP) was supplemented with growth medium containing (g/L): yeast extract (2), NH4Cl (1), KH_2PO_4 (1), $MgSO_4.7H_2O$ (0.3) and xylose (30) and inoculated with 1 g/L of S. cerevisiae F12. Fermentations were run in 100 mL Erlenmeyer for 48 h at 35 ºC and 150 rpm. Samples were taken periodically and centrifuged at 10,000 g.

The washed pretreated WIF-EOTP (WWIF-EOTP) was used as substrate for enzymatic hydrolysis using Cellic Ctec2 (Novozymes, Denmark) at a loading of 0.12 mL/g of dry WWIF-EOTP. Presaccharification step was performed at 18% (w/w) substrate (WWIF-EOTP) loading and 50 ºC in a 15 L bioreactor (Terrafors-IS, Infors HT, Switzerland) for 48 h. After presaccharification, temperature was reduced to 35 ºC and the hydrolysate (PWWIF-EOTP) was supplemented with nutrients as in the growth medium and inoculated with S. cerevisiae F12. Samples were withdrawn after 24, 48 and 72 h and analyzed for ethanol and glucose. All tests were carried out in triplicate.

To investigate the best process configuration to obtain a complete transformation of both xylose and glucose sugars into ethanol, different fermentation schemes were developed (Figure 1).

1. Separate fermentation of prehydrolyzate (C5 sugars) and hydrolyzate (C6 sugars).
2. Combined fermentation of prehydrolyzate (C5 sugars) and hydrolyzate (C6 sugars).
3. Sequential fermentation of prehydrolyzate (C5 sugars) and hydrolyzate (C6 sugars).

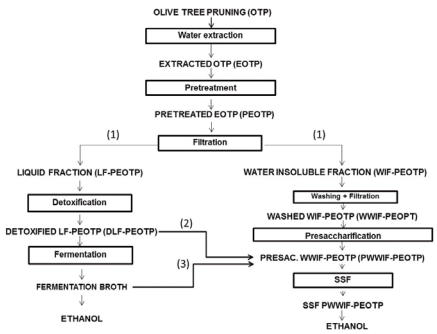

Figure 1.- Scheme of different strategies for ethanol production from olive tree pruning biomass. (1) Separate, (2) Combined and (3) Sequential fermentation.

3. Results.

3.1. Extracted and Pretreated OTP Composition

Table 1 shows the composition of extracted olive tree pruning (EOTP) and the composition of solid (WWIF-PEOTP) and liquid (LF-PEOTP) fraction after phosphoric acid steam explosion pretreatment Glucans (as glucose) is the main component (31.3%) of EOTP, corresponding 26.9% to cellulose and 1.4% to starch. Hemicellulose content is 20.1%, with xylose (15.7%) as the main sugar. Galactan, arabinan and mannan content are 2.6%, 3.7% and 1.1%, respectively. Total lignin (as sum of soluble and insoluble) is about 26% and ash 4.8%. It should be noticed that in spite of extraction process, 9% of extractives are still present in the water extracted material.

Regarding pretreated material, glucan is the main component (about 43%) in WWIF-PEOTP, followed by lignin (47.9%). Hemicellulose sugars such as galactose, arabinose and mannose were almost completely solubilized during pretreatment; although,

a small quantity of xylose (1.3%) remains in WWIF-PEOTP. LF-PEOTP is composed mainly of sugar and degradation compounds. Xylose, coming from hemicellulose solubilization, is the main sugar (15.9 g/L). Arabinose and galactose concentrations of 4.2 and 4.1 g/L respectively were also found in the LF-PEOTP. It is also worth noting the relative high glucose concentration in the liquid fraction (6.6 g/L). This unusual high amount of glucose in prehydrolyzates from lignocellulosic biomass could be due to the hydrolysis during pretreatment of starch and, to a lesser extent, glucosides present in the leaves such as oleuropein [15]. LF-PEOTP contains also degradation compounds originated during pretreatment such as acetic acid (3.9 g/L), formic acid (0.3 g/L), furfural (2.3 g/L), hydroxymethylfurfural (HMF) (0.5 g/L) and minor content of phenolic compounds (0.02 g/L of vanillin and 0,04 g/L syringaldehyde).

Table 1. Composition of extracted olive tree pruning (EOTP) and pretreated solid fraction (WWIF-PEOTP) and liquid (LF-PEOTP) fraction

	EOTP (%)	WWIF-PEOTP (%)	LF-PEOTP (g/L)			
Component			Sugars		Inhibitors	
Cellulose	26.9	42.8	Glucose	6.6	Furfural	2.3
Hemicellulose	20.1	1.3	Xylose	15.9	5-HMF	0.5
Lignin	26.0	47.9	Arabinose	4.2	Acetic acid	3.9
Ash	4.8	---	Galactose	4.1	Formic acid	0.3
Extractives	9.0	8	Mannose	0.9	Vanillin	0.02
					Syringaldehyde	0.04

Figures 1, 2 and 3 show the fermentation profiles of separate, combined and sequential configurations, respectively.

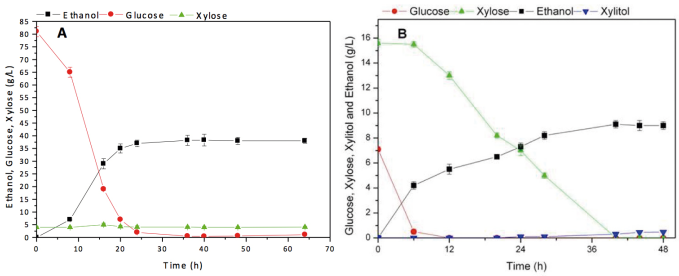

Figure 1. Ethanol production and sugar consumption during separate fermentation of PWWIF-PEOTP (A) and DLF-PEOTP (B).

In separate fermentation scheme, 80 g/L of glucose were produced in the presaccharification step of WIF-PEOTP (PWWIF-PEOTP) (Fig. 1A). 5 g/L of xylose was also detected in the enzymatic hydrolysate, showing that the commercial cellulases cocktail used in this work contains enzymes that degrade the residual hemicellulose in the pretreated WWIF-PEOTP. After 24 h form fermentation, F12 produces about 37 g/L of ethanol, which corresponds to 85 % of SSF theoretical yield (Fig 1a).

Regarding fermentation of prehydrolyzate (DLF-PEOTP) all the glucose was exhausted in the first 6 hours of fermentation. Xylose consumption started after glucose depletion. Xylose was consumed with a linear rate and totally consumed by S. cerevisiae F12 after 42 h of fermentation. Considering the total sugars in the media (glucose and xylose), 76.7 % of theoretical ethanol yield was obtained.

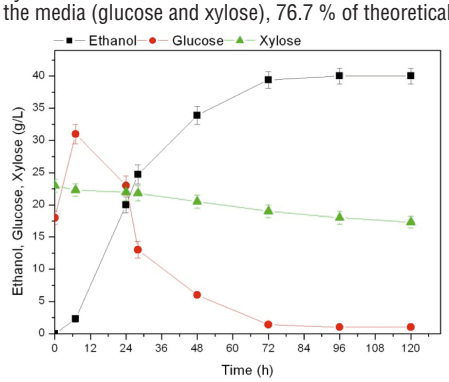

Figure 2. Ethanol production and sugas consumption during combined fermentation of prehydrolyzate and hydrolyzate.

In the combined prehydrolyzate and hydrolysate fermentation, simultaneous conversion of xylose and glucose into ethanol production was not observed. Glucose was completely assimilated after 72 h from SSF process, reaching an ethanol concentration of 40 g/L. However, F12 was not able to consume significant amounts of xylose, even when glucose was completely exhausted. Considering all sugars in the media, 73% of SSF theoretical yield was obtained. Consequently, in order to obtain efficient fermentation of the xylose contained in the pretreated OTP material with recombinant S. cerevisiae F12, it is necessary to keep low glucose concentrations in the fermentation media.

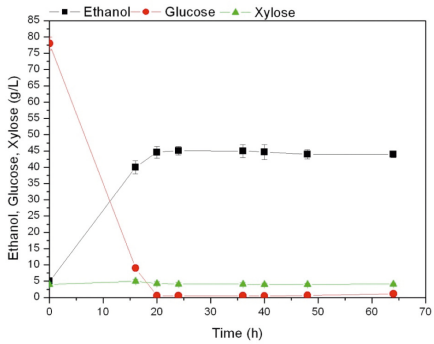

Figure 3. Ethanol production and sugar consumption during sequential fermentation of prehydrolyzate and hydrolysate.

Better results were obtained using the sequential fermentation. In this scheme, the prehydrolyzate (DLF-PEOTP) was used as media for cell propagation and then was added to the hydrolysate (PWWIF-PEOTP). At this configuration, all xylose contained in the prehydrolyzate is consumed to produce more cells and ethanol (8 g/L), providing a higher inoculum for fermentation on PWWIF-PEOTP. More cells in the fermentation of hydrolysates provide higher ethanol yield and productivity. Sequential configuration permits to shorten fermentation time to 20 h and obtain a final ethanol concentration of 45 g/L, corresponding to 82% of SSF theoretical yield. Sequential fermentation scheme permits to transform into ethanol all sugars contained in the lignocellulosic biomass, reaching an overall process yield of 154 g of ethanol per kg of EOTP biomass.

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Second generation bioethanol from *Eucalyptus globules labill* and *Nothofagus pumilio* using ionic liquids

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Abstract
Bioethanol was produced from lignocellulosic residues of a Chilean native tree, Lenga (*Nothofagus pumilio*) and Eucalyptus globulus Labill. As the first step, wood-chips originating from Lenga residues were pretreated with the ionic liquid (IL) 1-N-ethyl-3-methylimidazolium chloride (C_2mimCl) for 15 minutes at 150oC, followed by subsequent fermentation of the solid fraction using both the strategy of Simultaneous Saccharification and Fermentation (SSF) as well as Separate Hydrolysis and Fermentation (SHF). The procedures were carried out in parallel, using a commercial cellulase preparation (Celluclast and Novozyme) boosted with *Saccharomyces cerevisiae* strain Ethanol Red®. Fresh and processed carbohydrate content of Eucalyptus and Lenga residues were analyzed gas chromatography. The liquid fraction was analyzed for glucose content using a commercial kit, whereas total reducing sugars were analyzed using the dinitrosalicilic acid reagent and ethanol was analyzed by HPLC. The SHF process yielded 0.134 g ethanol/g glucose (26.3 wt-% of the theoretical yield) compared to the SSF process which yielded 0.173 g ethanol/g glucose (33.9 wt-% of the theoretical yield) within the first 24 h of fermentation. In case of Eucalyptus residues, another IL, 1-N-ethyl-3-methylimidazolium acetate (C_2minOAc) was applied during the 30 min pretreatment carried out at 150°C. Herein, the SSF process was applied for a period of three days. As a result, 3.7 g ethanol/L was obtained at 72 h (corresponding to a yield of 0.19 g of ethanol/g of glucose or 38.0 wt-% of the theoretical maximum). When fresh Lenga and Eucalyptus residues were fermented without any pretreatment, the SSF process yielded 0.017 and 0.002 g of ethanol/g of glucose, respectively (3.33 wt-% and 0.48 wt-% of the theoretical maximum, respectively). Thus, the pretreatment procedures resulted in a significant increase in ethanol production, therefore justifying the need of pretreatment prior to the co-enzyme hydrolysis and fermentation for this type of biomass. Further, the combination of IL pretreatment and use of SSF process demonstrated the high potential for bioethanol production from Lenga and Eucalyptus residues. Nevertheless, further improvement by optimization of operational conditions is required to maximize the ethanol yield. Likewise, further characterizations and a study of the IL recycling should carry out in future.

1. Introduction
Bioethanol is among the most produced biofuels worldwide and could be obtained from various types of bioresources. Alternative to the use of edible crops as raw material, the use of lignocellulosic materials or production of the so called second generation biofuels, has seen ever increasing research efforts during the last decade. The Chilean market is adaptable for the use of lignocellulosic biomass as the raw material in bioethanol production, since it does not interfere with the agricultural industry. Further, in terms of using lignocellulosic biomass, forest industry is one of the most important activities in the country [1,2]. An efficient conversion of lignocellulosic materials into bioethanol and other energy supplies is necessary to yield reasonably prized renewable energy. Consequently, the production of bioethanol from lignocellulosic material usually requires incorporation of an efficient pretreatment, followed by saccharification of the carbohydrates to achieve satisfactory efficiency [1]. The pretreatment is needed to render cellulose and hemicellulose, which are embedded in the lignin matrix, more accessible to the enzymes in the saccharification step, whereupon they are broken down into the constituent simple sugars [1,3,4]. Different pretreatment strategies have been developed throughout the years for lignocelluloses, including physical, biological, chemical and physicochemical processes. After the pretreatment in the bioethanol production process, the saccharification and fermentation steps can be carried out via different configurations: a) Separate Hydrolysis and Fermentation b) Simultaneous Saccharification and Fermentation c) Consolidated BioProcessing d) Simultaneous Saccharification and Co-Fermentation of hexoses and pentoses and e) Solid State Fermentation [5,6,7].
Ionic liquids (ILs) are organic salts able to melt under 100oC, but some even at significantly lower temperatures [2,8]. Due to their versatile chemical nature, there is almost an unlimited number of anion and cation combinations that yield ILs [9]. In terms of chemical processes, they show excellent physical characteristics such as the ability to dissolve polar and non-polar organic or inorganic substances, as well as polymers. Multitude of studies have demonstrated that cellulose is soluble in several hydrophilic ionic liquids such as: 1-butyl-3-methylimidazolium chloride [10,11], 1-ethyl-3- methylimidazolium-chloride [12,13] and 1-ethyl-3-methylimidazolium-acetate, to mention a few classical examples [14,15, 16,17]. The literature examples have demonstrated that the use of ILs for extraction of cellulose from wood avoids the use of toxic and hazardous chemicals, and can be carried out under mild conditions [18,19].
Eucalyptus is the second most abundant lignocellulosic material in Chile, corresponding to a 23 % of the total of forest plantations in the country, equivalent to 483 Mha. Lenga is a Chilean native tree that grows preferably in the extreme south of the country, covering around 3,600,000 ha, representing 26.5% of native forests [20].
Lenga formations are regarded, in general terms, as over-mature forests due to the high proportion of existing trees under decay and aging [21]. This situation implies low yields of wood for industrial purposes. On the other hand, in the case of Eucalyptus, this type of tree is widely used in the forest industry, therefore there are tons on Eucalyptus residues that are usually considered as waste or just burned [22] these reasons, Lenga and Eucalyptus residues could constitute an interesting source of biomass for second generation biofuel production, especially in a country that is characterized by its extreme dependence on foreign sources of energy and where the conversion of lignocellulosic resources could contribute to diversify the power matrix, based on the utilization of widely available agricultural and forestry derived residues [23].
The aim of the present work was to study different process configurations and pretreatment strategies (ILs) upon saccharification and fermentation (SSF and SHF) of Eucalyptus and Lenga residues aiming for second generation ethanol.

2. Experimental
2.1. Materials
Residual Lenga (*Nothofagus pumilio*) was cut into about 1- 2 mm high and 1-3 mm wide as well as 5-7 mm long chips and residual Eucalyptus (*Eucalyptus globulus Labill*) was cut into 0.5-1 mm wide, 0.5-1 mm high and 10-20 mm long.
Ionic liquids: 1-ethyl-3-methylimidazolium-chloride (C2minCl) from Merck and 1-ethyl-3-methylimidazolium-acetate (C2minOAc) from Sigma.
Enzymes: A commercial cellulase enzyme complex (Celluclast) supplied with additional β- glucosidase from Sigma.

2.2. Methods
2.2.1. Lignocellulosic materials dissolution with IL
Lenga and the ionic liquid (1:3 wt wood:wt IL) were loaded into test tubes. Two different pretreatments were compared: a) reference case when no pretreatment was applied b) C2minCl at 150°C for 15 minutes. After incubation, the solid material was filtered, washed and freeze-dried overnight.
In the case of Eucalyptus residues, biomass loadings of 1:3 (wt wood:wt IL) were utilized and three different pretreatments were compared: a) reference case when no pretreatment was applied b) C2minOAc at 150°C for 30 min and the residues were washed with distilled water 2 times c) C2minOAc at 150°C for 30 min and the residues were washed with distilled water 6 times. Subsequently, the remaining solid material was filtered, washed and freeze-dried overnight.

2.2.2. Separate Hydrolysis and Fermentation (SHF)
Enzymatic saccharification
1 g of IL pretreated raw material or Lenga without pretreatment in 20 ml of solution in sodium acetate buffer (50 mM, pH 4.8) was incubated at 50°C. The enzymatic hydrolysis reaction was initiated by adding the following enzyme loadings: 37 [FPU/g of cellulose] of cellulase and 4.9 [CBU/g of cellulose] of β-glucosidase.

Fermentation
SHF Fermentations were carried out in anaerobic sterilized Erlenmeyer flasks by adding 20 mL of hydrolyzate from the saccharification with 5 g/L yeast extract; 0.5 g/L of $(NH_4)_2HPO_4$ and 0.025 g/L of $MgSO_4 \cdot 7H_2O$. Thereafter, the Erlenmeyer flasks were incubated with S. cerevisiae Ethanol Red® at 40oC for 72 h.

2.2.3. Simultaneous Saccharification and Fermentation (SSF)
When Lenga residues were utilized, a suspension containing 1 g of IL pretreated biomass or Lenga without pretreatment plus fermentation media and cellulase mixture was used. SSF was carried out in Erlenmeyer flask with 20 mL of media. The enzyme loadings were 37 [FPU/g cellulose] of cellulase and 4.9 [CBU/g of cellulose] of β-glucosidase. The Erlenmeyer flasks were incubated with *S. cerevisiae* Ethanol Red® at 40oC for 72 h.
When Eucalyptus residues were utilized, a suspension containing 1 g of IL pretreated biomass or Eucalyptus without pretreatment was used. SSF was carried out in Erlenmeyer flask with 20 mL of media, using the same fermentation media described for Lenga residues. The enzyme loadings were 37 [FPU/g cellulose] of cellulase and 4.9 [CBU/g of cellulose] of β-glucosidase. The Erlenmeyer flasks were incubated with *S. cerevisiae* Ethanol Red® at 40oC for 72 h.

2.2.4. Carbohydrate analysis in the solid phase
Acid methanolysis method: The content of hemicelluloses and pectins in fresh and pretreated Eucalyptus and Lenga biomass were determined by acid methanolysis method [24].
Acid hydrolysis method: The cellulose content in fresh and pretreated Eucalyptus and Lenga biomass were determined by acid hydrolysis method [25].

2.2.5. Analysis of monosaccharide, sugars and ethanol in liquid phase
Monosaccharide analysis from Lenga and Eucalyptus: The monosaccharides profiles were determined by Gas Chromatography (GC). The analysis was performed

in a column (J&W HP-1). Ethanol was quantified by high performance liquid chromatography (HPLC). Biorad Aminex HPX-
87H column was used with 0.005 M H_2SO_4, flow rate of 0.5 mL/min and column temperature of 45°C. Standard of ethanol was prepared (1 to 10 gr/L).

3. Results and discussion

3.1. Composition of Lenga biomass and comparison between SSF and SHF processes

The main carbohydrate components in Lenga are glucose 434.02 mg/g dry mass (67.4 wt-%) and xylose 146.78 mg/g dry mass (22.8 wt-%) of total carbohydrates.
The differences of SSF and SHF protocols upon ethanol production from Lenga residues are shown in Table 1. We can see that when applying the SSF strategy, more ethanol was produced. The reason for the better performance of the SSF strategy might be related to the lower amounts of inhibiting compunds [5,26].

Table 1. The yield of ethanol production for different fermentation and saccharification processes (SHF and SSF) from Lenga.

Pretreatment	Ethanol yield [gr ethanol/gr glucose]	Percentage relative to theoretical yield (wt-%)
Theoretical yield	0.510	100.0
Without pretreatment/SHF*	0.020	3.92
Pretreatment with C_2mimCl/ SHF*	0.134	26.3
Without pretreatment/SFF*	0.017	3.33
Pretreatment with C_2mimCl/ SSF^	0.173	33.9

(*) Fermentation in 4 hours and (^) fermentation in 24 hours.

3.2. Eucalyptus as the lignocellulosic material and results of the SSF

In case of Eucalyptus residues, the SSF process was selected. The results after fermentation of non-pretreated and pretreated Eucalyptus residues for a period of three days are shown in Table 2. The overall concentration of ethanol achieved after pretreatment was 3.7 g /L, at 72 h (a yield of 0.194 g of ethanol/g of glucose, corresponding to 38.0 wt-% of the theoretical maximum). When the non- pretreated Eucalyptus residue was fermented, only 0.48 % of the theoretical maximum was obtained. Thus, a 79-fold increase in the amount of ethanol produced was reached, justifying the need of pretreatment to co-enzyme hydrolyze and ferment this type of biomass. In fact, it has been postulated that C2minOAc can disrupt the inter- and intra-molecular hydrogen bonding [27,28].

Table 2. Influence of washing cycles on the yield of ethanol production for simultaneous saccharification and fermentation (SSF) of Eucalyptus after 72 h.

Pretreatment	Ethanol yield [gr ethanol/gr glucose]	Percentage relative to theoretical yield (wt-%)
Theoretical yield	0.510	100.0
Without pretreatment	0.002	0.48
Pretreatment with C_2minOAc/ fibers washed two times	0.083	16.5
Pretreatment with C_2minOAc/ fibers washed six times	0.194	38.0

This is the first time bioethanol was produced from Lenga and Eucalyptus residues pretreated with ionic liquids. However, the IL C_2minOAc has previously been successfully used to pretreat lignocellulosic biomass subsequently fermented to ethanol. Goshadrou et al. (2013) treated aspen wood with C_2minOAc at 120°C (1 to 5 h) and subsequently used the SHF process using commercial cellulases and *S. cerevisiae*. They claimed an improvement in the fermentation of aspen wood up to 82 wt-% of the theoretical yield in 24 h [29]. The differences in the yield obtained by us (38.0 wt-% theoretical yield) could be at least partially explained by the fact that in the above study intensively milled (particle size 177-840 µm) raw material was used whereas in our case relatively large (1-7 mm) chips were used. Naturally, also the wood species was different. Shafiei et al. (2013) pretreated Native spruce wood (*Picea abies*) in C2minOAc (both chips and powder) at 120°C in 1 to 15 h. The subsequent SHF process using commercial cellulases and *S. cerevisiae* gave 23.5 wt-% at 120°C in 1 h and 66.8 wt-% of the theoretical ethanol yield when the pretreatment time was prolonged to 15 h. As expected, when wood powder was used, 72.1 and 81.5 wt-% of the theoretical ethanol yield was achieved for pretreatments times of 1 and 15 h, respectively [30].

4. Conclusions

The use of C_2minCl and C_2minOAc as "structure-disruptive" solvents upon pretreatment of Lenga and Eucalyptus residues was demonstrated. In case of IL-pretreated Lenga, when the SSF strategy was applied and 0.173 g of ethanol/g of glucose was obtained (33.9 wt-% of the theoretical maximum). In case of C_2minOAc pretreated Eucalyptus residues after SSF, 0.194 g of ethanol/g of glucose was produced (38.0 wt-% of the theoretical maximum). Consequently, the combination of pretreatment with an appropriate ionic liquid followed by the SSF process is a potential process for bioethanol production from Lenga and Eucalyptus residues. Still, improvements are possible by further optimization of the operational conditions.

Acknowledgments

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Ethanol production from CMC and Avicel using ethanologenic *Escherichia coli* expressing a novel endoglucanase

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Abstract
Lignocellulose is a promising feedstock for ethanol production. The conversion involves three major steps: pretreatment, saccharification and fermentation of released sugars. Several enzymes are involved in the saccharification step, such as glucanases, glycosidases and xylanases. Nowadays, these enzymes have to be added to the process and are usually produced and purified by a dedicated supplier. In this work, we applied functional metagenomics to screen for new enzymes that could confer to ethanologenic *Escherichia coli* the ability to grow on cellulose as the sole carbon source while producing ethanol in the process.
A fosmid library of 30,000 clones was constructed from metagenomic DNA isolated from microorganisms inhabiting the rumen of Uruguayan cows (1). The library was screened for the expression of cellulases and xylanases using carboxymethyl-cellulose (CMC), avicel and xylan from birch wood as substrates. 27 clones were positive for cellulolytic activity and 11 showed xylanase activity, while 11 more showed both activities. Some of these clones conferred to E. coli strains the ability to grow on minimal media supplemented with cellobiose, filter paper (FP) or sugarcane bagasse as sole carbon sources. It was also found that the cellulolytic activity was inhibited by glucose and xylose, but not by cellobiose or avicel. Some of these fosmids were transferred into the ethanologenic strain MS04 (2), and ethanol production was confirmed under anaerobic conditions using CMC as sole carbon source (1).
Full sequencing was performed on these fosmids. In some of them no obvious genes for glycolytic enzymes were found, while others contained full operons that combined endoglucanases and xylosidases with enzymes also involved in lignocellulose deconstruction such as laccases. An endoglucanase encoded in one of these fosmids, was cloned and overexpressed on *E. coli* BL21 strain. The enzyme, named EndoG, was fully characterized, and its biochemical features were determined showing maximal activity at pH of 5.5 and 50ºC. The enzyme was not inhibited in the presence of cellulose degradation products (glucose and cellobiose), solvents (methanol, propanol and acetone), metal ions (Fe, Ca, Cd, K, Ni, Mg, Mn), chelating agents (EDTA), or salt (up to 4M NaCl).
EndoG was expressed in MS04 ethanologenic strain and, in co-culture with MS04 cells expressing a recombinant beta-glucosidase (3); 3.5 g/L and 2.0 g/L of ethanol was obtained from CMC or Avicel as sole carbon sources, respectively, after 12 hours of incubation at 42ºC and pH 6.0.

Introduction
Cellulosic materials are a sustainable feedstock for chemical and fuel production because of their relative low cost and plentiful supply. The main impediment to widespread utilization of this resource is the absence of low-cost technology for overcoming the recalcitrance of cellulosic biomass (4). To date, enzymatic hydrolysis is the most convenient and eco-friendly method for cellulose hydrolysis resulting in appreciable sugar yields (5) but the industry demands better cellulases with higher catalytic efficiency on insoluble cellulosic substrates, wider temperature range, increased stability, and higher tolerance to end-product inhibition (6).
Based on their catalytic action modes, there are three major types of cellulase activities: β-1,4-endoglucanase (EC 3.2.1.4), which cut internal sites on crystalline cellulose surfaces; exoglucanase, including cellobiohydrolase (EC 3.2.1.91) and cellodextrinase (EC 3.2.1.74), which remove cellobiose on the reducing or non-reducing ends of cellulose chains and β-glucosidase (EC 3.2.1.21), which hydrolyze soluble cellodextrins and cellobiose to glucose (7).
For lignocellulosic ethanol production, the enzymatic hydrolysis may take place in a separate step followed by fermentation (SHFprocess), or it may take place together with the fermentation in a simultaneous saccharification and fermentation (SSF) process. The ultimate objective would be a one-step consolidated bioprocess (CBP) of lignocellulose to bioethanol, in which all of these steps occur in a single reactor, where a single microorganism or microbial consortium converts pretreated biomass into products without added enzymes (8).

Experimental
Metagenomic library construction
Metagenomic DNAwas isolated from cow ruminal fluid fed with natural pastures as previously described (9). The 30–50-kbp fraction was isolated and cloned into the fosmid vector pCC1FOS. EPI300-T1R cells were transformed and selected in Luria broth (LB) solid media supplemented with chloramphenicol (Cm).

Library Screening
For the identification of clones with cellulase activity, the metagenomic library was replicated onto LB-Cm agar plates containing 0.5% carboxymethyl-cellulose (CMC) sodium salt or xylan from birch wood. Plates were incubated at 30°C for 5 days, and then were washed and stained with Congo red (10). Clones expressing CMCase activity were identified by the formation of clear orange halos around the colony. Fosmids were isolated from positive clones in primary screening and were re-introduced into fresh electro-competent E. coli EPI300-T1R cells to confirm their phenotypes.

Sequencing and gene annotation
Fosmid Csd4 sequence was determined by a shotgun library construction followed by Sanger sequencing of individual clones performed and assembled by Macrogen Inc. (Seoul, South Korea). Primer walking was used to close the gaps between contigs. Bioinformatic tools were run on the WebMGA server (11). Open reading frame (ORF) prediction was done by MetaGene (12) and annotation by RPS Blast (NCBI, USA). Comparison with the rumen metagenome from JGI (13) was carried out using IMG/Mand Blast. The DNA sequence of Csd4 is available in the GenBank database under the accession number KP843855.

Cloning, expression of endoG and protein purification
ORF Csd4_23 encode EndoG was amplified by polymerase chain reaction (PCR). PCR fragments were cloned into pET28a creating a C-terminal 6xHis Tag fusion. Recombinant EndoG-6xHis, was expressed in Escherichia coli BL21 (DE3) pLysS cultivated in 2xYT medium, induced during 4 hours at 30ºC in the presence of 0.1 mM isopropyl thio-β-D-galactoside (IPTG). Cells were lysed by sonication and rEndoG was purified using a Ni-NTA resin. Purity and integrity were checked by SDS-PAGE.

Enzymatic assays
Substrate 4-methylumbelliferyl-β-D-cellobioside (4-MUC) was used for enzymatic characterization. Exoglucanase activity was determined using 0.5 µg of EndoG, and 2 mM of 4-MUC. Reactions were carried out at 45ºC for 15 min. The release of 4-methylumbelliferone was followed by fluorescence (excitation 365 nm, emission 445 nm). The pH effect on EndoG activity was evaluated by carrying out enzymatic reactions in a mix of 0.05 M acetic acid, 0.05 M MES and 0.1 M Tris to ensure constant ionic strength (14). The effect of temperature on EndoG activity was determined between 20 and 80ºC in 50 mM sodium phosphate pH 5.5. For the thermostability assay, EndoG was pre-incubated at 50, 55 or 60ºC for 10–60 min in the absence of substrates. The residual activity was determined at pH 5.5 and 45ºC. The effect of metal ions (5 mM) on the activity of EndoG were determined at pH 5.5 and 45ºC. Analogously, acetone, methanol, propanol, butanol, toluene, Tween 20, glycerol, SDS and Triton X100 were evaluated at a final concentration of 5% (v/v), EDTA at 50 mM, and phenylmethanesulfonyl fluoride (PMSF) at 5 mM. To determine the effect of glucose (up to 1 M), cellobiose (up to 10 mM), acetate (up to 2 M), ethanol (up to 15%), furfural (up to 150 mM) or NaCl (up to 4M) on rEndoG activity the enzymatic reactions were carried out at pH 5.0 and 45ºC using 2mM 4-MUC as substrate.

Determination of substrate specificity
EndoG specific activity against 1% CMC sodium salt, lichenan, xylan from birch wood, avicel, pre-treated sugar bagasse or PASC was assayed at 45ºC and pH 5.0. Reaction products were quantified by determining the concentration of reducing sugars by the method of the dinitrosalicylic acid (DNS) (15). One unit (U) of glucanase activity was defined as the amount of enzyme that released 1 µmol of reducing sugars per min. Specific activity was defined as the units of enzyme per mg of protein.

Conversion of cellulose into ethanol
Ethanologenic E. coli strain MS04 (2) was complemented with Csd4 fosmid or pTrc99A-endoG. To provide β-glucosidase activity, MS04 cells expressing BglC (3) were used too. Fermentations were performed in minimal salt medium AM1 at 45°C and 150 rpm. For mixed fermentation, glucose and CMC were added. On single fermentation assay, CMC or avicel were added at 7,5 g/L as carbon sources. The enzyme expression was induced with 10 µM IPTG. Sampling was done every 2 h, cells and solids were removed by centrifugation and ethanol was analyzed by gas chromatography using n-butanol as the internal standard. All assays were performed in triplicates.

Results and discussion
Metagenomic library screening
A 30.000 clones metagenomic library was obtained from ruminal liquid. From those, 48 showed cellulose or hemicellulase activity in agar plates. 27 clones were able to breakdown carboxymethyl-cellulose (CMC), 11 showed xylanase activity against xylan from birch wood as substrate, while 11 showed both activities. Complements assay were performed for all clones and nine showed that could confer to *Escherichia coli* the ability to grow on minimal media supplemented with cellobiose, filter paper (FP) or sugarcane bagasse as sole carbon sources. Five of the nine positive clones showed 4-MUC activity in cellular extracts too. One of these, which also displayed this activity in intact cells, named Epi300(Csd4), was selected for further characterization.

Characterization of the Epi300 (Csd4) clone
Epi300 (Csd4) growth was evaluated on minimum media with avicel, FP, sugar cane bagasse or xylan as carbon source. The strain was able to growth after 5 days of incubation at 30ºC, demonstrating that cellulolytic genes were successfully expressed on *E. coli*. To assess the regulation of enzyme expression, 4-MUC activity was evaluated using complete cell and diverse substrates. The 4-MUCase activity was inhibited when glucose or xylose were added to media, but not when cellobiose or avicel were incorporated (Figure 1).

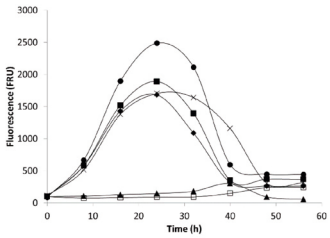

Figure 1. 4-MUC hydrolysis by Epi300 (Csd4) during growth in LB media supplemented with glucose (white squares), xylose (black triangles), cellobiose (black squares), CMC (cross), avicel (black circles), or with no supplement (diamonds). Initial OD 0.05. FRU fluorescent relative units. Experiments were conducted in triplicate, and averages were plotted.

Epi300(Csd4) clone growth in CMC as carbon source was evaluated. The strain was able to reach 0.6 OD after 120 hs at 37ºC, but wen exogenous β-glucosidase (BglC) was added, the same OD was achieve at 48 hs without lag phase (Figure 2).

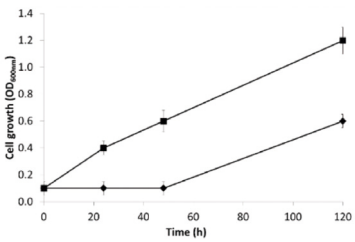

Figure 2. MS04 (Csd4) grown in minimal media containing CMC with (squares) or without (diamonds) BglC at a concentration of 10 U/g of substrate. Experiments were conducted in triplicate, and averages were plotted. Error bars indicate standard deviations.

Ethanol production by MS04 (Csd4)

To evaluate if Csd4 confer to an E. coli ethanologenic strain the ability of using cellulose as carbon source, the fosmid was transferred to MS04 strain and fermentation assay with glucose and CMC as carbon source were carried out. When only glucose was added, MS04 with and without Csd4 growth and ethanol production were similar, However, when CMC was present MS04 (Csd4) strain produce 27% more ethanol than parental strain after glucose exhaustion. To assess if Csd4 was also able to sustain ethanol production by MS04 from CMC in the absence of glucose, fermentations were conducted in minimal media with 1% CMC supplemented with exogenous BglC (Figure 3). MS04 carrying a control fosmid was not able to produce ethanol from CMC as the sole carbon source, while MS04 (Csd4) produced up to3.7 g/L of ethanol by 48 h of incubation, representing 65.4% of theoretical maximum yield. These data confirmed that Csd4, supplemented with a β- glucosidase, was sufficient to confer the ability to ferment CMC into ethanol by MS04 in the absence of any other fermentable carbon source and without the addition of exogenous endocellulase or exocellulase.

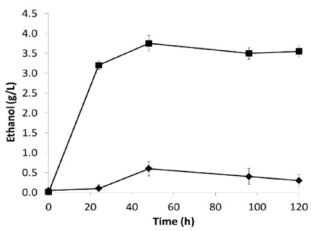

Figure 3. Ethanol production in mini-fermenters in minimal media containing CMC by MS04 carrying a randomly picked control fosmid (diamonds) or by MS04 (Csd4) (squares). BglC was exogenously added to the culture media at a concentration of 10 U/g of substrate. Initial OD620 0.1. Experiments were conducted in triplicate, and averages were plotted. Error bars indicate standard deviations.

Fosmid Csd4 sequence analysis

Full sequencing was performed on Csd4 fosmid. 28 ORFs were assigned on it 34,406 pb. 3 ORFs with predicted protein sequences putatively associated with lignocellulose degradation were identified: Csd4_16, Csd4_22, and Csd4_23.The ORF Csd4_16 encoding a putative β-xylosidase/arabinosidase belonging to CAZy glycoside hydrolase family43 (GH43). GH43 includes mostly enzymes that have β-1,4-xylosidase activity (EC 3.2.1.37). They are part of an array of hemicellulases that are involved in the final breakdown of plant cell wall. They hydrolyze β-1,4glycosidic bounds between two xylose units in short xylooligosaccharides. The ORF Csd4_22 deduced protein sequence has 59% identity with a polyphenol oxidase laccase belonging to COG1496. This cluster also includes RL5, a confirmed multicopper polyphenol oxidase with laccase activity (EC 1.10.3.2) isolated from cow rumen. Laccases are able to oxidize a wide variety of phenolic and non-phenolic compounds and are widely distributed among both prokaryotes and eukaryotes. It is worth noting that the screening that elicited Csd4 was not designed to detect either xylanases or laccases.
The ORF Csd4_23 was assigned into COG2730. This cluster has been clearly linked to endo-β-1,
4-glucanases (EC 3.2.1.4) belonging to GH5 family (16) that also includes endo-β-1,4-xylanases (EC 3.2.1.8) and β-glucosidases (EC3.2.1.21), among others. GH5 enzymes are abundant in different ecological niches, which have been evidenced by their frequent identification in various metagenomes studies (17,13).

EndoG biochemical characterization

EndoG was cloned and overexpressed on E. coli BL21 strain. The enzyme showed it maximal activity at pH of 5.5 and 50ºC. The enzyme was not inhibited in the presence of glucose or cellobiose, the cellulose degradation products. When solvents (methanol, propanol, ethanol and acetone), metal ions (Fe, Ca, Cd, K, Ni, Mg, Mn), chelating agents (EDTA), or salt (up to 4M NaCl) were added the enzyme activity was unaffected (Figure 4). Similarly, when inhibitors generated during biomass pretreatment were added, like furfural, acetate or ionic liquids, EndoG performance was slightly affected.

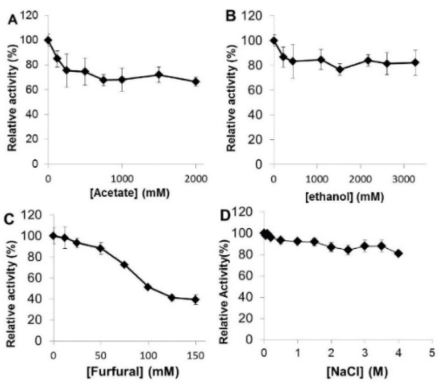

Figure 4. Activity of rEndoG in presence of common inhibitors. The effects of acetate (A), ethanol (B), furfural (C), and NaCl (D) on rEndoG activity were measured at standard reaction conditions using 2 mM 4-MUC as substrate. All data points represent the mean ± SD of experiments performed in triplicate

Ethanol production by MS04 (EndoG)

EndoG was expressed in MS04 ethanologenic strain and, in co-culture with MS04 cells expressing a recombinant beta-glucosidase (3); 3.5 g/L and 2.0 g/L of ethanol was obtained from CMC or Avicel as sole carbon sources, respectively, after 12 hours of incubation at 42ºC and pH 6.0 (Figure 5).

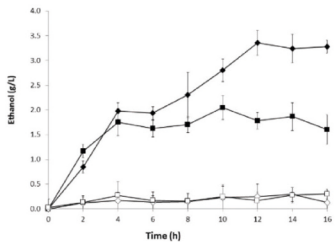

Figure 5. Ethanol production from 7.5 g/L CMC (♦) or avicel (□) by co-culture of MS04 cells expressing EndoG and BglC in 200 mL mini-fermenters incubated at 45°C and 150 rpm. Data points represent the mean ± SD of experiments performed in triplicate

Conclusions

Using a functional metagenomics approach, 48 clones with cellulolytic activity were identified. Nine of those conferred to E. coli strains the ability of use filter paper, cellobiose, sugarcane bagasse or avicel as carbon source. One fosmid from those clones, named Csd4, was fully sequenced and three enzyme involved in lignocellulose degradation were identified. An endoglucanase (EndoG) was characterized and its biochemical properties were determinated. The results showed that solvents, chelating agents, ion metals, glucose or cellobiose did not affect cellulose degradation ability of EndoG. The incorporation of Csd4 or EndoG to Escherichia coli ethanologenic strain MS04 allow the ethanol production from CMC and avicel on CBP configuration process.

Acknowledgements

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Sequential thermochemical hydrolysis, enzymatic saccharification and fermentation to ethanol of stover from white corn with ethanologenic bacteria

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Abstract

One of the main agricultural residues in Mexico is the stover obtained from the production of white corn, which is widely produced in this country for human consumption in the manufacture of *tortillas, tamales, tostadas* and a wide array of food products.

In the present study, the stover from white corn was characterized, and the conditions for maximizing xylose recovery and minimizing the formation of the toxic compounds known as furans during the diluted acid thermochemical hydrolysis were studied. These conditions were obtained by combining three variables: pretreatment temperature, holding time and H_2SO_4 concentration. Furthermore, the glucan contained in whole slurries from two selected pretreatment conditions was hydrolyzed with commercial enzymatic cocktails. The stover was subjected to a sequential thermochemical hydrolysis (with an initial load of 15% w/w), and enzymatic saccharification and at the end whole slurries were fermented with the metabolically engineered ethanologenic strain *Escherichia coli* MS04 to produce ethanol.

The thermochemical hydrolysis was designed to release the highest amount of xylose, but with a concomitant formation of low amounts of furans. It was found that 29.0% or 93.2% of the xylan was recovered as free xylose at 130°C, 8 min and 1% or 2% w/w of sulfuric acid, producing only 0.06 or 0.44 g/L of total furans, respectively. The enzymatic saccharification of slurries from thermochemical hydrolysis was performed with 15 FPU/g_{glucan}, pH 4.8 at 50 °C. After 24 h of enzymatic saccharification, 76 to 77 g/L of pentoses plus hexoses were obtained. These slurries, containing 0.03 to 0.26 g/L of total furans and 5.14 to 5.91 g/L of acetate, were fermented with the ethanologenic *E. coli* to 24.5 - 23.5 g/L of ethanol in 24 – 36 h. Furthermore, it was found that no detoxification or extra nutrient substrates are required to efficiently use the slurries as a culture medium for this bacterium. Additionally, the presence of solids, acetate and a small amount of furans in the slurries did not affect the bacterial metabolism to produce ethanol. Sequential processing allowed the recovery of approximately 92.5% of the polymerized sugars present in the stover and the production of the equivalent of 225 L of ethanol per metric ton of dry stover from white corn in the laboratory.

Key words: Ethanol, Corn Stover, Ethanologenic Escherichia coli, Sequential Process, Furans

Introduction

Bioethanol is one of the most important liquid biofuels for ground transportation. Lignocellulosic residues, such as corn stover, are widely recognized as suitable feedstocks for biofuel production. White corn is one of the main crops in Mexico and each year approximately 42 million tons of stover are produced from this crop, which can be used as a feedstock for bioethanol production.

Hemicellulose and cellulose are the main fractions containing polymerized sugars in lignocellulosic materials; and pentoses and hexoses are the most abundant monomeric sugars in such fractions. Widely speaking, pretreatment and saccharification are the main methods use to release sugars from hemicellulose and cellulose, respectively. The dilute acid thermochemical pretreatment method is used to hydrolyze sugars present in hemicellulose and enhances the cellulose digestibility [1]. However, concomitantly this method produces furfural and 5-hydoxymethyl furfural (HMF), two compounds that completely inhibit the *Escherichia coli* cellular growth at concentrations above 3 and 4 g/L, respectively [2].

In this study, to maximizing xylose recovery with low furans concentration, the conditions that affect the thermochemical hydrolysis with sulfuric acid were assessed: temperature, holding time and acid concentration. The material obtained from best thermochemical hydrolysis conditions (mainly glucans), was hydrolyzed with commercial enzymes without detoxification; and whole slurry (with solids) was fermented with the ethanologenic *E. coli* strain MS04 [3] to produce ethanol.

Experimental

The corn stover was obtained from a local feed store. Solids at a humidity of 4.7 % (w/w) were milled. Material selected for the study was the one retained between mesh 20 and 80 in a manual sieve. The macromolecular composition was determined using the reported online methodologies of NREL. The stover used contains (as percentage of dry material): Ashes 11%; extractives 7%; acetyl moieties 5%; glucan 30%; xylan 20%; arabinan 3%: and lignin 22%. The temperature effect was studied using microwave cooking and a Teflon bomb. The effects of time and sulfuric acid concentration were performed using an autoclave and 50-mL Schott flasks; these flasks were perfectly sealed to avoid steam loss during the pretreatment. Solid loads were 15% w/w for all pretreatments.

Saccharification of slurries pretreated with different concentration of sulfuric acid (130°C and 8 min) was carried out using commercial enzymes during 24 h in mini-reactors fitted with a peg mixer and the conditions described elsewhere [4]. Slurries containing solids and sugars from corn stover that was sequentially hydrolyzed with sulfuric acid (1%) and further saccharification with commercial enzymes were conditioned at pH 7 and 37°C to perform fermentation with the ethanologenic *Escherichia coli* strain MS04 [3]. Xylose and glucose were quantified using an enzymatic analyzer (YSI model 2700), furans (furfural and 5-hydroxymethyl furfural), arabinose and acetate by HPLC, and ethanol by gas chromatography as previously reported [4].

Results and discussion

Figure 1 shows the effect of two different temperatures on the release of xylose, arabinose and glucose from thermochemical hydrolyzed corn stover using the Teflon bomb and microwave radiation. Also, the formation of furans, furfural and 5-hydroxymethyl furfural, is shown. As the temperature was changed from 130°C to 170°C (with a holding time of 8 min and 2% of sulfuric acid), the release of glucose increased 75%. However pentoses consistently were reduced at temperatures above 130°C when microwave radiation was used and such conditions promoted the formation of furans, mainly furfural from the dehydration of pentoses, reaching values above 13 g/L at 170°C and 5-hydroxymethyl furfural concentrations close to 4 g/L. This amount of furans undoubtedly would inhibit the growth of ethanologenic microorganism [2], either bacteria or yeast. Hence, we decided to use 130°C and change the pretreatment method to indirect vapor using an autoclave to study the effect of holding time and acid concentration.

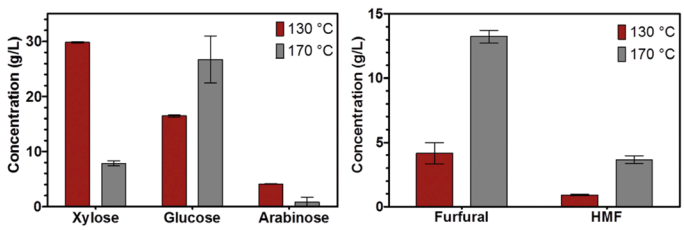

Figure 1 Production of sugars and furans (furfural and 5-hydroxymethyl furfural) from corn stover pretreated at different temperatures (holding time 8 min and 2% sulfuric acid).

The holding time effect on sugar release and furans production (Figure 2) was performed using an autoclave and holding the temperature at 130 °C during 0, 8 and 60 min (H2SO4 2%). The xylose released increased as the holding time was increased. At 0 min of holding time, the xylose concentration was 24.5 g/L, and 3.3 g/L of glucose and arabinose were detected with the production of 0.09 g/L of furfural and 0 g/L of HMF. Compared to 0 min, with 8 min of pretreatment, the xylose and arabinose concentrations increased 21% and 50% and 0.27 and 0.32 g/L of furfural and HMF were produced, respectively. When the holding time was increased to 60 min, the xylose concentration increased slightly and the arabinose concentration was very similar to values obtained with 8 min of holding time. However, furfural and HMF concentrations substantially increased to 1.2 and 0.5 g/L respectively. Holding times of 8 min allow releasing 93% of xylose present in the material with a low furans concentration; therefore we selected this time to continue the study.

The sulfuric acid concentration was studied at 130 °C and 8 min of holding time, and using 0%, 1% and 2% of sulfuric acid (Figure 2). No xylose release was detected when 0% of sulfuric acid was used, but 28.9% and 92.5% of the xylans were hydrolyzed to xylose with 1% and 2% of sulfuric acid, respectively. No furfural was produced when 0 and 1% of sulfuric acid were used and only 0.28 g/L were detected when the thermochemical hydrolysis was performed with 2% of

sulfuric acid. The study showed that 1% or 2% of sulfuric acid, 8 min and 130°C, are proper conditions to maximize the xylan hydrolysis with the added advantage producing total furans concentrations lower than the inhibitory concentration reported elsewhere [2].

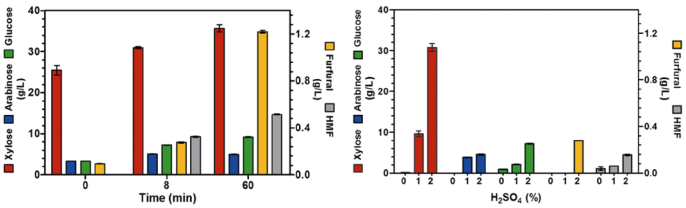

Figure 2 Production of sugars and furans (furfural and 5-hydroxymethyl furfural) from corn stover pretreated at different holding times (130°C and 2% sulfuric acid) and sulfuric acid concentrations (holding time 8 min and 130°C).

The slurries, containing solids and released sugars from thermochemical hydrolysis pretreated with 0%, 1% and 2% sulfuric acid, were processed by saccharification in sequential form with commercial enzymes (NS22086 Novozymes) at a load of 15 PFU/g_{Glucan} (50°C, pH 4.8 and 150 rpm) in a mini-reactor fitted with a peg mixer [4]. After 24 h of saccharification, 32%, 95.4% and 100 % of xylans were recover as free xylose from materials pretreated with 0%, 1% and 2 % sulfuric acid, respectively (Figure 3), and glucose was obtained at 26.8, 43.8 and 47.9 g/L of glucose, respectively. These values correspond to 53.8%, 87.9% and 96.2% release of glucose from the glucan fraction contained in the corn stover. After saccharification, no furans were detected in the slurries from materials pretreated with 0 and 1% sulfuric acid. The higher percent of glucose released suggest that the thermochemical hydrolysis with 1% and 2% of sulfuric acid improves glucan accessibility for the enzymes [5]. To evaluate the production of ethanol, we selected the slurry obtained from the sequential thermochemical hydrolysis of hemicelluloses with 1% of sulfuric acid and enzymatic hydrolysis of glucans with commercial cocktails.

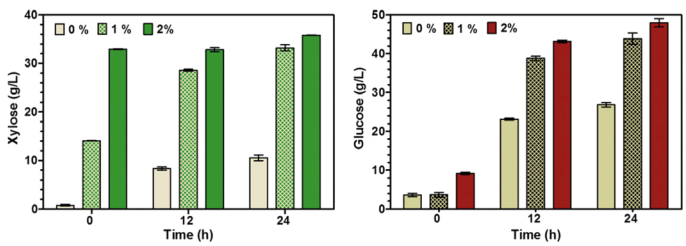

Figure 3 Increase of xylose and glucose at different saccharification times of slurries pretreated with different concentrations of sulfuric acid at 130°C and a holding time of 8 min.

The stover hydrolyzed with 1% H2SO4 (130°C, 8 min) and 15 PFU/g_{Glucan} was fermented to ethanol with 3.7 g/L of the ethanologenic strain E. coli MS04. Only the pH of the slurry was adjusted to 7, but the solids were not removed and no nutrients were added to perform the fermentation step, i.e. to follow the sequential concept. The slurry contained 35.4, 25.2 and 3.3 g/L of, glucose, xylose and arabinose respectively; furans were not detected. All sugars were consumed at 24 h and produced 25 g/L of ethanol, with an ethanol yield on sugars consumed of 76% of the maximum theoretical and a volumetric productivity of 1 g_{EtOH}/L.h. These data allow proposing the sequential process for industrial applications. Furthermore, together with the thermochemical hydrolysis at 130°C, 8 min and 1% H_2SO_4, these results shows that the detoxification step is not necessary in the sequential process developed in this study.

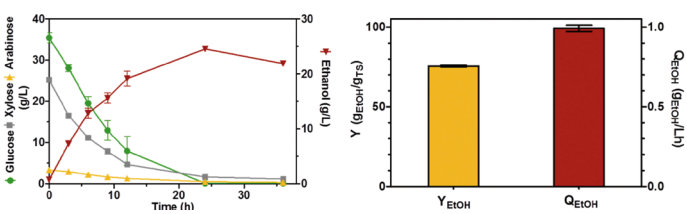

Figure 4 Kinetics of xylose, arabinose and glucose consumption and ethanol production from syrups (containing solids) produced from the sequential thermochemical and enzymatic hydrolysis of corn stover pretreated at 130°C, holding time of 8 min and 1% sulfuric acid. Ethanol yield (Y_{EtOH}) as percentage of the maximum theoretical based on the consumption of total sugars and volumetric ethanol productivity (Q_{EtOH} obtained at 24 h of elapsed fermentation time).

Conclusions

Results from this study demonstrate that is possible to perform the sequential thermochemical hydrolysis, enzymatic saccharification and fermentation to ethanol of stover from white corn with the metabolic engineered bacteria E. coli strain MS04. Furthermore, due to the conditions used, no detoxification process was necessary and the ethanologenic bacteria was able to ferment all sugars, including hexoses and pentoses, in the presence of solids and reached a yield of ethanol on sugars consumed of 76% of the maximum theoretical.

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Sustainability analysis of lignocellulosic bioethanol production and electricity generation. Western Mexico case study

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Abstract
A multi-feed/multi-task biorefinery design (MPB10) is presented as a solution for producing energy (lignocellulosic biofuels and electricity) and simultaneously treating agro-industrial wastes (cheese whey and tequila vinasses), providing a solution for the environmental pollution caused by the vinasses. MPB10 exhibits a higher process complexity than simpler conventional lignocellulosic biofuel biorefineries. However, the design increases the overall energy productivity and achieves similar environmental and economic sustainability values than a single-feedstock biorefinery producing lignocellulosic ethanol and electricity.

Keywords: lignocellulosic biofuels, sustainability analysis, biorefinery design.

1.Introduction

This paper presents a biorefinery design that exploits the use of multiple raw-materials for producing energy in the form of biofuels and electricity and, simultaneously, providing a solution for the environmental pollution caused by local agro-industrial wastes in a Western Mexico region. The biorefinery produces bioethanol from wheat straw and uses waste streams from the dairy (cheese whey) and tequila (vinasses) industries as raw materials for enhanced electricity production, making the biorefinery almost self-sustainable in electricity. Under this multi-feed/multi-task scheme, water consumption and TPC per unit of energy produced are substantially reduced compared with similar schemes found in the literature [1]. This biorefinery would be hypothetically located in a specific agro-industrial region of western Mexico with sufficient supplies within an 80 km radius of the main feedstock [2] and waste streams for a 500 ton DB (dry basis)/day capacity plant. Available amounts of cheese whey and tequila vinasses wastes in this region are approximately 100 ton/day (50% w/w) [3] and 3,900 ton/day [4] respectively. Vinasses treatment and its associated biogas production is currently being adopted by most large-scale tequila producers. However, small and medium size tequila factories may not have access to these technologies. A multi-feed/multi-task biorefinery that could be used in the region to deal with this environmental problem and simultaneously providing other benefits (i.e., energy production) could be an affordable solution.
This 500 ton DB/day multi-feed/multi-task biorefinery (termed MPB10) was designed to be as (economically and environmentally) sustainable as a similar (but less complex) single-feed biorefinery (SPB) producing bioethanol from the same feedstock (i.e., wheat straw) with a competitive TPC. Metric and indicator values taken from the sustainability analysis of SPB were employed as design guidelines for MPB10 focusing on improving energy productivity and reducing water consumption whilst maintaining the amount of bioethanol produced as close as possible to SPB production.
The following section presents a brief description of the MPB10 design as well as its single-feed biorefinery (SPB) counterpart. Section 3 then provides a quick introduction to the sustainability analysis method chosen for this work. Section 4 presents the values of the environmental, economic indicators and global sustainability employed as the basis for the design of MPB10 and its comparison with SPB. The sustainability of both designs is discussed, showing how MPB10 is almost sustainable in electricity (producing 99% of the electrical duty), consuming half of the process water per energy-unit-produced of SPB, with a similar TPC per energy-unit-produced. The paper closes in Section 5 with a summary of the pros and cons of MPB10 as an integral solution for the treatment of the agro-wastes considered.

2. Description of Biorefinery Designs

The block diagram of the multi-feed/multi-task biorefinery (MPB10) is depicted in Fig. 1. 10% of the pentoses-rich liquid stream is derived from the pretreatment to the dark fermentation stage to improve biohydrogen and organic acids co-production. These acids, together with the high Chemical Oxygen Demand (COD) vinasses stream, are fed to the wastewater treatment (WWT) stage, producing biogas. Biohydrogen and biogas, together with solid residues from the separation stage, are fed into the co-generation stage.

The SPB includes an overliming stage instead of the dark fermentation as shown in Fig. 1. Feedstock produces bioethanol from the available polysaccharides based on a conventional biochemical platform. WWT receives only lignin and biomass residuals for biogas production. Both designs include energy integration and 80% of process water recirculation. A detailed description of both biorefineries can be found in [5]

3. Sustainability Analysis Method

The method chosen in this work (i.e., [1]) is based on a Sustainability Framework (SF) constructed using the Process Analysis Method (PAM) [6] in which the process stages of the biorefinery can be clearly related to those stakeholders

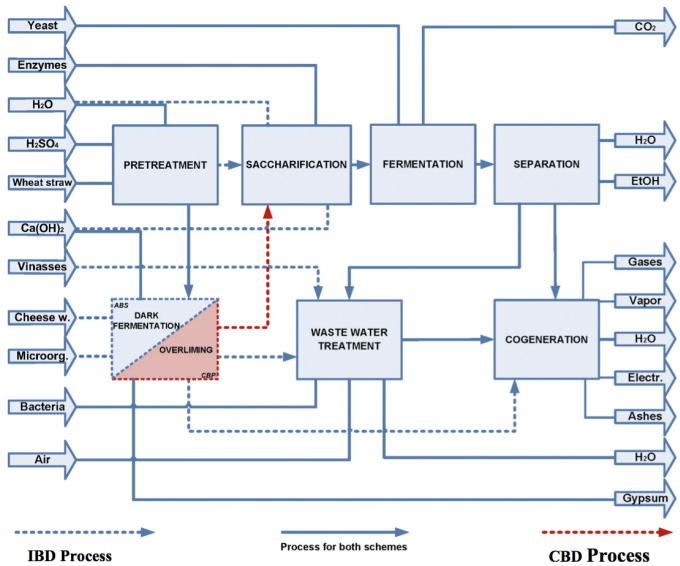

Figure 1: Block diagram of SPB and MPB10. Overliming substitutes dark fermentation in SPB.

receiving the impacts of the production process. A graphical representation of this sustainability analysis method is shown in Fig. 2.. Details of the method and calculation tools can be found in [1].

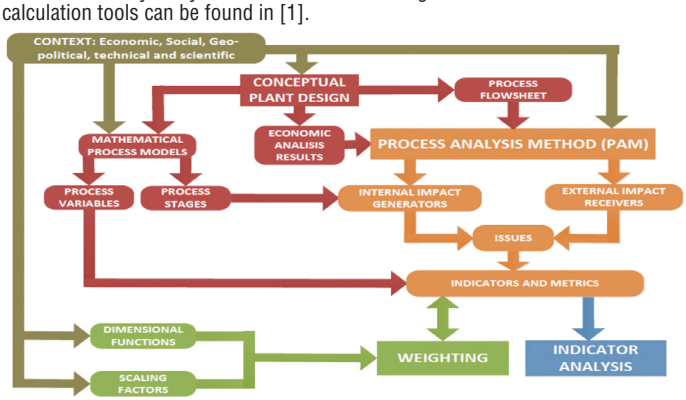

Figure 2: Sustainability Analysis Method

4.Results

Table 1 shows the results of the indicator calculations. The left-hand side column lists the 12 indicators obtained from the SF, followed by their associated metrics, the coefficient from the dimensional functions to monetize the indicators and their corresponding values for SPB and MPB10. The monetized values for each indicator are shown in the right-hand side of the table. EER and TPC indicators were identified as the most important contributors to the sustainability.

4.1 Analysis of global sustainability

Figure 3 shows the resulting values for environmental, economic and global sustainability, asserting that both designs can be considered equally sustainable. The values obtained for the EER indicator of both designs, once weighted, contribute around 90% to the global environmental indicator. For the economic domain, TPC and Yield indicators are, at least, two orders of magnitude higher than the rest of the economic indicators. However, TPC indicator values are about four times higher than Yield indicator, with a contribution to the global economic sustainability indicator of 76.6% and 81.3% for SPB and MPB10, respectively.

4.2 Analysis of environmental indicators

The normalized indicators for the environmental domain are shown in Fig. 4. For the emitted GHG indicator, the largest impact was obtained for MPB10 which is 10% larger than the corresponding SPB indicator. Notice that, in terms of CO2 production only, MPB10 produces 29% more CO2 than SPB (21,844 kg/h vs. 16,905 kg/h). The co-generation stage is the main contributor to CO2 production with three main sources: residual solids (lignin and non-converted sugars), biohydrogen and biogas. Approximately 80% of the GHG emissions come from the co-generation stage followed by the alcoholic cofermentation stage with 21.06% and 15.66% in SPB and MPB10, respectively.

Emitted non GHG refers to the emissions of PM2.5, PM10, NOx, SO2 and other pollutants [7]. Since these biorefinery designs only produce SO2, the other three metrics were not included in the SF. A 17% difference SO2 production was obtained between the designs (35.04 kg SO2/MJout and 30.19 kg SO2/MJout for SPB and MPB10, respectively).
Water consumption (WC) in MPB10 is around 50\% of SPB, as shown in Fig. 4. MPB10 only requires 0.8 L of fresh water per MJout against 1.57 L per MJout for SPB. This is because more process water is treated in the MPB10 WWT stage coming from the cheese whey and vinasses streams (about 1,790 ton/day and 210 ton/day of MPB10 and SPB, respectively). Therefore, in MPB10 a larger amount of water is recycled into the process, maintaining the recirculation percentage (80% fixed by design).

Environmental Domain								
Indicator	Metric	Value		Coefficient	Value		Monetized Indicator (USD/MJ_{out})	
		SPB	MPB10		SPB	MPB10	SPB	MPB10
Emitted GHG	$M_{CO_2}, \frac{gCO_{2eq}}{MJ_{out}}$	4.26E+02	4.68E+02	$C_{CO_2}, \frac{USD}{gCO_{2eq}}$	-3.50E-06	-3.50E-06	-1.49E-03	-1.64E-03
Emitted non GHG	$M_{SO_2}, \frac{gSO_{2eq}}{MJ_{out}}$	3.50E+01	3.00E+01	$C_{SO_2}, \frac{USD}{gSO_{2eq}}$	-1.50E-04	-1.50E-04	-5.26E-03	-4.53E-03
Water Consumption	$M_{W_C}, \frac{L_{FreshWater}}{MJ_{out}}$	16.00E-01	8.00E-01	$C_{W_C}, \frac{USD}{L_{FreshWater}}$	-1.69E-04	-1.69E-04	-2.66E-04	-1.35E-04
Water Quality	$M_{COD}, \frac{kgCOD}{MJ_{out}}$	1.92E-03	1.80E-03	$C_{COD}, \frac{USD}{kgCOD}$	-3.25E-02	-3.25E-02	-2.81E-04	-3.75E-04
	$M_{W_P}, \frac{kg_{DissolvedPollutants}}{MJ_{out}}$	2.30E-03	8.00E-04	$C_{W_P}, \frac{USD}{kg_{DissolvedPollutants}}$	0.00E+00	0.00E+00		
	$M_{W_T}, \frac{MJ_{thermalenergy}}{MJ_{out}}$	-9.10E-02	-1.3E-01	$C_{W_T}, \frac{USD}{MJ_{thermalenergy}}$	2.39E-03	2.39E-03		
	M_{pH}, pH	5.93E+00	6.31E+00	$C_{pH}, \frac{USD}{MJ}$	0.00E+00	0.00E+00		
Amount of Produced Solid Wastes	$M_S, \frac{kg_{SolidWastes}}{MJ_{out}}$	15.70E-02	13.4E-02	$C_S, \frac{USD}{kg_{SolidWastes}}$	-1.73E-02	-1.73E-02	-2.72E-03	-2.32E-.03
End use Energy Ratio (EER)	$M_{EER}, \frac{MJ_{out}}{MJ_{in}}$	4.80E-01	5.00E-01	$C^*_{EER}, \frac{USD}{MJ_{out}}$	1.40E-01	1.48E-01	-7.36E-02	-7.36E-02
Economic Domain								
Indicator	Metric	Value		Coefficient	Value		Monetized Indicator (USD/MJ_{out})	
		SPB	MPB10		SPB	MPB10	SPB	MPB10
Yield	$M_Y, \frac{MJ_{out}}{kg_{polysaccharides}}$	3.07E+00	3.6E+00	$C^{**}_Y, \frac{USD}{kg_{polysaccharides}}$	-1.21E-01	-1.21E-01	-3.94E-02	-3.35E-02
Production Cost	$M_{TPC}, \frac{USD}{MJ_{out}}$	1.40E-01	1.48E-01	C_{TPC}, -1	-1.00E+00	-1.00E+00	-1.40E-01	-1.48E-01
Reduction of Fossil Fuel Imports	$M_I, \frac{\Delta USD_{import}}{USD_{TotalImport}}$	2.90E-04	2.80E-04	$C_G, \frac{USD_{TotalImport}}{MJ_{out}}$	1.79E-02	1.46E-02	5.16E-06	4.05E-06
Fraction of the Energy Demand (for transport) that the Plant can cover	$M_t, \frac{MJ_{out}}{MJ_{transport}}$	2.10E-04	1.50E-04	$C_t, M_{TPC}, (\frac{USD}{MJ_{out}})$	1.40E-01	1.48E-01	2.94E-05	2.21E-05
Fraction of the Total National Bioenergy that is Produced in the Plant	$M_B, \frac{MJ_{out}}{MJ_{bioenergy}}$	3.50E-03	4.00E-03	$C_B, M_{TPC}, (\frac{USD}{MJ_{out}})$	1.40E-01	1.48E-01	4.86E-04	6.03E-04
Plant's Electrical Productivity	$M_E, \frac{Electricity_{out}}{Electricity_{in}}$	7.02E-01	9.91E-01	$C_E, \frac{USD}{MJ_{Electricity}}$	-4.40E-03	-1.00E-04	-3.06E-.03	-9.59E-05

Table 1: Metrics, coefficients and weighted indicators calculated for SPB and MPB10.

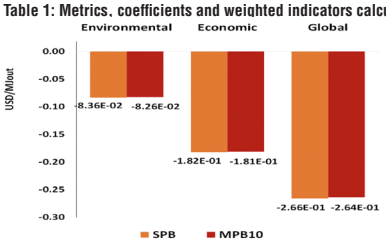

Figure 3: Sustainability values for SPB and MPB10

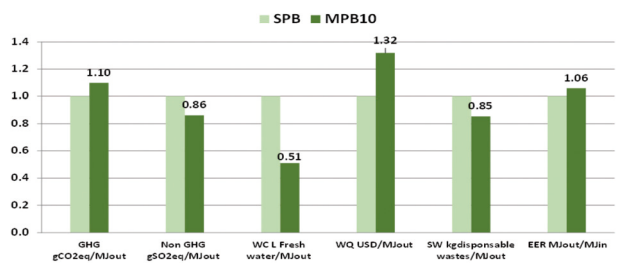

Figure 4: Normalized environmental indicators values. WC= Water Consumption, WQ= Water Quality, SW= Solid Wastes, EER= End-use Energy Ratio.

The obtained value for the water quality (WQ) indicator is the sum of its four monetized metrics (COD, dissolved pollutants, discharged water temperature and pH). The highest value of this indicator was obtained for MPB10, since the dissolved pollutants, the thermal energy and pH metrics values were higher for MPB10 than for SPB, due to the additional streams included in this scheme for the hydrogen and biogas production. Note that coefficients for dissolved pollutants and pH metrics are zero because the metric values are within accepted ranges of local regulations. The difference between SPB and MPB10, however, is about 32% larger for MPB10, as shown in Fig. 4.
The MPB10 solid wastes (SW) produced indicator was 15% lower than the value obtained for SPB, as shown in Fig. 4 and Table 1. This is because SPB and MPB10 produce the same amount of solid wastes in the overliming stage.
The calculated values for EER stem from the fact that the total energy produced by the ethanol LHV, steam and electricity in both biorefineries is smaller than their total energy demand. In terms of energy balance, SPB (producing 4% more bioethanol than MPB10), generates about 48% of the energy required by the process. However, SPB does not cover its electricity demands, falling short by 30%. MPB10 produces only 50% of its energy demand, but satisfies 99% of its electricity duty (5,119.36 kW-h/h) thanks to the extra burning fuel obtained from the cheese whey and vinasses streams. None of the designs meet their heating/cooling demands.

4.3 Analysis of economic indicators

Half of the indicators of the economic domain are very similar for both designs (see Fig. 5). Yield, TPC per energy unit produced and electricity productivity are discussed in this paper.
The total energy produced by each kilogram of polysaccharide fed is calculated by the yield indicator. The obtained value for MPB10 was 18% higher than SPB. Since a 10% of the pentoses-rich stream from MPB10 pretreatment is derived to biohydrogen production, the amount of produced bioethanol in SPB is slightly (4%) higher than that produced with MPB10. However, MPB10 produces 80% more electricity than SPB because it generates more biogas, in addition to bioethanol and biohydrogen, thus increasing the energy yield.
MPB10 TPC per energy unit is 6% larger than SPB TPC (0.148 USD/MJout vs. 0.140 USD/MJout) because this indicator strongly depends on the bioethanol production. In addition, the capital cost and the total investment for MPB10 is higher than for SPB due to the equipment required for the dark fermentation stage and the higher capacity WWT equipment.
With respect to electrical productivity, the calculated values of co-generated electricity for SPB and MPB10 are lower than the required electricity (70% and 99% for SPB and MPB10, respectively). However, including the cheese whey and vinasses streams to MPB10 considerably improved the electricity co-generation compared to SPB (from around 35% to 99%). The solid residues (non-converted sugars and lignin) from the separation stage are the main source of fuel for the electricity co-generation. The biogas from WWT is the second contributor to electricity co-generation for MPB10.

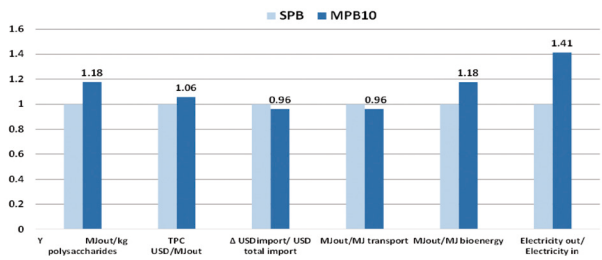

Figure 5: Normalized economic indicators values. Y= yield, TPC= Total Production Cost

5. Conclusions

The use of multiple raw materials for biofuel co-production to increase electricity productivity in MPB10 levels its economic and environmental sustainability with SPB. MPB10 also exhibits lower water consumption (51% of SPB), fewer solid wastes (85% of SPB), similar EER, higher yield and electricity productivity (41% higher than SPB) with only 4% lower production of bioethanol than SPB and the added benefit of solving a severe environmental pollution problem. Nevertheless, SPB TPC and TPC per energy unit produced are 18% and 6% smaller than MPB10 values, respectively.

Acknowledgements

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Biorefineries

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Thermochemical conversion

Upgrading of low-grade biogenic feedstock by innovative screw pyrolysis

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Introduction

Biomass is recognized to play a fundamental role in the energy systems of the future [1]; moreover, it is the only carbon-based renewable resource. Thus, it appears evident that biomass can contribute to energy system for heat and power generation, as well as raw materials for the production of liquid biofuels and valuable chemicals. Biogenic feedstocks include a wide spectrum of biomass, ranging from high valuable wood, down to inhomogeneous agricultural residues, organic municipal waste and manure.

Upgrading by pyrolysis and optionally further refining are major steps to convert low grade materials into valuable gaseous, liquid and solid fuels, respectively. Pyrolysis is the thermochemical decomposition of organics occurring in absence of oxygen [2]. The pyrolytic processes are suitable for small scale plant, addressing the widespread distribution of low grade biomass.

Taking into account the aforementioned issues, the Karlsruhe Institute of Technology (KIT) has developed a new pyrolysis reactor -STYX-, with integrated recleanable hot gas filters for the long-term stable production of particle and ash free vapors and condensates, respectively.

This work will at first introduce the technology, unfolding its flexibility in terms of feedstock and process parameters, with major experimental work on wood biomass as a reference. Afterwards, a detailed explanation of the sequential extraction of water and CO2 will be addressed. Some examples on the conversion of low grade materials will be provided, assessing finally the potential of the direct coupling of the pyrolysis reactor with high temperature CHP units.

STYX Reactor Technology

The STYX is a trough screw reactor with integrated hot gas filters. In contrast to conventional screw rotary drum reactors, it has a U-shaped design, which enables the displacement of the ceramic filter candles directly within the vessel. The bench scale unit has an electrically heated length of 2 m, a diameter of 0.15 m and a freeboard height of 0.20 m and a maximum capacity of 75 kWtherm. Intermediate pyrolysis is performed at reactor temperatures between 250°C and 550°C, while the residence time of the solid may be adjusted in the range of 5 to 25 minutes.. Moreover, the eight electric heater sections may be adjusted independently to different temperatures, thus enabling the emulation of a hot gas stream. The feeding system is provided with four independent inlets for the co-feeding of different feedstocks and of additives. After a major reconstruction process, the actual configuration is provided with 6 horizontal filter candles, equally distributed along the reactor length. The candles are grain ceramic filter elements Schumalith, with a coarse-grained support made of silicon carbide associated with fine aluminosilicate filter membranes. Both sides of the elements have openings, which enable the recovery of the vapors, on the one hand, while the other side provides recleaning gas for online re-cleaning. Once the vapors suction side is closed, nitrogen is applied from the other side on a slight overpressure; it flows through the candle matrix and detaches the filter cake, which falls on the bottom of the screw and is transported to the char outlet. The re-cleaning system may be manually adjusted or operated in an automated mode. A condensation train is associated to the reactor, made of 3 double pipe condensers, where the cooling medium is a water-glycol mixture. The condensate is a mixture of a water-like phase with a separated bottom oil-like phase, which may be easily removed by mechanical separation. The non-condensable organic aerosols are finally recovered adopting an appropriate electrostatic precipitator. Figure 1 reports the flow sheet of the STYX reactor.

The reactor may be operated for the production and characterization of the pyrolysis products, as well as a research tool for the evaluation of specific parameters and for fundamental work on the pyrolysis process. The STYX is equipped with several sampling ports along its length, thus the progression of the primary degradation of the solids may be investigated at 4 intermediate local positions, equidistant between the feedstock dosing system to the char recovery barrel.

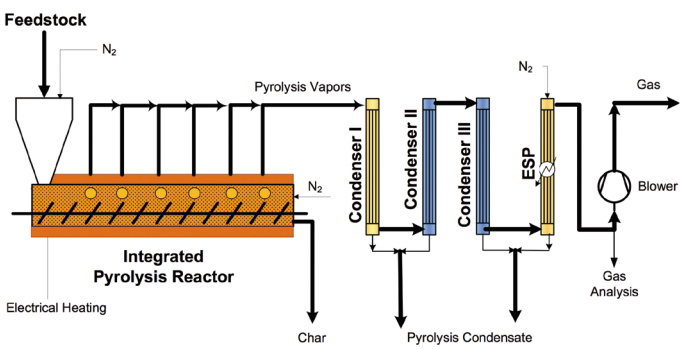

Fig. 1: Flow sheet of the STYX Reactor

Furthermore, the evolution of the pyrolysis vapors, i.e. the secondary homogeneous gas-phase reactions and heterogeneous reactions on the filter cake kinetics, may be investigated in steady state operation at 6 positions, before and after each filter element.

Finally, a procedure called sequential filtration and extraction has been integrated with the target of concentrating organic pyrolysis vapors by locally extracting the moisture water steam and the CO2, which are released in the early steps of the process [3].

Process and Feedstock Flexibility

The STYX reactor has been operated continuously for more than 1500 hours between 2012 and 2014, with the main target of producing pyrolysis products from a wide spectrum of feedstocks under different process conditions. More than 20 different biomass and other low-grade materials have been successfully tested in steady-state conditions for several hours of operation each. The spectrum spans from lignocellulosic biomass, such as beech wood and poplar wood or wheat straw, down to low-grade materials such as residues from agricultural and forestry industries like coffee dust and cherry stones and further down to very low-rank fuels such as manure, sewage sludge and oil sand. Some results are reported in [4], while other examples will be treated later on this paper. Potential stand-alone plant configuration for the decentral conversion of such low-grade materials into heat and power adopting pyrolysis are presented in [5].

However, extensive work has been carried out adopting a high valuable, bark and ash free, beech wood as a reference. Such a clean material enables an accurate description of the process, allowing the characterization of fundamental mechanical, thermal and chemical mechanisms within the reactor. Figure 2 shows some selected results from these investigations with beech wood, specifically the reproducibility of the experiments at fixed process parameters (a), the mass yields distributions before and after the reconstruction (b), the carbon and energy content of the char (c) and the mass yields of selected organic liquid markers (d) for pyrolysis temperatures between 350°C and 500°C.

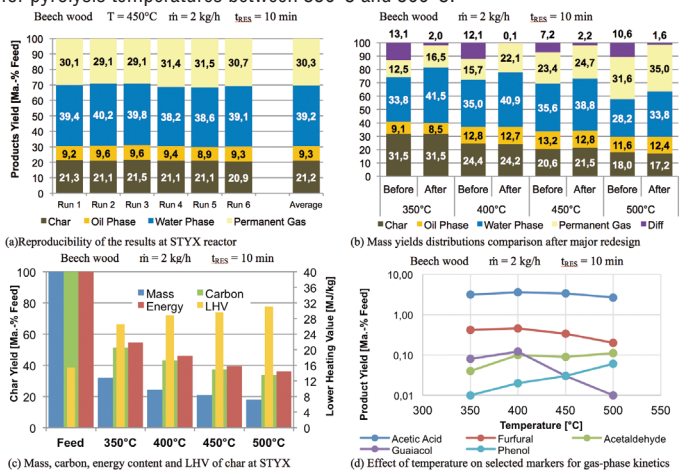

Fig. 2: Examples of experimental work at STYX adopting the reference biomass beech wood.

Reproducibility is of fundamental relevance for the characterization of the pyrolysis process. In Figure 2(a) it is highlighted that, during a preliminary test campaign of 6 runs and total 30 hours of operation and 60 kg of feedstock, the variation of the results is ±0.4% for the solids and for the oil phase, and ±1.0% for the water phase. The permanent gas yield was, in those cases, balanced by difference.

In early 2015, a major reconstruction aimed at the improved placement of the filter elements, as well as at a general optimization of the reactor. Exemplary results of the improvement in the mass balance are reported in Figure 2(b).

Figure 2(c) reports the calorific properties of the char as function of the temperature. It is shown that the lower calorific value of the solids increases with the increasing release of volatile matter and, that the carbon content very well approximates the energy content. Finally, Figure 2(d) shows the effect of the reactor temperature on some specific condensable organics. Details are reported in [3]. Briefly, the vapors released during pyrolysis are made of several hundreds of single substances, thus the description of the vapors composition, as well as the modelling of the gas-phase reactions, requires a reduction or lumping process. One approach may be the definition of specific markers, representing the reaction path for a class of substances. In this case, furfural, acetaldehyde and acetic acid are considered for the cellulosic components; guaiacol and phenol have been adopted as primary and secondary products of lignin.

Besides the mass and energy balances, the characterization of the pyrolysis process plays an important role in the procedure of parameter optimization. Properties such as the calorific values of the products and the viscosity of the oil have to be experimentally determined in order to design the pyrolysis process and to tailor its products to the preferred application. The results of such characterization are highlighted in Table 1.

Table 1: Calorific values of the pyrolysis products and dynamic viscosity of the pyrolysis oil at STYX

Temperature [°C]	Char [MJ/kg]	Oil Phase [MJ/kg]	Water Phase [MJ/kg]	Permanent Gas [MJ/kg]	Viscosity Oil [mPa s]
350	26.5	19.0	5.0	3.4	75
400	28.9	20.6	6.3	6.5	106
450	29.5	21.0	3.2	8.5	163
500	31.0	22.4	1.5	12.6	132

Sequential Extraction and Filtration

The moisture content of biomass has always been a limitation for pyrolysis processes, since the drying has high heat requirements and because the water ends up in the pyrolysis condensate. Typically, the biomass is pre-dried in a separated reactor down to a moisture content of about 10 Ma.-%, which is a suitable value for the screw pyrolysis.
The characteristic displacement of the filters, as well as of the outlets, may enable the extraction of the water in the first section, together with a fraction of the CO2, which is also released during the initial stage of the pyrolysis. A procedure for the control of the extraction process is currently under development targeting at the increase of the calorific value of the pyrolysis vapors by extracting the non-burnable components CO2 and water vapor. The first results have been described in detail elsewhere [6]. However, Figure 3 reports the compositions as well as the mass yields of the two vapors streams. There is a significant reduction of the water vapor content in the main stream. The calorific value of the main stream (about 11 MJ/kg) is increased by a factor 2 in comparison to that of the extracted one, which still contains much condensable organics in the form of acids and aldehydes.

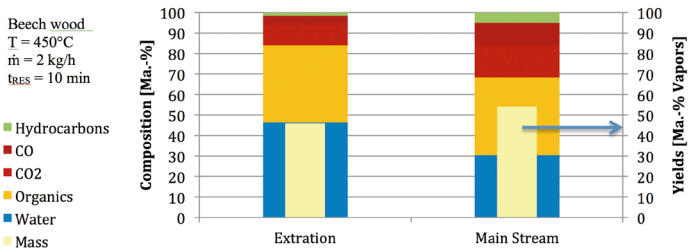

Fig. 3: Compositions and yields of extracted and main vapors streams

The current results are encouraging for the further exploitation of the sequential extraction and filtration procedure.

Low-Grade Biomasses Conversion

Pyrolysis at STYX reactor was designed for the treatment of low-grade feedstocks, since previous work already proved that the screw technology was well suited to convert difficult residual materials. As already mentioned, the STYX reactor reliability have been tested adopting more than 20 different feedstocks. Exemplary mass balances are reported in Table 2.
The yield of the char increases with the content of inorganics. Specifically potassium, calcium and phosphor species quantitatively remain in the char. The presence of phosphor in relatively high concentration (about 10 Ma.-% of the char for manure), associated to the high nitrogen content (about 5 Ma.-% in the chars) and a stable carbon matrix may open new markets for the effective application of pyrolytic char of agricultural and industrial residues.

Table 2: Mass balances of selected feedstock at STYX (450°C, 2 kg/h, 10 min)

Feedstock	Char	Oil Phase	Water Phase	Gas (diff)
Coffee residues	28.0	16.4	25.0	30.6
Wheat straw	30.9	11.3	32.6	25.2
Chicken manure	44.8	10.2	23.7	21.3
Sewage sludge	50.0	13.5	21.3	15.2

Concerning of the volatile products, some options are foreseeable. Because of the effective high temperature filtration, the vapors and the condensate from STYX are particle and ash free. However, there are issues related to the nitrogen and sulfur content of the vapors; see Figure 4 as an example for residues from the coffee industry and chicken manure. Pyrolysis condensates from agricultural residues have pH of about 9.5 to 10. The water phase, in particular, has high ammonia content, which may lead to environmental concerns, but it may also unfold interesting application opportunities. On the other hand, the most of the sulfur is found in the non-condensable gases. The emissions may be, therefore, maintained under control. One other chance to reduce the sulfur emissions may be the in-situ sorption of sulfur adopting additives during the pyrolysis process [4].

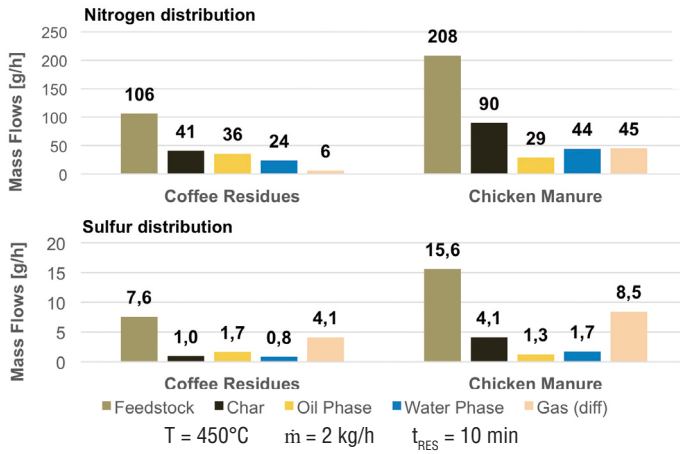

Fig. 4: Nitrogen and sulfur distributions among the pyrolysis products at STYX

Despite the content of potential pollutants, CHP in decentralized units is often mentioned as a suitable option for pyrolysis applications. Other possibilities are hereby enlisted:

- Direct application of the oil as fuels in internal combustion motors and turbines, if the emission limits can be met;
- Conversion of the vapors without condensation in combustion chambers and externally fired thermal engines;
- Refining of the oil including removal of nitrogen (HDN), sulfur (HDS), etc.

Although CHP applications appears to be the most suitable in the near future, the introduction of pyrolysis oil in the major streams of actual refineries and its inclusion in foreseeable biorefineries may not be underestimated.

Conclusion

Major investigations have been carried out adopting high quality wood for fundamental understanding of the effects of process parameters in a new trough screw reactor with integrated hot gas filtration. Future work will focus on kinetics and modelling issues.
Intermediate pyrolysis of low-grade feedstock at STYX reactor has been investigated thoroughly, with the aim of demonstrating the suitability of the technology in stand-alone applications for the production of upgraded solid and liquid fuels, as well as in combination with CHP. However, the effective and clean combustion of pyrolysis vapors and condensate are essential for high efficient and reliable CHP systems based on screw pyrolysis.

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Selective production of formic acid from aqueous phase bio-oil by catalytic oxidation using heteropoly acids (for bio-oil hydrodeoxygenation)

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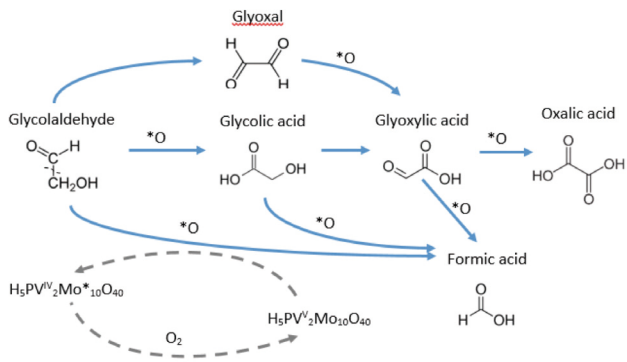
Abstract
In this work we show that catalytic oxidation of aqueous phase bio-oil with molecular oxygen and a Keggin-type polyoxometalate ($H_5PV_2Mo_{10}O_{40}$) produces formic acid with high selectivity. Bio-oil was obtained from sawdust (pinus radiata, size of 1-3 mm, moisture content of 9.1% on dry-basis), in a fast pyrolysis pilot plant using a fluidized bed of quartz sand at 530°C. The main aqueous-soluble compounds detected in 100 g of bio-oil were: 5.98 g of glycolaldehyde, 3.58 g of levoglucosan, 2.26 g of acetol, 1.39 g of acetic acid and 1.06 g of formic acid. The catalytic oxidation of the aqueous phase bio-oil was conducted at 90°C under 30 bar O_2 for 7h and produced 13.27 g of formic acid per 100 g of bio-oil. The process uses air or molecular oxygen as a cheap and green oxidant. The enriched product in formic acid will be used as H-donor for bio-oil hydrodeoxygenation at mild conditions.

1.Introduction
Pyrolysis of lignocelulosic biomass is one of several biorefinery strategies currently in development to reduce World's reliance on crude oil. Due to the high oxygen content of pyrolysis liquids (bio-oil), upgrading is needed to convert bio-oil into transportation fuels, for example, by hydrodeoxygenation (HDO). HDO requires hydrogen and severe operating conditions (350-450°C, H_2: 5-15 MPa) [1]. To minimize external hydrogen supply, one approach is to fractionate bio-oil by addition of water and to produce hydrogen via aqueous-phase reforming. However, the temperature of this process is above 200°C, which favors the formation of solid or tar by-products in the aqueous phase bio-oil [2]. A recent approach proposes hydrogen donor compounds (e.g. formic acid or alcohols) as source of hydrogen [3, 4]. In general, hydrogenation with H-transfer is often carried out under much less severe conditions than those applied to HDO processing of bio-oils, but the success of this technology depends on the source and cost of H-donor compounds.
Formic acid – a hydrogen donor – is present in small amounts in bio-oil. The chemical transformation of holocellulose or derived compounds in organic acids is a subject matter of long standing. Since early twentieth century it is known that it is possible to obtain formic acid as a by-product of the production of D-arabinonic acid from D-glucose [5]. Formic acid is also a byproduct of the production of levulinic acid from cellulose (Biofine process) [6]. Under basic conditions and in the presence of hydrogen peroxide (18 mmol glycolaldehyde, 200 mmol H_2O_2, 0.5N KOH) glycolaldehyde is converted almost quantitatively to formic acid in 1 h and 38°C [7]. Using glucose as substrate, 25% yield of formates are obtained in absence of base, and 75% in the presence of 1M NaOH at 1 min and 250°C, and peroxide excess (240% stoichiometric) [8]. The peroxide excess is necessary to avoid the formation of dehydration products of glucose, which are oxidized giving mainly acetic acid. Recently, it was shown that using heteropoly acid catalysts, formic acid can be selectively obtained from carbohydrates derivatives (glucose, xylose, cellobiose, xylan, glycolaldehyde and glyoxal), using oxygen or air as oxidizing agent at moderate temperatures and pressures. The complete conversion of glucose to formic acid (48% yield) and CO_2 is reported using the kegging-type polyoxometalate $H_5PV_2Mo_{10}O_{40}$ at 90°C and 30 bar of O_2. CO_2 is not produced by formic acid decomposition [9]. Li et al., using air as the oxidant (5 MPa) at 100°C and the same catalyst (5 mol %), obtained 52% yield formic acid from glucose in 3 h [10]. Besides the possibility of using air as the oxidizing agent, the oxidation catalyzed by polyoxometalates does not require raising the pH of the substrate (aqueous phase bio-oil: pH ~ 2), unlike the methods mentioned above.
In this contribution, we show that formic acid can be obtained with high selectivity from the carbohydrates present in the aqueous phase bio-oil using the Keggin-type polyoxometalate (POM) $H_5PV_2Mo_{10}O_{40}$ catalyst and molecular oxygen as the oxidizing agent.

2. Experimental
2.1. Materials and preparation
The POM catalyst $H_5PV_2Mo_{10}O_{40}{\cdot}35H_2O$ was synthesized according to the literature [11]. Analysis by ICP-OES revealed a P/V/Mo ratio of 1/1.98/9.17. The FT-IR spectra showed the same signals as published by [11, 12].
Pinus radiata sawdust was dried to a moisture content of 9.1% (dry-basis), and was sieved to obtain a particle size in the range of 1-3 mm. Fast pyrolysis process was conducted using a 20 kg/h pilot plant The system consists of a biomass feeder, an injection auger, a fluidized bed reactor of quartz sand, a cyclone, hot vapor filter made of sintered Inconel 6.10 steel (pore diameter: 22 µm), and a quench, a cyclone, a condensing tower plus an electroprecipitator for condensing pyrolysis vapors. The temperature of the fluidized bed was hold at 530°C, and the mean residence time of pyrolysis vapors was 1.7 s.
Aqueous phase bio-oil was obtained by dissolving bio–oil in butyl acetate (1:1) and extracting the solution with water (1:1), and washing the aqueous phase twice with butyl acetate (1:1). Ratios expressed in (wt:wt.).

2.2. Catalytic oxidation reactions
All oxidation experiments were carried out in a 1200 mL Parr reactor of AISI-316 stainless steel. The mixture of substrates and catalyst (100-150 mL) was stirred at 400 rpm in oxygen atmosphere and heated to the desired temperature in approximately 30 min. After holding for the specified time samples were extracted from the reactor and analyzed by HPLC using 2 Phenomenex ROA- Organic Acid H^+ columns in series at 75°C and a eluent flow of 0.6 ml/min H_2SO_4 0.0075 M.

3. Results and discussion
Table 1 shows the oxidation of glycolaldehyde at different oxygen pressures and reaction times. The selected reaction temperature was 90°C. Albert et al., [13] reports that under 70°C the catalyst has low catalytic activity, and about 100°C the decomposition of formic acid is favored. Without the catalyst formic acid selectivity was low. The presence of glycolic and oxalic acids in the reaction product is an indication that in aqueous medium and presence of molecular oxygen, glycolaldehyde oxidation follows the steps shown in Scheme 1. Higher oxygen pressures favored formic acid production (entries 1, 7, 9 and 10), which reached up to 50% after 7h with 30 bar of oxygen (entry 10). Similar yield were obtained with POM catalyst at 12 bar and 3 h (entry 2). The highest yield of formic acid was obtained at 20 bar oxygen pressure and 3 h reaction time (entry 5).. The long reaction time in presence of POM catalyst affected adversely the formic acid production (entry 5 and 6). In general, it can be observed that in all experiments with the POM catalyst glycolaldehyde conversion and formic acid selectivity increased significantly. This reflects the catalytic ability of the POM catalyst to facilitate oxygen insertion into C-C bonds of carbohydrates [14] (see Scheme 1).



Scheme 1. Possible steps for glycolaldehyde oxidation with molecular oxygen.

Table 1. Oxidative conversion of glycolaldehyde to formic acid (FA).

N°	Catalyst	Pressure [bar]	Time [h]	Selected compounds after reaction [mmol/100g]					Substrate Conv. [%]	FA-Yield [%]	Selectivity* [%]
				Glycolaldehyde	Formic Acid	Glycolic Acid	Glyoxilic Acid	Oxalic Acid			
1	-	12	12	1.72	8.06	0.62	N.D.	0.03	89.3	25.3	28.3
2	POM	12	3	3.45	16.16	1.01	N.D.	0.07	78.4	50.7	64.7
3	POM	12	7	0.80	19.51	0.43	N.D.	0.01	95.0	61.2	64.4
4	POM	12	12	N.D.	20.44	N.D.	N.D.	0.09	100	100	64.1
5	POM	20	3	N.C.	24.20	0.20	N.D.	0.29	100	75.7	75.7
6	POM	20	7	N.D.	22.66	N.D.	N.D.	N.D.	100	70.9	70.9
7	-	30	3	4.56	14.47	N.D.	N.D.	0.02	71.5	45.3	63.3
8	-	30	7	2.36	15.69	0.14	N.D.	0.15	85.2	49.1	57.6
9	POM	30	3	3.20	14.66	0.64	N.D.	0.06	79.9	46.0	57.6

Reaction conditions: substrate 15.96 mmol glycolaldehyde and 1.54 g of POM catalyst dissolved in 100.0 mL H_2O, temperature 90 °C with 400 rpm stirring. N.C.: Not quantified; N.D.: Not detected; FA-Yield: Formic acid yield per mol C substrate. *Selectivity = [(FA-Yield)/(Substrate Conv.)]·100%.

The reactivity of levoglucosan in acid aqueous medium in presence of oxygen is not well known. Table 2 shows oxidation experiments using levoglucosan as a substrate. Without the POM catalyst (entries 1 and 2), low conversion of levoglucosan was observed and only some acetic acid formed. In the presence of the POM catalyst (entries 3 and 4), levoglucosan conversion approached 80% after 7 h of reaction, but formic acid yield was only moderate.

Table 3 shows the results of aqueous phase bio-oil oxidation. In this case, formic acid production increased 3.0-6.1 times in the presence of the catalyst compared to the experiments without catalyst (entries 3 and 6 compared to entry 1). Acetic acid was the main by-product, probably derived from acetol oxidation. Considering

Table 2. Oxidative conversion of levoglucosan to formic acid (FA).

N°	Catalyst	Pressure [bar]	Time [h]	Selected compounds after reaction [mmol/100g]								Substrate conv. [%]	FA-Yield [%]	Selecti-vity* [%]
				Levoglu-cosan	Formic acid	Acetic Acid	Glycolic acid	Glyoxilic acid	Oxalic acid	Glyoxal	Glycolaldehyde			
1	-	20	3	2.21	0.20	0.28	N.D.	N.D.	N.D.	N.D.	N.D.	10.5	1.3	12.6
2	-	20	7	2.25	N.D.	0.28	N.D.	N.D.	N.D.	N.D.	N.D.	8.8	-	-
3	POM	20	3	-	0.91	N.D.	N.D.	2.00	N.D.	N.D.	N.D.	-	6.2	-
4	POM	20	7	0.51	3.35	N.D.	N.D.	0.97	0.18	0.17	N.D.	79.3	22.6	28.5

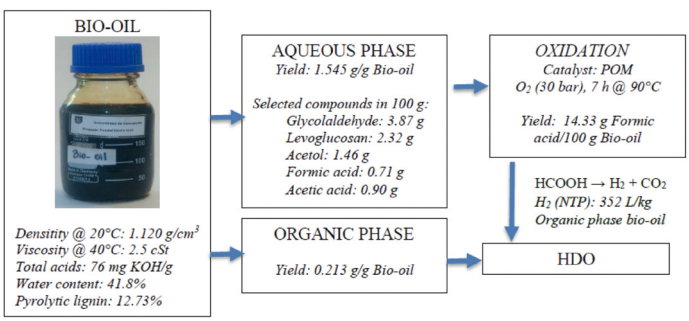
Reaction conditions: 2.47 mmol levoglucosan and 1.54 g of POM catalyst dissolved in 100.0 mL H2O, temperature 90 °C with 400 rpm stirring. N.D.: Not detected; FA-Yield: Formic acid yield per mol C substrate. *Selectivity = [(FA-Yield)/(Substrate Conv.)]·100%.

Table 3. Oxidative conversion of aqueous phase bio-oil.

N°	Catalyst	Pressure [bar]	Time [h]	Selected compounds at indicated time [mmol/100g]								Substrate conv.[a] [%]	FA-Yield[a] [%]	Selectivity* [%]
				Glycolaldehyde	Levoglucosan	Acetol	Formic acid	Acetic acid	Glycolic acid	Glyoxilic acid	Oxalic acid			
			0	15.38	3.42	4.70	3.66	3.58	N.D.	N.D.	N.D.	-	-	-
1	-	20	3	8.09	2.37	2.40	11.01	6.79	N.D.	1.99	0.32	44.4	14.4	35.3
2	-	20	7	10.99	3.43	3.91	9.43	11.96	N.D.	3.16	0.30	23.3	11.3	66.3
3	POM	20	3	N.D.	0.86	N.D.	25.90	11.79	N.D.	N.D.	0.68	95.4	43.4	48.3
4	POM	20	7	N.D.	0.80	N.D.	29.98	12.69	N.D.	N.D.	0.70	95.7	51.4	56.7
5	POM	30	3	N.D.	1.36	N.D.	43.60	16.15	N.D.	1.16	1.34	92.8	77.9	84.0
6	POM	30	7	N.D.	0.73	N.D.	48.32	16.30	0.46	1.19	1.18	96.1	87.1	90.6

Reaction conditions: 31.53 g of aqueous phase bio-oil and 1.54 g of POM catalyst dissolved in 100.0 mL H_2O, temperature 90 °C with 400 rpm stirring. N.D.: Not detected. FA-Yield: Formic acid yield per mol C substrate. a per mol C of glycolaldehyde and levoglucosan. *Selectivity = [(FA-Yield)/(Substrate Conv.)]·100%.

results of the entry 6 and the mass balance of the global process (see Scheme 2), 14.33 g of formic acid was obtained per 100 g of bio-oil (13.27 g produced by oxidation). The decarboxylation of formic acid yields approximately 352 L H2 (NTP) per kg of organic phase bio-oil for the hydrodeoxygenation of the organic phase bio-oil (21 wt. % of bio-oil). This value is comparable to those reported by Elliot et al., [15] for hydrodeoxygenation of various samples of bio-oil and bio-oil fractions using a Pd/C catalyst. They reported a consumption of H_2 in the range of 76-252 H_2 L/ L of bio-oil. Using a commercial Co-Mo catalyst can raise the hydrogen consumption in the hydrotreatment up to 500 L/L bio-oil [16].



Scheme 2. Mass balance of oxidation process.

4. Conclusions

Efficient conversion of carbohydrates present in aqueous phase bio-oil to formic acid was achieved using molecular oxygen and a POM catalyst. Glycolaldehhyde oxidation to formic acid was optimal at an oxygen pressure of around 20 bar. However, levoglucosan was not selectively converted to formic acid at this condition. The amount of hydrogen available from the product by formic acid decarboxylation is comparable to the demand reported in the literature for bio-oil hydrodeoxygenation. The formic acid enriched product will be used as H-donor for organic phase bio-oil hydrodeoxygenation.

Acknowledgements

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Catalytic hydrodeoxygenation of pyrolysis oil over nickel-based catalysts under H_2/CO_2 atmosphere

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1 Introduction

Pyrolysis oils from biomass are considered to play a key role in future of biorefineries. Due to their higher-energy density (approx. 15-30 MJ/kg) and moderate level of biomolecule decomposition they are an attractive platform feedstock in the bioeconomy [1]. The main problems in use and handling of pyrolysis oils are their high viscosity, high acidity, lower heating value then fossil fuels and low chemical stability. These negative properties are mainly caused by the high oxygen content present in the organic components. For this reason an upgrading step like catalytic hydrodeoxygenation (HDO) is often necessary and has to be investigated and optimized.
Nickel is quite often considered attractive for the industrial application of the HDO process. Nickel catalysts have reached a satisfactory deoxygenation degree in many studies [2, 3]. Ruthenium was found to have a higher HDO activity than nickel, but its application is limited by the relative low abundance and the high price.

Mortensen et al. (2013) performed a wide screening with a total of 23 different catalysts on phenol at 100 bar hydrogen pressure and 275 °C [4]. Nickel has been tested on a variety of supports and among them zirconia showed the highest activity as a carrier material. Therefore the nickel-based catalysts seem to be promising for a successful deoxygenation of raw pyrolysis oil, but only few of them were tested on real bio oils. The influence of the support also needs to be investigated further.

For this reason the systematic testing of nickel-based catalysts on raw pyrolysis oil was the objective of our previous work. The mild HDO at 250°C is up to now fully investigated. In summary our work showed that nickel-based catalysts are promising for the hydrotreatment process for upgrading raw pyrolysis oils. Ru/C is still superior but too expensive and rare (table 1). The results from nickel-based catalysts were satisfying. A phase separation into polar and apolar groups took place which favors a densification of energy and an isolation of platform molecules. At 250°C the pyrolysis oils are mildly hydrogenated and stabilized. Some hydrodeoxygenation reactions could be detected. No big influence of the support or promoters on the hydrodeoxygenation was found. This indicates that the role of the support is less important for the hydrogenation than for the hydrodeoxygenation.

Another approach is a physical enhancement of the HDO reaction by the use of high CO2 pressures. Under near critical conditions the solubility of a gas in a liquid can increase by several orders of magnitude. The liquid phase can be expanded and changes its physical properties significantly. This physical state is called gas expandes liquid (GXL). H. Zang (2015) carried out a number of revealing experiments with carbon dioxide and pyrolysis as GXL system [5]. The rheological behavior of the GXL and the volume expansion under near critical, supercritical, and liquid carbon dioxide were measured. The influence of carbon dioxide on the viscosity is also very strong. At 52°C and a CO_2 pressure of 5 bar the viscosity is reduced by 30%. With 40 bar of CO_2 the viscosity decreases by 60%. Thus the characteristics of pyrolysis by the expansion of carbon dioxide are mutable. This is why influences on the catalytic deoxygenation are conceivable. Due to these facts the catalytical HDO was also investigated under elevated pressures of CO_2 over Ni/Al_2O_3 catalyst.

Tab. 1 - H_2 consumption at 250°C from several Ni-based catalysts

catalyst	H_2 consumption at 250°C	
	NL/kg	mol_H/kg
blank	25	1.1
Ru/C	108	4.8
$NiCu/Al_2O_3$	71	3.2
Ni/SiO_2	59	2.6
Ni/ZrO_2	50	2.2
NiW/AC	60	2.7
Ni/TiO_2	54	2.4
Ni/Al_2O_3	62	2.7

2 Experimental

2.1 Catalyst

A supported nickel catalyst that was found active for the HDO reaction was chosen. For this purpose, an industrial nickel catalyst (METH® 134, 25±1% nickel oxide on alumina, C&CS) which is actually a commercial methanation catalyst was used. The reduction was accomplished in a heated reaction tube at 500°C for 4 hours by a gas mixture of 50% hydrogen in nitrogen at a flow rate of 2.6 NL/min. The catalyst raw material was crushed and sieved to obtain a uniform particle size fraction in the range of 0.25 0.50 mm.

2.2 HDO reaction

For the HDO experiments two 200 ml batch reactors were used. Both were built in the KIT-IKFT workshop. For each run we used 50 mL pyrolysis oil and 2.5 g catalyst powder. The reactor was always flushed with nitrogen for 10 min (Air Liquid Alphagaz™ 6.0) to remove oxygen and avoid explosive mixtures with hydrogen. After flushing, the reactor was pressurized. The reactor was charged with CO_2 (Air Liquid Alphagaz™ 6.0) first. The set pressure corresponds to the partial pressure of carbon dioxide in the mixture (0 bar, 20 bar, 40 bar). Next, hydrogen (Air Liquid Alphagaz™ 6.0) was added. The absolute total pressure was always 80 bar at room temperature.
The reaction time was 2 hours, including the ramp (5°C/min for 250°C, 15°C/min for 340°C). The agitator was running during the reaction time at 1000 rpm. The experiments were run all isochorically and under autogenous pressure. The pressures reached 150-205 bar at 250°C and 250-325 bar at 340°C. After the reaction time the reactor was rapidly quenched.

2.3 Analytics

2.3.1 Gas production and determination

Gas chromatography (GC) was used to determine the mol fraction of the process gases (H_2, CO_2) and side products (e.g. CH_4) in the gas phase after the reaction. The GC we used was a 6890 Agilent with two switchable columns (Restek 57096 Hayesep Q and Resteck Molsieve 5A). Two detectors were build in: a FID (flame ionisation detector) and a TCD (thermal conductivity detector).

2.3.2 Water content

The water content of the liquid products is expressed as mass fraction, and was determined by the Karl Fischer titration determined. The titrator used is an 841 Titrando from Metrohm.

2.3.3 Elemental analysis

For this purpose, an elemental analyzer (CHN628, Leco) was used which measures the content of carbon, hydrogen, and nitrogen. The mass fraction of oxygen is calculated as the difference between 100% and the percentage of the other elements.

2.3.4 Quantitative ^{1}H-NMR spectroscopy

For a more detailed analysis of the chemical structures in the liquid samples a quantitative hydrogen-nuclear magnetic resonance spectroscopy (^{1}H-NMR) was applied. For pyrolysis and deoxygenated oils the method is appropriate and is proposed as a method of analysis of pyrolysis oils in the literature [6].
We used a Bruker Biospin spectrometer. The measurement parameters were set a the following: 90° pulse program (4.95 µs), acquisition time (AQ) = 10.0663 s, relaxation delay (D1) = 1.0 s, number of scans (NS) = 24, spectral width (SW) = 3255.2 Hz, time domain (TD) = 32K.
The spectra were integrated in specific regions as proposed in literature [7], each representing a certain chemical groups .

2.3.5 GC-MS

In order to identify components and reaction products, a gas chromatography with coupled mass spectrometry (GC-MS) was applied. For this purpose, the solutions of the samples from the NMR measurements were used. Since the column of the gas chromatograph can be blocked and damaged by fine particles, the samples were filtered before the measurement (Teflon® Ø0.2 µm). We used a HP 1800A Gas Chromatograph with a GCD Stabilwax® column. The mass spectrometer used was an HP 5971.The mass spectra were analyzed with a computer and were compared to a database to identify individual chemical compounds.

3 Results and discussion

3.1 Hydrogen consumption

The activity of the different catalysts is reported as normal liters of hydrogen consumed during the reaction per gram of pyrolysis oil. The values were all quite similar and have an average of 53.8 NL/kg at 250°C, and 71.7 NL/kg at 340°C. No trend could be observed when using CO_2, but NMR and elemental analysis indicate a slightly weaker activity for hydrogenation (see also 3.4).

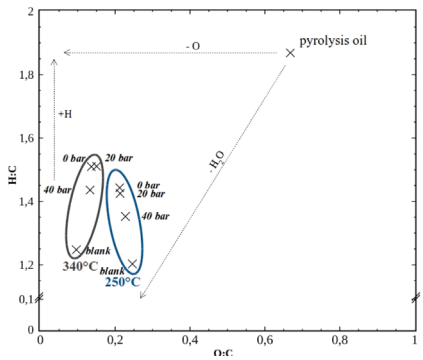

Fig. 1 - Van-Krevelen-plot of raw pyrolysis oil (feed) and the HDO reaction products from different temperatures and CO_2 atmospheres (catalyst: Ni/Al_2O_3)

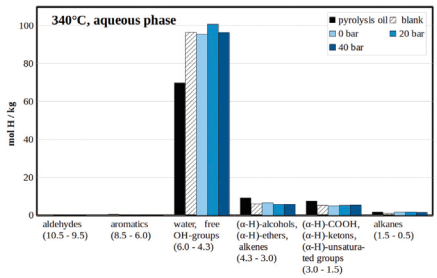

Fig. 2 - Content of H-species in the aqueous phase obtained at 340°C (measured by ^{1}H-NMR spectroscopy) under various CO_2 pressures. Ranges in ppm.

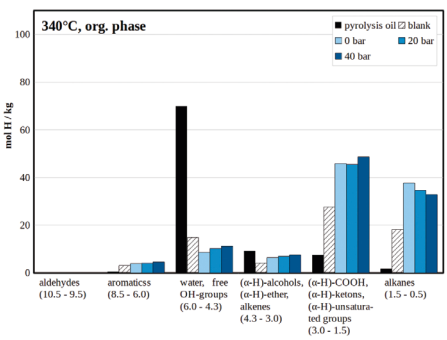

Fig. 3 - Content of H-species in the oil phase obtained at 340°C (measured by ^{1}H-NMR spectroscopy) under various CO_2 pressures. Ranges in ppm.

3.2 Products, yield and water content

The main products at 250°C were an aqueous phase (80-82%) and an oil phase (7-9%). The oil phase had a higher density than the aqueous one. At 340°C the yield shifts to more oil phase (12-13%) and less aqueous phase (73-77%).

The losses during the recovery were in the range 3-8%. The production of gases and solids was limited to small percentages (1-2%), mainly coke, CO_2 and negligible amounts of lower hydrocarbons (e.g. CH_4). The aqueous phases of all the reactions at 250°C had a similar content in water (70.3-72.1%) while the oil products contained 9.2-14.5% water. The pyrolysis oil initially contained 58.3% water. At 340°C the water content increases in the aqueous phase (80.3–82.3%) with regard to 250°C. This can be interpreted as a more effective deoxygenation/hydrogenation and therefore more intensive phase separation. The yield of oil dry mass is significantly higher at 340°C than at 250°C (45% vs. 25% in average).

The expansion with CO_2 showed no effect on yield or water content at both temperatures. Further, we detected no relevant amounts of methane. We conclude that the catalyst did not hydrogenate CO_2 to methane although the conditions can favor the methanation.

3.3 Elemental composition

The results from elemental analysis and Karl Fischer titration are reported in a Van Krevelen plot which is used to judge and interpret the quality of fossil fuels like crude oil. With higher temperature the values shift to higher H:C ratios and lower O:C ratios (figure 1). Taking the separation in two phases into account we observed a combination of hydrodeoxygenation reactions and of the repartition of more apolar compounds in the upgraded oil. The blank test shows lower content of hydrogen, but the oxygen content is similar to the nickel-based catalysts.

The only observed effect when using CO_2 was a weakening of the hydrogenation with decreasing partial pressure of hydrogen in the mixture, both at 250°C and at 340°C.

The deoxygenation with nickel-based catalysts was successful, but was not improved in comparison to earlier works. The oxygen is reduced from 43.5% in the feed to 20-23% in the oil (dry basis).

3.4 Changes in chemical structure

The chemical nature in the aqueous phases is not significantly changed by the HDO reaction at both temperatures. Only the concentration of water and free hydroxyl groups increases sharply. They increased at 250°C in all experiments on about 90 mol/kg and at 340°C even further, to about 95 mol/kg. At 250°C all chemical groups remain almost unchanged in their concentration. At 340°C a slight decrease oxygen-containing groups (alcohols, ethers, ketones and carboxylic acids) can be seen (figure 2).

The chemical composition of the oil phase changes significantly in comparison to the raw pyrolysis oil. The concentration of water and free hydroxyl groups is reduced to about a quarter. Alkyl groups and α-H atoms of carbonyl groups form the majority of the hydrogen species. Also aromatics are present in the organic phases with 5-10 mol/kg. With increasing temperature the concentration of polar groups is reduced, and the concentration of aliphatic and polar groups increases.

There is only a weak trend in chemical nature with increasing partial pressure of CO_2. The concentration of aliphatics decreases at 340°C in the oil phase due to the lower partial pressure of hydrogen (figure 3). Similarly, the concentration of oxygen-containing groups increases (water, free OH-groups, alcohols, ethers, α-H atoms of carbonyl groups).

With GC-MS we found mainly formic acid, acetic acid, propionic acid, hydroxypropanone, and levoglucosan. All these substances are typical pyrolysis products from cellulose and hemicellulose. Some furans and furfurals were also present in the investigated oil. Typical lignin fragments could be found, like phenol, guaiacol and syringol. Also numerous other phenol derivatives were present. Many unidentifiable compounds had molecular weights between 200 u and 300 u.

In the organic phase we found many phenol derivatives having two to three methyl or ethyl groups. The organic phases, which were obtained at 340°C with a catalyst, still show further reaction products. It has been found a number of substances, which are formed by ring-opening reactions of heterocyclic compounds (such as furfurals).

4 Conclusions

In summary, a successful hydrodeoxygenation was achieved. Only the expansion with CO_2 was not effective as expected. No significant GXL effect could be observed. Classical GXL catalysis is usually performed under milder conditions. We suspect that the chosen temperature was to high to obtain a significant physical enhancement. Further studies will check this theory. However, no negative effect could be observed, except an indirect weakening of the hydrogenation reactions due to the lower partial pressure.

A positive finding of our work is that nickel-based catalysts are not active for the methanation of CO_2 when they are used together with pyrolysis oil.

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Concept for combined heat and power production from wood via gasification followed by catalytic gas cleaning

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Abstract
To overcome the disadvantageous of wet synthesis gas cleaning (scrubbers) in combined heat and power production from biomass gasification, a dry gas cleaning system with catalytic tar reforming was developed and demonstrated in full stream downstream a 500 kW fuel input circulating fluidized bed gasifier. Recently, catalysts are further developed for application with lower temperature on one hand and for lower residual tar concentration for chemical utilization of cleaned gas. This development is carried out in a test rig with simulated synthesis gas with controlled dosing of model tar components and selected catalyst poisons.

Introduction
Based on a detailed technology survey on different gasification technologies for woody bio-mass, Fraunhofer UMSICHT started its process development in 1994 with the fundamental decision to use a fluidized-bed reactor as core component for a CHP plant, because back then most of the existing fixed-bed gasifier systems showed severe problems in gas quality and gas cleaning; many of these reported difficulties are even today hindering market entry to a larger extent [1]. Also in fluidized-bed gasification the producer gas contains contami-nats that need to be removed before gas utilization in gas engines in order to produce power and heat. These contaminants are primarily dust (fly ash and attrited bed material) and tar [2]. The original intention of the development was to modify or replace the bed material (silica sand) in a way that the resulting producer gas has a tar concentration below the en-gine's threshold value. For this purpose, a circulating fluidized-bed gasification pilot plant with a fuel capacity of 500 kW was built and operated with a large variety of bed materials from pure silica sand over silica sand with additives to other natural and artifical minerals. The tar content of the producer gas could be greatly reduced by these changes from about 4,250 to 10,000 mg/m^3(s.T.p.) for pure silica sand down to 120 mg/m^3(s.T.p.) for the best material used in the test runs [3,4]. On one hand this value still was above the engine's treshold value of 50 mg/m^3(s.T.p.), which implied secondary tar removal downstream the gasifier, and on the other hand these materials were expensive and in comparison to silica sand very prone to attrition, so that a large amount must be fed to the gasifier to keep up the solid inventory, which turns the whole process uneconomic. The final choice fell on olivine as bed material, which showed moderate activity in tar reduction inside the gasifier (2,500 mg/m3(s.T.p.)), is cheap and very resistant to attrition. The remaining research task was to develop a dry tar removal system to avoid all known disadvantages of wet tar scrub-bing systems [1].

Conceptual Design of Combined Heat and Power Station
With the results from bed material testing and the aim, to avoid wet tar removal systems, the design of a CHP station based on biomass gasification is straight forward and shown in fig. 1.

The core component is a fluidized-bed gasifier (either bubbling or circulating) with olivine as bed material, operated with air as gasifying agent for the ease of operation. The producer gas is fed to a catalytic reactor to reduce the tar content and subsequently cooled down to around 120 °C to allow dedusting with cheap and simple baghouse filters. Afterwards, the cleaned producer gas can be cooled down to engine entry temperature (40 °C) and the occurring condensate can be disposed into sewerage. Electricity can be fed to the grid and heat can be collected from the engine, the flue gas and the producer gas cooler.

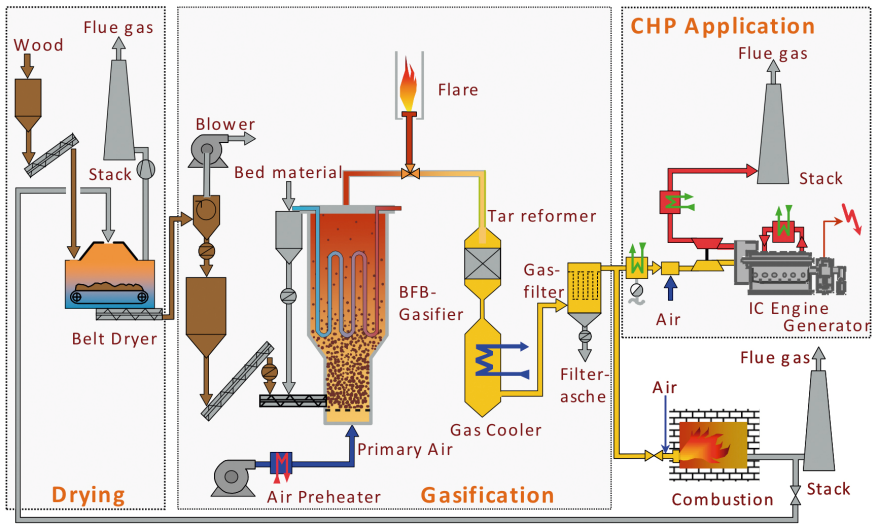

Figure 1: Process scheme of combined heat and power plant [9]

Tar Reformer Development
Material Testing in Side Stream
To test a variety of catalytically active materials for the reduction of tar content in producer gas, the 500 kW pilot plant was equipped with a small scale reactor in a side stream. The majority of materials were bulk solids applied as fixed bed. These materials ranged from minerals (silica sand, bentonite, bauxite, dolomite) over metals (stainless steel swarf) and coke (activated charcoal, activated lignite) to supported reforming catalysts (Ni, Ni/Co, Co/Mo). All materials besides silica sand, bentonite and stainless steel swarf showed sufficient activity to reduce the tar content of the producer gas below the engine's threshold value at least for a limited time. Due to the high reforming temperatures of around 900 °C the carbon based materials not only converted the tar at their surface but also underwent gasification reactions with steam and carbon dioxide. This led to a continuous loss of material from the reactor. The best results were achieved with different types of dolomite and a supported Ni-catalyst [3].
The main disadvantage of the fixed bed reactor system was the fact that the dust coming from the gasifier together with the producer gas accumulated in the fixed bed and by that increased the pressure drop of the fixed bed tar reforming reactor constantly. Similar find-ings led to the development of a tar reforming system based on a moving bed with continu-ous catalyst-dust separation and recycle of catalyst by Fraunhofer IFF [5]. However, the choice for further development of tar reforming at Fraunhofer UMSICHT was the use of supported Ni-based catalysts on honeycomb-type monoliths, as these systems only need a single reactor, where the catalyst can stay inside the whole lifetime. Here, only reforming catalysts are contemplable as natural minerals cannot be shaped with sufficient mechanical strength, and Ni was chosen because of its superior performance compared to all other re-forming catalysts in the bulk material tests [3]. A long-term stability test with 100 operating hours was performed and a naphthalene conversion and conversion of higher hydrocarbons of above 95 % could be achieved over the whole time [3].

Operational Experience from Full Stream Reformer
The promising results from the tests in the side stream reactor encouraged the institute to build a full stream catalytic tar reforming reactor, which is shown in fig. 2.
The tar reformer was connected directly to the exit of the primary cyclone of the gasifier to maintain the highest possible temperature of the producer gas. The monoliths used in the full stream reactor had dimensions of 15x15x30 cm. For the treatment of the whole producer gas, 9 monoliths were needed in a single layer and in total two layers were necessary (although the reactor was designed for three layers). As the reforming reactions taking place at the catalytic surface were all endothermic, the temperature of the gas reduces along the monolith axis. Ni catalysts are known to be active only at elevated temperatures. Therefore, the temperature of the gas mixture was increased between the first and second layer of monoliths by controlled addition of air. A test run over 150 h was performed with a fresh set of monoliths and later accumulated in additional tests a total of 486 h with varying gasification conditions and biomass fuels (even with very high sulfur contents). The dust displacing on the monoliths needed to be blown

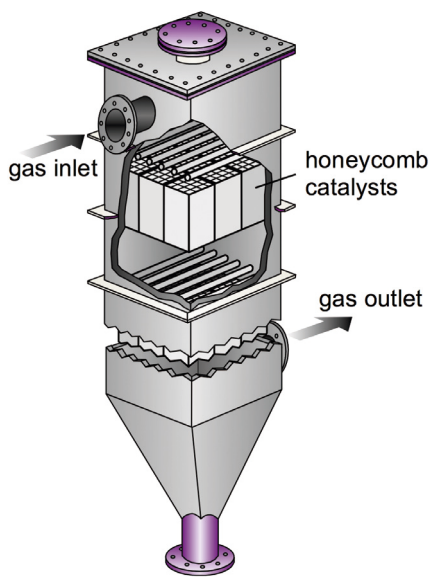

Figure 2: Reactor Design for Full Stream Tar Reformer [7]

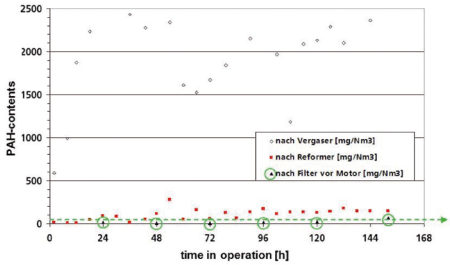

Figure 3: Content of PAHs in mg/m³(s.T.p.) behind gasi-fier (◊), behind reformer (■) and behind filter (▲) [8]

off periodically and the forming carbon deposits had to be burnt off from time to time together with the conversion of inactive NiS to NiO. With these measures, the original catalyst activity could be maintained over the whole accumulated time on stream [6,7].

Figure 3 shows the concentration of polyaromatic hydrocarbons (PAH) in the producer gas for the 150 h test run. As can be seen clearly, the output concentration of the gasifier is fluctuating, but the average value is very constant around 2,000 mg/m³ (s.T.p.). The value behind the catalytic tar reformer is slowly increasing over time due to carbon deposition and sulfur poisoning. Behind the baghouse filter prior to the engine's entry valve the concentration of PAHs is always below 50 mg/m³ (s.T.p.). After that time the catalyst needed regeneration. For the test conditions it could be concluded, that a regeneration procedure of roughly 4 to 5 hours is needed once a week of continuous operation [8].

Greenhouse Gas Reduction and Cost Calculations

Four different power station capacities from very small plants to industrial size were investigated and greenhouse gas emissions were calculated for two options: for variation 1 the electrical auxiliary power was taken from the German power grid with an overall emission of 597 g_{CO_2}-Equivalent/kWhel, while for variation 2 it was assumed to use electrical power from own production for auxiliary electricity. The results are shown in fig. 4. Certainly, the greenhouse gas emissions coupled with biomass production do

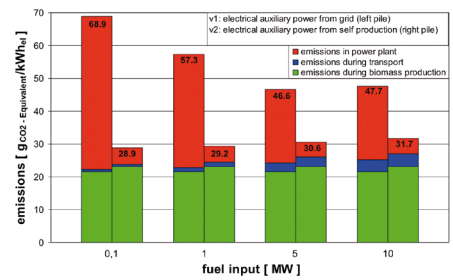

Figure 4: Greenhouse gas emissions in biomass CHP as a func-tion of fuel input capacity [9]

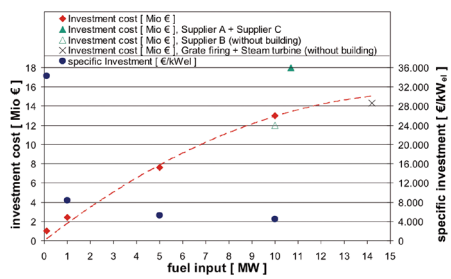

Figure 5: Calculation of investment cost for biomass gasification CHP [9]

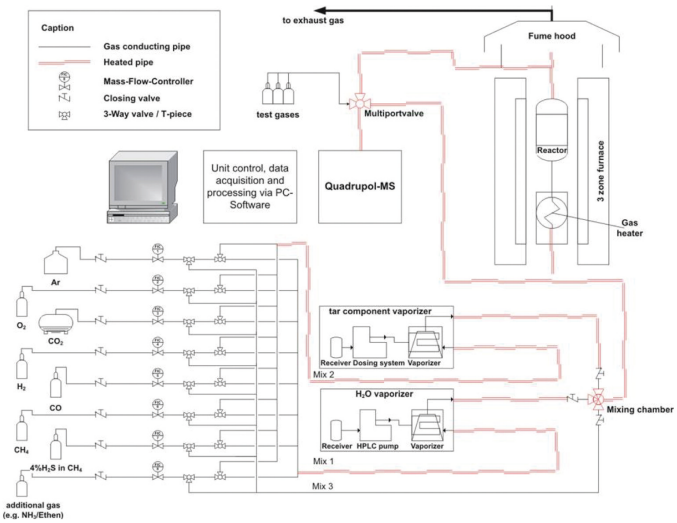

Figure 6: Test rig for reforming catalyst investigation [10]

not change with plant capacity. Only the additional biomass needed for electrical self supply in variation 2 increases these emissions a little bit. As for larger plant capacities the transportation distance in¬creases, the greenhouse gas emissions coupled with biomass transport to the power station increases. Otherwise, the relative losses of energy decrease with plant capacity and therefore the emissions within the power station decrease with increasing capacity.

The investment costs for small to larger scale CHP plants were estimated. The costs for main equipment were assessed by quotations and full plant costs were calculated by applying Lang factors. The results are given in Fig. 5 in comparison with competing technologies and suppliers. For the larger plants with capacities between 5 and 10 MW fuel input (equivalent to 1.5 to 3 MW electrical output) the electricity production cost are in the range of 0.20 €/kWh_{el} for the case where no heat is sold and 0.16 €/kWh_{el} for the case of complete heat utilization as district heating [9]

Recent Research on Tar Reforming Monoliths

During the last five years the focus of work lay on the testing of different catalytic materials for tar re¬forming. A test rig (Fig. 6) was built to investigate bulk and monolithic catalysts in different synthesis gases with changing contaminant compounds and concentrations. The synthesis gas composition of any gasifier can be simulated by preparing a mix¬ture from gas bottles and evaporating water and tar model compounds (naphthalene and xylene). The gas is analyzed by a quadrupole-MS upfront and downstream the reactor. This setup allows the fast test of newly developed catalysts under controlled conditions and their response to certain levels of poisons and contaminants like H_2S or NH_3. Standard Ni-based catalysts were compared to PGM-based catalysts. Also the influence of preparation procedure is under investigation. The first results show very even distribution of catalytically active material on the monolithic support surface going along with significant lower catalyst loading in comparison to commercially available reforming catalysts.

Conclusion

Fraunhofer UMSICHT developed a concept for the combined production of heat and power based on biomass gasification in a fluidized bed followed by dry gas cleaning and a gas engine. After testing a large variety of bed materials, olivine was chosen for the further development, as it is partially active in primary tar reduction, cheap and resistant against attrition. Final tar conversion into synthesis gas compounds like CO, CO_2, H_2 and CH_4 was achieved with Ni catalyst supported on monolith, which made them applicable in high dust containing environment. Latest work focuses on the development of new catalyst materials for higher tar conversion for chemical utilization of synthesis gas and tar conversion at lower temperature for alternative gasifiers with lower synthesis gas temperature.

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The Bio-SCWG project: Integration of biomass supercritical water gasification with CHP units

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Abstract: The objective of this paper is to present some of the preliminary results of a joint project on integrated supercritical water gasification (SCWG) of biomass. The project is carried out as a collaboration between Aalto University, Åbo Akademi University (both in Finland) and the University of Sao Paulo (Brazil). The gasification of biomass in supercritical water is an emerging technology which offers the opportunity to produce methane (CH_4) and hydrogen (H_2) from high-moisture biomass and waste streams. The obstacles of the technology owe to an early development stage but can also be attributed to the thermally challenging process conditions (>375 °C, > 220 bars). The scope of our project is to better understand the process of biomass SCWG, by simultaneous laboratory tests, chemical and process modelling. One goal of the work was to define a reaction model that can sufficiently well describe the process' mass and species balance for different real-life feedstock. This model was used to describe the SCWG process within a heat and power-integrated plant model. With that, the potential product yield and optimal process set-up for different inputs can be determined. Those will form input to further economic evaluation and to the investigation of the social acceptability. Two conceptual plant models have been developed and represent integrated production of H_2 and CH_4 via SCWG, respectively. Both H_2 and CH_4 production require complex downstream processing in order to achieve reasonable production rates and high purity. Therefore different process lay-outs, aiming for energetic self-sufficiency have been synthesized and compared to each other. The most promising options have been further scrutinized by means of pinch analysis. Simulation results show that the overall process efficiency of CH_4 and H_2 production via SCWG can be as high as 0.61 and 0.46, respectively. For H_2 production, a lean feedstock solution with a solid content of approximately 5% should be used, whereas for CH_4 production the feedstock concentration is limited to 18% by pump ability. Although the energetic efficiency of the SCWG favours CH_4 production, for economic feasibility other factors such as equipment size and cost and feedstock and product market prices will play an important role. By using the mass- and energy balances established in this work, our future work will assess the economic feasibility of the proposed processes.

Introduction: Supercritical water gasification (SCWG)

Renewable and sustainable energy systems must play a major role in the future in order to mitigate global CO_2 emissions and societies' fossil fuel dependency. Being the only renewable carbon source, biomass processes in particular will be of high importance. Offering high specific area yields and photosynthetic efficiency, aquatic biomass such as algae and cyanobacteria are currently discussed as feedstock for so-called 3rd generation biorefineries. However, being of aquatic origin this feedstock has a high moisture content and cannot be upgraded efficiently by conventional thermo-chemical processing. One alternative are hydrothermal treatment processes that apply water as chemical reactant and/or reaction medium such as supercritical water gasification. The supercritical point of water is reached when the pressure exceeds 221 bars and the temperature is above 374 °C. Above the critical point, a decreased dielectric constant, thermal conductivity, ion product and viscosity can be observed and water acts as a non-polar solvent. Organic material is hence fully soluble and inorganic material is practically insoluble which can be used to simplify phase separation. As water is the reaction medium, highly wet feedstock can be processed without prior drying [1].

A basic SCWG process consists of the main reactor and a subsequent heat exchanger in where the feed suspension, or slurry, is heated as close as possible to the critical temperature. In the heated SCWG reactor, solid biomass is converted to syngas and, subsequently, is separated into hydrogen (H_2) / methane (CH_4)-, carbon dioxide (CO_2) - and water-rich phases in a two-stage separation process prior to further upgrading [2]. The SCWG yields a practically tar-free syngas at high pressures, rich in H_2 and/or CH_4. The syngas composition is influenced by reaction temperature and feedstock concentration. H_2 production is favoured by both, higher temperature interval (550-800 ˚C), and lower solid content in the reactor, below 10 wt-%. Lower temperature intervals and higher solid content favour a CH_4-rich syngas [3-6].

Considering the reactor's heating demand, on the one side, high solid contents would be favourable, as this means that less heating is required to raise water to reaction conditions, but on the other side a solid content which is too high, hampers carbon conversion and H_2 formation (if desired). For all cases, metal-catalysts have proven to enhance the reaction rate and product yield; however some possible biomass components (alkali, chloride and sulphur) can deactivate the catalyst material [1,2]. Another major concern related to SCWG are fouling and corrosion issues of heat exchange equipment at sub- and supercritical conditions. This paper presents and compares fully heat and power-integrated plant models for H_2 and CH_4 production from spirulina algae via SCWG. Based on a reaction model that calculates valid mass and energy balance of the SCWG process, the potential product yield and optimal process set-up for different in-and output scenarios can be determined.

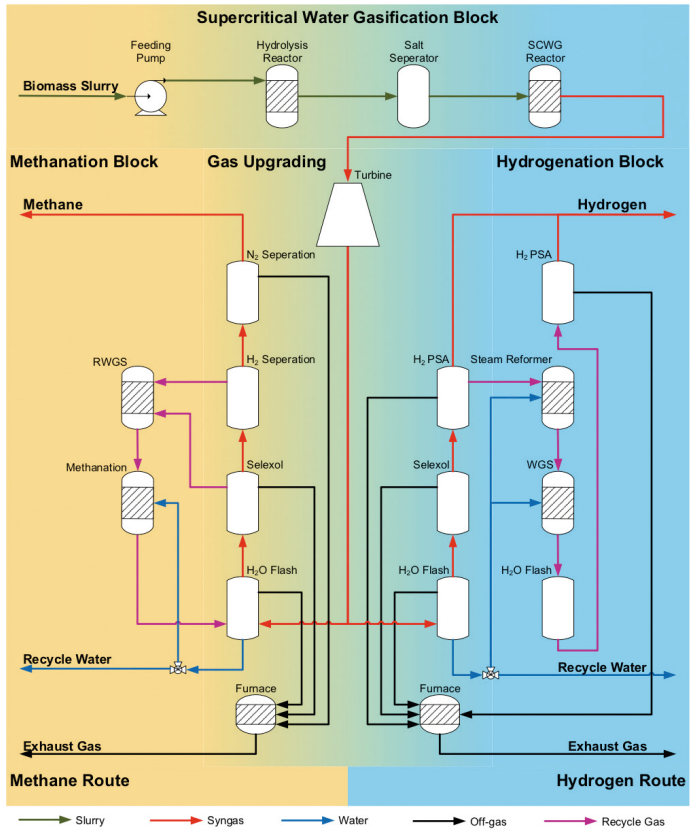

Figure 1: Simplified process scheme of integrated CH4 (left side) and H2 (right side) production via SCWG of lipid-extracted spirulina algae. Abbreviations: PSA: pressure swing adsorption; RWGS: reverse water-gas-shift; WGS: water-gas-shift.

Conceptual plant design and simulation model for CH_4 and H_2 production

The SCWG of lipid-extracted spirulina algae feedstock was modelled with Aspen Plus®. In the following the simulation models for H_2 and CH_4 production are briefly described and the major assumptions are given. A simplified illustration of the two processes (heat-exchange- and pressure-change equipment excluded) is presented in Fig. 1. For a more detailed description of the simulation models please refer to [7, 8]. The feedstock contains (dry basis) 48.6% of carbon, 7.1% of H_2, 32% of oxygen, 0.5% of sulphur and 7.1% of nitrogen, with the rest being ash. The thermo-physical properties of the non-conventional feedstock have been calculated by using Aspen Plus®' built-in correlations HCOALGEN and DCOALIGT.

Common units – Feed pre-treatment, SCWG reactor and syngas purification

As can be seen from Fig. 1, the upstream treatment of the biomass (regardless of the final product being CH_4 or H_2) comprises of pressurising the slurry, subcritical hydrolysis reaction with subsequent salt separation and the actual SCWG reaction. The feedstock is pressurised to 250 bars by means of a rotary lobe pump [9]. In order to facilitate salt separation, the biomass slurry is hydrolysed at 350 °C forming so-called bio-crude, a viscous organic liquid, comparable to pyrolysis oil. The hydrolysis reaction is described with a yield reactor in which the biomass is decomposed to its elemental constituents. The reactor has been heat-coupled with the SCWG reactor in order to compensate for the energetically over-estimated heat of the decomposition reaction. When reaching supercritical conditions, hydrocarbons mix with water and form a reverse flow upwards exiting from the top at 400 °C while the insoluble salts precipitate at the bottom and exit as brine. Gassner et al. estimated conservatively a loss of 10 wt-% of the organic feed. Finally, the slurry enters the SCWG reactor in where the organic material is converted at 600 °C to form mainly CH_4, H_2, CO_2, carbon monoxide (CO) and water. The reactor has been modelled as a Gibbs reactor (equation-of-state package: Peng-Robinson-Wong-Sandler, PRWS) implying that chemical equilibrium is achieved. Shown by others, this approach has produced experimentally validated results [11-13].

Subsequently the product gas enters a supercritical steam turbine where it is expanded to 65 bar at an isentropic efficiency of 92.5%. After expansion, the gas is further cooled to 28 °C before a two-step water flash separation. In the first step (at 60 bar) the syngas is separated. The liquid stream is further flashed at atmospheric pressure yielding liquid water and a small depleted gas flow which is co-fired for

heat recovery. The dry syngas is then fed into a Selexol acid gas removal unit, operated at 60 bar. The SELEXOL technology utilizes Methyldiethanolamine as solvent for selective extraction of CO_2 and H_2S at 96.5% and 91% removal rate, respectively. The off-gas released during solvent recovery is co-fired for energy recovery.[14]

Downstream process for H_2 production: H_2 adsorption and hydrogenation

The syngas leaving the Selexol unit is subjected to pressure swing adsorption at 20 bar where the H_2 purity is increased to 99.99% [15]. The off-gas exiting at 5 bar is either send to the hydrogenation process (H_2 base case) in order to increase the H_2 yield or co-fired for energy recovery (H_2 Case 2). Therefore the CH_4-rich off-gas is hydrogenated, i.e. CH_4 and water are catalytically (NiMg, 650 °C, 5bar) reformed to H_2 and CO. Subsequently, for maximised H_2 yield the hydrogenated stream is fed to the water-gas-shift reactor in which CO and water are reformed to H_2 and CO_2. After water-removal the H_2 enriched stream is subjected to pressure swing adsorption in order to obtain the required purity of 99.99%.

Downstream process for CH_4 production: CH_4 purification and methanation

The syngas leaving the Selexol unit is fed to a continuous H_2 membrane separator [18]. The final removal of remaining nitrogen is achieved by three-stage membrane separation [19] which yields CH_4 at grid specification. For increased CH_4 yields, the H_2-rich off-stream from the H_2 separation unit is, together with CO_2 exiting the Selexol unit, fed into a reverse water-gas-shift reactor (operating at 20 bar) in where the CO/H_2 ratio is adjusted. Finally, the stream is recompressed (60 bar) and enters, enriched with steam for avoiding coke formation, the methanation reactor [20] in which CO, CO_2 and H_2 are reformed to CH_4 and water, before entering the H_2 separation unit.

Heat integration aspects and modelled cases.

Basic Pinch analysis was carried for the cases modelled and it was found that for neither of the production pathways enough high-temperature heat can be recovered in order to fulfil the SCWG units' heat demand. In fact, there is only a very small amount of heat available above the Pinch, originating from combusting the off-gases. This means that for all cases additional heat needs to be supplied. Depending on the desired product, different strategies can be chosen. In the case of CH_4 production, the SCWG reaction temperature was decreased to 450 °C and the turbine was removed (CH_4 –case 2). In the case of H_2 production, the hydrogenation block was removed which results in increased heat generation by also combusting the CH_4-rich off-gas (H_2 – case 2).

Results and Discussion

The results of the plants' energy balances for the different cases are given in table 1. The results show, that on an energy base, more feedstock energy can be converted into H_2 than into CH_4, if the indirect hydrogenation option is chosen for H_2 production. Simulation results further suggest that the lower direct yield of CH_4 (produced in the SCWG reactor) for the base case can be compensated by indirect methanation. However, this pathway is penalised with 3-fold increase of the heating demand, representing the indirect process' lower efficiency. Also the H_2 production's specific heat demand more than doubles the CH_4 production's heat demand. This is a result of the lower feedstock concentration which is required in order to reach high direct H_2 yields. In fact, forcing H_2 production at 18% feedstock concentration (results not presented in table 1) would decrease the direct production down to approximately 17%. In turn this would also disproportionally increase the specific heat load due to the lower energetic efficiency of the indirect, hydrogenation-based, production pathway.

Table 1: Energy balance of integrated SCWG for the production of H2 and CH4, respectively.

	H_2 – Base case	H_2 – Case 2	CH_4 – Base case	CH_4 – Case 2
SCWG temperature [°C]	600	600	600	450
Feedstock concentration	0.05	0.05	0.18	0.18
Fuel conversion ratio	0.85	0.47	0.74	0.73
Direct production ratio	0.54	1.00	0.79	0.94
Heat demand ratio	1.48	1.34	0.61	0.17
Cooling demand ratio	0.90	0.93	0.23	0.10
Power ratio	0.29	0.29	0.06	-0.01
Energetic efficiency	0.46	0.32	0.50	0.61

The presented cooling demand ratios, do not need critical evaluation, since rather they can be seen as a potential energy resource that could be exploited for heating and cooling generation, with the latter one being achieved e.g. by absorption chillers. Assuming that 1/3 of the cooling demand could be utilised for district heating, the energetic efficiencies could be improved to 0.58, 0.45, 0.54 and 0.64 for H_2 base case, H_2 case 2, CH_4 base case and CH_4 case 2, respectively. Thus, if the SCWG reactor is operated at 600 °C, the efficiency of H_2 and CH_4 production are almost comparable and with a given low temperature heating demand, H_2 production could be even more efficient, than the production of CH_4. The higher power ratio for H_2 production, which is calculated as the ratio of net power generated to feedstock input, can be contributed to two facts. Firstly, as the net power output of the processes is almost identical, the lower ratio stems from the higher feedstock input of the CH_4 case. However, secondly, the power consumption of the methanation equipment is considerably higher due to the gas re-compression requirement before the reverse water-gas-shift- and the methanation reactor. The values for the processes' overall energetic efficiencies suggest that CH_4 production at low temperatures is most beneficial due its low additional heating demand. However, it needs to be considered that full carbon conversion at those conditions would require either high catalyst loading (or new more efficient catalysts) or long to very long residence times. Another aspect to be considered is the market value of the targeted products; CH_4 being a major commodity has market price, when sold as transport fuel in Finland, of approximately 1.35 €/kg [21], whereas H_2 is traded at prices ranging from 6-15 €/kg [22]. Based on the simulation results, it can be calculated that the turnover for H_2 would be the same as for CH_4, if H_2 could be sold at a price of approximately 10 €/kg. Certainly, those values are only a very rough estimation, but still, they demonstrate, that H_2 production has a high potential to be produced at the same profitability via SCWG than CH_4.

Conclusions and future work

Integrated simulation models for H_2 and CH_4 production via SCWG have been developed. The results show that the overall process efficiency of CH4 and H_2 production via SCWG can be as high as 0.61 and 0.46, respectively. For H_2 production, a lean feedstock solution with a solid content of approximately 5% should be used, whereas for CH_4 production the feedstock concentration is limited to 18% by pump ability. For final evaluation, a feasibility study needs to be carried out, which will form part of our future work. Also optimised models and approaches for CH_4 production with focus on improving the conversion efficiency at low temperatures and optimised H_2 production at possibly high feedstock concentrations will be developed and supported by experimental research.

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Activated biochar derived from agricultural residual biomass pretreated with alcaline agent

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Abstract
The objective this work was to increase the specific surface area of a biochar derived from agricultural residues (oat hull), using KOH to treat both biomass before pyrolysis and biochar after pyrolysis. A factorial design 2^3 with 4 central replicates was used to study, the effect of KOH/oat hull or KOH/biochar mass ratio (2, 1.25, 0.5), temperature (400°C to 600°C) and residence time (1 to 3 h), were evaluated. The activated biochars obtained were characterized in terms of specific surface area, total pore volume and pore size distribution. FTIR was used for the identification of functional groups in the products. The structure of activated biochars were analyzed by using SEM. Moreover, an elemental analysis of biochar was performed. The results show that activated biochar from agricultural wastes increased its surface area from 49 m^2/g to 386 m^2/g. Moreover, porosity is influenced by all factors (temperature, residence time and KOH impregnation ratio) evaluated.

1. Introduction

Biochar is a carbon-rich material produced by slow pyrolysis of lignocellulosic biomass. Recently, biochar has been used in diverse applications, such as soil amendment, global warming mitigating material, support material for enzymes and microorganisms immobilization, catalyst and contaminants adsorbent, among others [1]. Biochar characteristics (e.g. specific surface area, porosity, surface functionality, ash content) depend on the pyrolysis conditions (temperature, heating rate, nitrogen flow rate), as well as biomass characteristics. Slow pyrolysis of waste agricultural biomass produces mainly biochar. However, the physical and chemical characteristics of the biomass and the low process temperature can influence biochar properties such as specific surface area and porosity, limiting its applications.

Activated carbon is a commercial carbonaceous material with high specific surface area (about 800 m^2/g) used as adsorbent in several industrial applications [2]. In addition, it is prepared from a large number of carbonaceous raw materials (e.g. coal, lignite, wood, some agricultural and industrial waste products) [3]. Therefore, biochar can be seen as a precursor for activated carbon production, by a further physical or chemical activation. Physical activation consists of a two-step process. First, biomass is pyrolyzed or carbonized to produce biochar, and in a second step, biochar is treated at high temperature in the presence of an oxidanting agent, steam, CO_2 or a combination of them [4]. The chemical activation consists of the impregnation of the raw material with alkaline agents, and the low temperature pyrolysis of the impregnated materials [4]. The objective this work was to increase the surface area of a biochar derived from agricultural residues (oat hull), using KOH to treat both biomass before pyrolysis and biochar after pyrolysis. Moreover the study presents the influence of different factors in chemical activation and pyrolysis. The studied factors were activation temperature, residence time during pyrolysis and KOH impregnation ratio both for the biomass and biochar.

2. Experimental

2.1. Biochar production

Oat hull (O) was dried and characterized before biochar production. A pilot-scale electric pyrolyzer designed at the University of La Frontera was used to produce biochar by slow pyrolysis. The operational variables were fixed according to optimal conditions defined previously to increase specific surface area (temperature 600°C, residence time 3 h and temperature increment rate 3°C/min).

2.2. Biochar activation

KOH activation was used to improve surface area of biochar (BO) derived from oat hull. The method consists in mixing biochar with 500 mL of distilled water with a desired KOH concentration. The mixture was kept 24 h at room temperature and vigorous agitation to ensure KOH and biochar contact. The suspension was filtered and dried at 105°C overnight. The impregnated biochar was placed in the pyrolysis reactor described in point 2.1, under inert atmosphere (N_2), and heated until the final temperature (400, 500 or 600°C), with a heating rate of 3°C/min, holding the final temperature for 1, 2 or 3 h (see Table 1). The biochar resulting was washed with 100 mL HCl 1M for 15 minutes to remove the residual activating agent, subsequently filtered and washed with hot distilled water several times until neutral pH. Finally, the activated biochar (AB) was dried at 105°C overnight.

2.3. Obtention of biochar activated during biomass pyrolysis

Oat hulls before pyrolyzation were impregnated with KOH. The resulting biochar after pyrolysis was called biochar activated from oat hull (BAO).

2.4. Characterization of activated biochars

Ultimate analysis of biochar samples were performed by an elemental analyzer (Elemental analyzer CHNS-O, Eurovector EA 3000), oxygen was determined by difference. Surface functional groups were determined using an Agilent Cary 630 FTIR diamond ATR. Specific surface area (BET), pore volume (BJH), and pore size distribution were determined using a NOVA 1000e porosimeter (QUANTACHROME) by adsorbing and desorbing nitrogen at 77 K on samples previously dried and out-gassed at 160°C for 16 h. Scanning electron micrographs (SEM) were recorded by scanning electron microscopy variable pressure (VP-SEM) to analyze structure and chemical contrast (SU-3500 Microscope Hitachi-Japan). Biochar samples were mounted on an aluminium sample holder 12 mm (stub) using carbon double-sided tape for accession. Ash contents of biochar were determined by combusting the samples in a muffle furnace at 650°C until constant weight (approximately 4 h) [5]. Biochar pHs were measured with an Orion 9512 electrode, using a biochar suspension sample/distilled water ratio of 1:5 (wt/wt) after stirring 1 h [6].

2.5. Design of experiments

A factorial design of 2-levels considering 3 factors with 3 central replicates was used in this study. The levels used were KOH/oat hull and KOH/biochar mass ratio (r = 0.50, 1.25, 2.00), activation temperature (400°C, 500oC and 600°C) and residence time (1, 2 and 3 h). Design expert software was used to generate randomized experimental designs. Table 1 shows the matrix of the randomly generated design for chemical activation of biochar from oat hull and Table 2 shows the matrix for chemical activation of biomass before pyrolysis.

Table 1: Experimental design for the biochar activation (AB).

Activation of biochar (BO) with KOH			
Run	Factors		
	Temperature (°C)	Residence Time of biochar (h)	KOH/biochar mass ratio
1	600	1.0	2.00
2	500	2.0	1.25
3	400	1.0	0.50
4	400	3.0	2.00
5	600	3.0	0.50
6	600	1.0	0.50
7	600	3.0	2.00
8	400	3.0	0.50
9	400	1.0	2.00
10	500	2.0	1.25
11	500	2.0	1.25

Table 2: Experimental design for the biomass activation process (O).

Activation biochar from biomass (O) with KOH			
Run	Factors		
	Temperature (°C)	Residence Time of biomass (h)	KOH/biochar mass ratio
1	600	1.0	2.00
2	400	1.0	0.50
3	500	2.0	1.25
4	400	3.0	2.00
5	400	3.0	0.50
6	500	2.0	1.25
7	600	3.0	0.50
8	500	2.0	1.25
9	400	1.0	2.00
10	600	3.0	2.00
11	600	1.0	0.50

3. Results and discussion

3.1. Characteristics of raw materials, biochar and activated biochar

The carbon content of oat hulls was 42.65% and the ash content was 6.2%. The lignin content was low (8%) and cellulose content was 34.3%. Biochar produced from oat hulls (BO) presented a specific surface area of 49.3 m^2/g and a total pore volume of 0.008 cm^3/g. These relatively low values can be related to the low lignin and cellulose content in oat hulls. Some authors mention that cellulose dehydration can generate porosity in biochar and volatiles production, while lignin directly correlates with char production and micropores formation at high temperatures [7]. Moreover, ash is a very important factor by blocking pores structure. However, the chemical activation by acid-washing can remove ash, unblocking pores [8].

Activated biochar obtained from biochar and oat hull impregnation presented higher specific surface area compared with the precursor in all cases. Ash content in activated biochar and activated oat hull derived biochar is similar to that of biochar from oat hulls (10%), showing that biochar washing after activation is efficient. The acid-washing process for biochar activation is used to remove metallic impurities and activation agent residues [8]–[10].

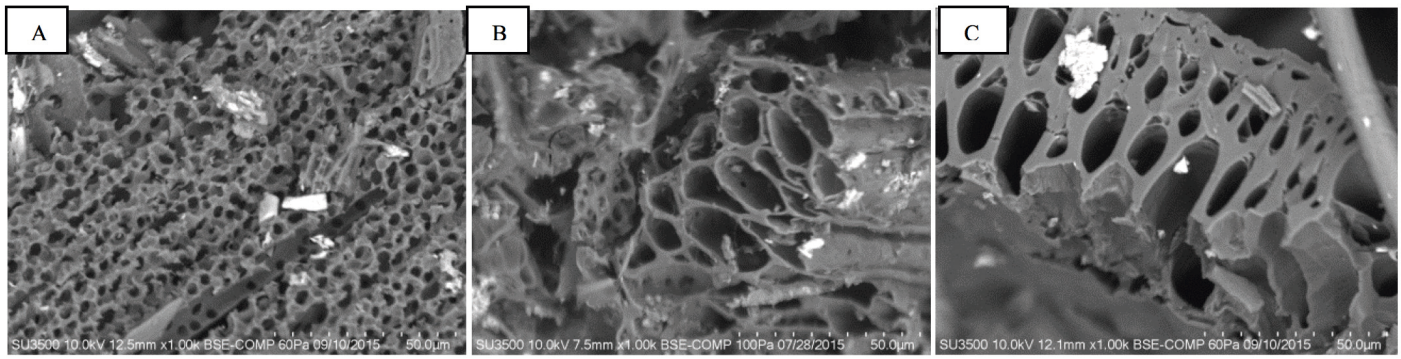

Figure 1: SEM images of activated biochar obtained from biochar impregnation with KOH at the following conditions A) 400°C, 3 h, r=2.0. B) 600°C, 1h, r=2.0. C) 600°C, 1h, r=0.5.

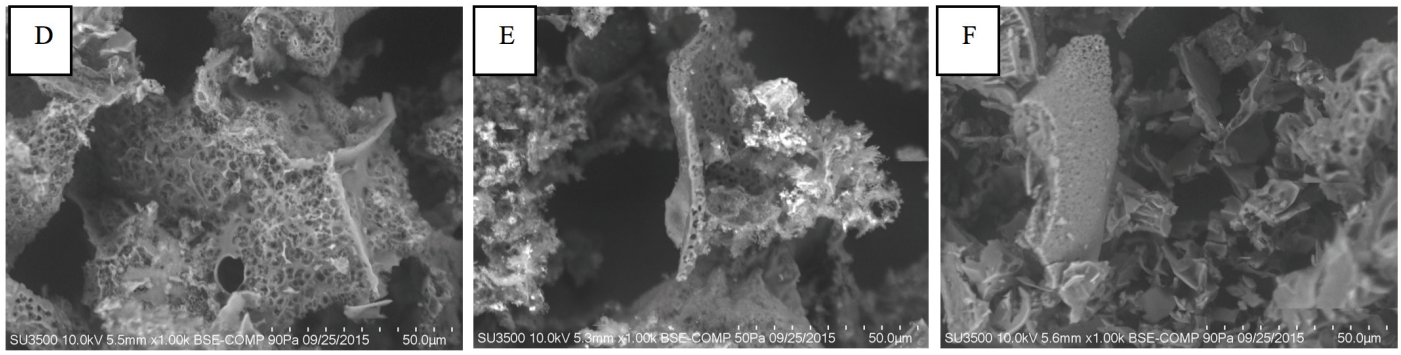

Figure 2: SEM images of activated biochar obtained from KOH impregnated biomass D) 500°C, 2 h, r=1.25. E) 600°C, 1h, r=2.0. F) 400°C, 1h, r=0.5.

3.2. Activation effect

Morphology of biochar and activated biochar obtained before and after pyrolysis process was studied by SEM images. Biochar porosity and structure is influenced by temperature, activation time and KOH impregnation ratio. The high porosity of activated biochar after pyrolysis (AB) is supposed to occur by the high temperature reaction of KOH with carbon, this reaction reforms pores from precursor biochar and create new pores by debilitating pore walls. Moreover, pores development is related to CO_2 generation at high residence time and temperature, resulting in the gasification and final breaking of pore walls. This ultimate stage decreases the specific surface area in activated biochars [4], [11]. This phenomenon is shown in Figure 1. Figure 1 shows an irregular pores structure, showing large pores and micropores of activated biochar.

The morphology of AB is very different compared to activated biochar obtained from impregnated oat hull pyrolysis (BAO). The structure of BAO presented a very high porosity. SEM images show that micropores are abundant on the surface. This effect is beneficial to the specific surface area and is associated to KOH-volatile biomass interaction. Figure 2 shows that when increasing the temperature and KOH impregnation ratio, the structure of BAO is more irregular.

3.3. Surface area

The specific surface area of activated biochar after pyrolysis (AB) is higher compared to biochar from oat hull unactivated (49 m^2/g). Specific surface areas of ABs ranged between 220 m2/g and 389 m^2/g. The effect of the 3 factors evaluated influenced these results. When increasing microporosity of activated biochar (AB-BAO), also the surface area increased. The microporosity increased with high residence time, but this effect can be prejudicial, because increasing residence time can break pores decreasing specific surface area. The micropores increased with high temperature of activation and KOH impregnation ratio, however, this impregnation ratio presented a maximum value in both cases. This result can be explained as by increasing KOH mass ratio, impregnation increased pores destruction by the action of KOH [9], [11], [12]. The maximum preliminary specific surface area was obtained with an operation temperature of 600°C and a low impregnation (KOH/BO) mass ratio (0.5), and 1 h residence time.

4. Conclusions

Oat hull biochar and biomass can be used as precursors of activated biochar through activation with KOH. The optimal conditions for maximum specific surface area in biochar activation is 600°C, 1 h residence time and a KOH/biochar impregnation ratio of 0.5. All factors analyzed are important parameters to produce activated biochar from waste agricultural biomass.

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Microalgae

Integral utilization of microalgae: Production of biofertilizers

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Abstract: Microalgae have been proposed for multiple applications, from pharmaceuticals to biofuels production. Moreover, based on the biorefinery concept it has been proposed that microalgae can be used at the same time to obtain products in largely different fields. In this work a microalgae-based biorefinery process is discussed. On the basis of this process it is concluded that the production of bio-stimulants/biofertilizers is one of best alternatives to valorise microalgae biomass. The fundamentals of the process are studied and then used to develop a feasible commercial. Additionally, a more general process considering not only the production of biofertilizers from microalgae biomass but also the production of microalgae biomass from wastewater is discussed, as an example of a sustainable biorefinery process.

Keywords: microalgae, nitrogen removal, biomass production

Introduction

Microalgae can be used in largely different fields, from the production of pigments and antioxidants, to the production of food grade biomass as nutritional supplement, and feed for animals and aquaculture (Spolaore et al., 2006). Additionally, it has been proposed that microalgae can be a promising source of biofuels and to contribute to the reduction of greenhouse effect emissions due to the capability of these microorganisms to uptake CO_2 (Chisti, 2007). This large number of potential applications is a consequence of the large amounts of different compounds found in microalgae biomass, in addition to their metabolic plasticity according to the imposed culture conditions. The high number of potential compounds potentially exploitable from microalgae has been used as argument to consider microalgae biomass as adequate raw material for the development of biorefinery processes obtaining multiple products (Murillo-Alvarado et al., 2013).

Major components in the microalgal biomass are carbohydrates (20-30%wt.), lipids (10-30%), and proteins (30-60%) (Rebolloso Fuentes et al., 2000). Carbohydrates have been reported to be useful for bioethanol production. However, to produce bioethanol from microalgae a sequence of complex steps are necessary, involving acid and thermal treatments that denaturalize the waste biomass remaining (Harun, Danquah and Forde, 2010). Regarding lipids, only the fatty acids fraction can be used to produce biodiesel (Azócar et al., 2010; Hidalgo et al., 2015). Two different processes are generally reported, the extraction of lipids from dry biomass or the direct transesterification of wet biomass. For the first method, a large amount of energy is necessary to remove the water from the paste biomass after harvesting, whereas in the second method substantial amounts of energy are also required to recover solvents after the reaction. In any case, the amount of biodiesel that can be produced is equal than the fatty acids content of the biomass, this representing approximately 50% of the total lipids contained into the biomass. Finally, proteins can be extracted from the biomass using adequate cell disruption methods and extraction procedures, even from wet biomass due to the high solubility of proteins in the aqueous phase. Different methods have been proposed to recover especial proteins from microalgae biomass mainly to be used as food colour (Bermejo et al., 2002).

Microalgae biomass has also been proposed as biofertilizer by its amino-acids profile and its content in other algae-derived natural substances (Ördög et al., 2004). Aminoacids-based fertilisation supplies plants with the necessary elements to develop their structures saving metabolic energy since the nitrogen is supplied in a -3 oxidation state and does not have to be reduced as happens in the case of nitrate, which must be reduced to ammonium prior to its incorporation and conversion to α-ketoacids in order to synthesize aminoacids. Protein hydrolysates can be obtained by different methods such as acid hydrolysis, exogenous proteases, or enzymatic autolysis. Enzymatic process based in microalgae biomasses could be performed on a commercial scale, since operating conditions have already been optimized for many enzymes laboratory scale (Romero et al. 2012).

Moreover, microalgae can be also produced in wastewater allowing the recovery of contaminants (COD, N, P) as nutrients for the production of the biomass (Posadas et al., 2013). This is a wider biorefinery concept not only producing "free" biomass but also obtaining revenue from the treatment service. The produced biomass can be also used as raw material for different purposes, the production of biofertilizers being one of most recommendable.

In this work an analysis of different biorefinery alternatives for the valorisation of microalgae biomass is performed. On the basis of this analysis it is concluded that the production of biofertilizers is one of most promising methods. The process to obtain adequate biofertilizers is presented and the optimal conditions for an industrial scale are discussed. Finally, the production of microalgae in wastewater is presented as a wider example of a microalgae-based biorefinery, the produced biomass being especially suitable to produce biofertilizers.

Material and Methods

Optimal conditions for the production of biofertilizers from microalgae biomass are determined at laboratory scale using 1 L stirred tank reactors at controlled conditions (stirring, pH and temperature). Freeze dry biomass is first used as standard material, then wet biomass form the reactors were used as raw material for the production of biofertilizers. Hydrolysis is performed using commercial enzymes, experiments being performed by evaluating the hydrolysis degree as a function of independent variables: (i) biomass strain, (ii) pH and temperature, (iii) enzyme.

Concerning wastewater treatment, the experiments are performed at indoor and outdoor conditions. Indoor experiments are done simulating outdoor conditions, in bubble column (0.3 L) and stirred tank reactors (1 L), to evaluate the capacity of microalgae for wastewater treatment under optimal and controlled conditions. Outdoor experiments are performed in three small raceway reactors (10 m^2), in addition to two larger reactors (32 m^2), a raceway and a thin-layer reactor. The reactors are operated in continuous mode modifying the hydraulic retention time, in addition to water depth, and weather conditions (light-temperature). The operation conditions (temperature, pH, dissolved oxygen), in addition to COD, N and P concentrations are determined at inlet and outlet flows. Biomass production and quality/composition of the biomass are determined. Additionally, the photosynthesis and respiration rates of the cultures were determined under standard conditions. Produced biomass is also used as raw material for the production of biofertilizers, the method for enzymatic hydrolysis being modified to be applied to this type of biomass.

Results and discussion

In this work the performance of a biorefinery scheme is described, including the production of (i) bioethanol, (ii) amino-acids concentrates, (iii) biodiesel, and (iv) biogas. The results demonstrate that, according to this scheme, from 100 g of biomass only 65 g are used to produce only a final 21 g of products (Figure 1). According to these numbers, up to 35% of the biomass is lost during the process, and moreover only 21% of the raw material used is obtained as products. Thus, a conservative calculation indicates that the price of the products must be at least five times higher than the cost of the microalgae biomass.

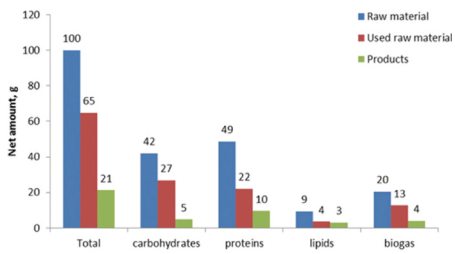

Figure 1.- Yield of each one of steps considered into the biorefinery scheme developed.

However, when considering the production of amino-acids concentrates and biogas the total amount of raw material used is higher, up to 73%, of which 29% is used to produce aminoacids concentrates and 44% for biogas. Amino-acids concentrates is a high value product whereas biogas is the cheaper biofuel, thus although the production of biogas is possible it is not of interest at commercial level. Bearing this in mind, it can be concluded that producing only biofertilizers by enzymatic hydrolysis of the whole microalgae biomass is the most promising valorisation process.

A method for the enzymatic hydrolysis of microalgae biomass has been previously described (Romero García, et al., 2012). The scheme of the developed process, showed in Figure 2, consists of a pre-treatment for cell disruption, followed by an enzymatic reaction under controlled pH and temperature to maximize the performance of the used enzymes, and finally a separation of the protein

hydrolysate from waste biomass remaining. In this work it is demonstrated that this process can be applied for different microalgae, from freshwater strains as *Scenedesmus*, to seawater strains and *Nannochloropsis*, and including cyanobacteria as *Anabaena*. Different enzymes can be used, such as that provided by Novozyme (Alcalase and Flavourzyme). The most critical factors for the process are the optimal biomass concentration and protein content of the biomass, in addition to the adequate control of operation conditions according to requested by the used enzymes. In this process the most complex step is the separation of waste biomass after enzymatic hydrolysis, but it can be avoided because the waste biomass can remain inside the biofertilizer for agricultural uses.

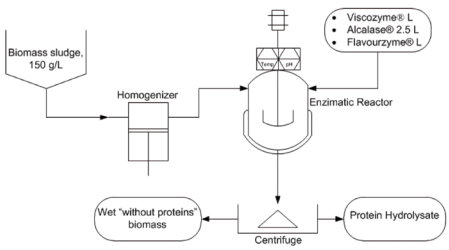

Figure 2.- Scheme of enzymatic process developed for the production of biofertilizers from microalgae.

To maximize the sustainability of biofertilizers production from microalgae biomass, the coupling with wastewater treatment can be considered. With this scheme, microalgae allow recovering the nitrogen and phosphorous contained into wastewater which is used to produce valuable biomass. Experiments performed outdoor in raceway and thin-layer reactors demonstrate that biomass productivities up to 35 g/m^2·day can be obtained only using wastewater as nutrients source, the productivity being higher in thin-layer compared to conventional raceway reactors, doubling the areal production rate for the same operation conditions (Figure 3). The improvement of the biomass production is a definitive advantage in terms of nutrients removal. Thus, in addition to carbon, the microalgae biomass consumes nitrogen and phosphorous from thře culture medium, the higher the microalgae biomass productivity the higher the depuration of culture media. Data from experiments performed using primary wastewater demonstrates that the optimal hydraulic retention time in raceway reactors range from 3 to 5 days, much lower than the of 7 to 10 days usually reported, if they are operated properly. Moreover, at this hydraulic retention time the removal of COD, N and P can be achieved at values of up to 80% (Figure 3).

The biomass produced from the wastewater treatment can be used to obtain valuable biofertilizers, but it must be first adequately harvested from the treated wastewater. For this, the utilization of flocculation + flotation has been reported as highly efficient recovery technique in both biomass removal and water purification. However, the biomass concentration of the sludge obtained after flotation is low, from 2 to 5%, which makes it necessary to increase it up to a minimum 10% d.wt content to produce biofertilizers. Moreover, the variability in the composition of the biomass produced is quite high, especially in terms of protein content. To cope with these problems, a standard procedure for the operation of the wastewater treatment was stablished in addition to a pre-concentration step in order to obtain biomass paste with up to 15% dry matter and 35-40% of proteins.

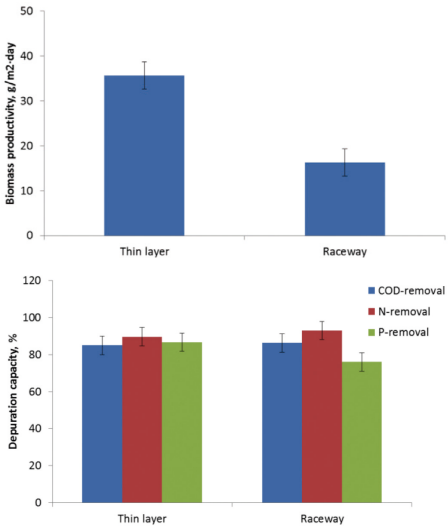

Figure 3.- Variation of biomass productivity and depuration capacity of thin layer and raceway reactors operated at 3 ad 5 days of hydraulic retention times, respectively.

Once the standard conditions to produce adequate paste biomass have been defined, the following step is to implement low energy/cost processes for the disruption of cells, previous to the enzymatic hydrolysis step. In this case mechanical and enzymatic cell disruption methods were compared, among others. The results demonstrated that homogenizers and enzymes were efficient for the cell breakage, but the enzymatic process was selected because of its lower complexity. The developed method was applied to fresh biomass harvested after wastewater treatment in real conditions. The results presented show that using the proposed process it was possible to obtain a paste biomass containing 120 g/L dry matter with up to 35% of proteins at the inlet of the reactor. At the outlet, once the enzymatic reaction is done, the remaining biomass is under 80 g/L, with a protein concentration in the liquid phase of 17 g/L, whereas the concentration of aminoacids was higher than 20 g/L, which is the limit allowed for agricultural uses.

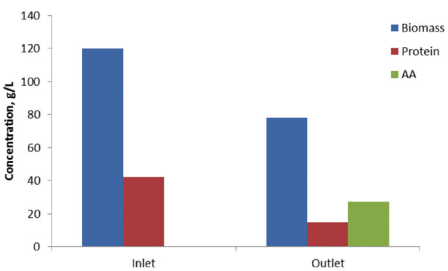

Figure 4.- Concentration of biomass, protein and aminoacids (AA) at the inlet and outlet of the process for the production of biofertilizers from microalgae biomass produced in wastewater treatment.

Conclusions

The sole production of biofertilizers is a promising strategy to maximize the utilization of the biomass and minimizing the release of wastes. An adequate methodology to transform microalgae biomass into biofertilizers has been presented. Moreover, for this purpose the microalgae biomass can be produced from wastewater treatment, thus obtaining profits from the recovery of nutrients from wastewater. The coupling ob biomass production with wastewater treatment imposes some differences into the process as the necessity of a pre-concentration and a cell disruption steps. On this way, the biomass thus produced was adequately transformed into valuable biofertilizers.

Acknowledgements

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Chilean Technological Consortium "Desert Bioenergy S.A."

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Abstract
Desert Bioenergy S.A. has carried out the start-up of a pilot plant to biodiesel and biogas production from microalgae biomass. Desert Bioenergy S.A. or "DBSA" is a Technological Consortium created on 2010 through the collaboration of the Chilean Government, 2 Universities and 3 enterprises. The Antofagasta University emplaced in the north of Chile take part of DBSA as the in charge of the research development on microalgae cultures and La Frontera University emplaced in Temuco city in the south of Chile is responsible of microalgae downstream processing. The enterprises partners of DBSA are the thermoelectric company E- CL, the milling company Empresas Gorbea S.A. and the enterprise of algae production Algas Prodalmar. With this strategic model Enterprise-University, DBSA has established as its main aim the research and technical development R&D, related to microalgae production to produce biofuels and high value products. In this context, DBSA has developed an integrate process of biorefinery through the research of microalgae cultures optimization yield, searching new strains, use of flue gases to feed microalgae cultures, biodiesel production, biogas production and high value products production, among others. After 3 years of research, in 2013 DBSA started the scaling processes with the start-up of a pilot plant to microalgae production in Tocopilla, Chile. The plant was located in the coast of Atacama Desert in the thermoelectric power plant E-CI. This zone is characterized by high irradiance during all year seasons and seawater availability. The total capacity of the microalgae production plant is 54 m^3 that include both raceways and photobioreactor systems, which generate a continuous production until date. Additionally, the plant included a biomass concentration system by using centrifugation and coagulants. A system to use flue gases produced from thermoelectricity plant was implemented in a second stage to feed with CO_2 the microalgae cultures. The operation have showed a very good performance by using filters to clean flue gases and using coal as raw material in thermoelectric plant. The effective operation of the system has showed the feasibility to get a sustainable process of microalgae production that allow diminishing of greenhouse gas emissions. Nowadays, in a third stage, DBSA is carrying out the start-up of a pilot plant to biodiesel and biogas production. The biofuel obtained in the biodiesel plant is produced by wet direct transesterification by using directly the microalgae biomass after harvesting. In this process are produced a crude biodiesel along with remaining microalgae biomass. After that, anaerobic digestion to produce biogas is carried out in the plant by using the remaining microalgae biomass generated during biodiesel production. The long way that DBSA has carried out has allowed getting an integrated and sustainable process to produce biofuels from microalgae in the implemented pilot plant. These is thank to the effort realized for the research group, administrative people and the working together between universities and enterprises that take part of DBSA.

Introduction
Interest in the use of algae, and particularly microalgae, as feedstock for biofuel production is at high extent the result of the capacity of some microalgae to accumulate lipids, suitable for biodiesel production. For biodiesel production from microalgae, the conventional technique is based on drying the biomass and after this, to carry out total lipids extraction. The lipids extraction procedure is usually carried out using non-polar solvents, such as hexane and/or petroleum ether, among others. Subsequently, the extracted lipids are used for biodiesel production through an acid transesterification reaction, which is carried out with sulfuric acid as catalyst, methanol as acyl acceptor at temperatures about 50°C during about 5 hours. As only approximately 10% of the microalgal biomass are transesterifiable lipids, the most of the biomass is lost as a residue in this process.
Although the high number of patents and papers related to microalgae culture, only few works have been published regarding the downstream processing of microalgal biomass to obtain valuable co-products, and in addition, no commercial process has been presented up to date. Nobre et al. (2012) recently reported the use of microalga *Nannochloropsis sp.* as feedstock in a biorefinery concept for fatty acids for biodiesel, hydrogen and high added-value compounds production. In the report the use of supercritical CO_2 for both lipid and pigment extraction was proposed. In addition, the use of the residual biomass for hydrogen production through dark fermentation was proposed. Although reported process seems to be interesting, CO_2 use as extraction solvent is a high cost alternative for biodiesel production, while a very low hydrogen yield has been reached. In a recent bibliographic revision Kleinegris et al. (2011) studied a two phase system to carry out *in situ* extraction of microalgal products. They established that this could be feasible through product excretion and cell permeabilization, however, the output was always the results of cell death. Anaerobic digestion from microalgae under a biorefinery concept has been also proposed. As part of the U.S. patent No 297749, the authors disclosed an alternative to produce biogas using the remaining biomass after lipid extraction; however, the lipid extraction could diminish biomass C/N relationship, which could negatively affect the anaerobic digestion performance.

In spite of efforts performed in downstream process to obtain co-products from microalgae, the production of each product has been optimized separately, and therefore, the overall downstream process has not been improved. In this sense, to use whole microalgal biomass, it is necessary to propose a downstream process which improves overall efficiency. This can be feasible adapting each process step to improve the following stage of the production process in an interconnected approach.

The present work relates to the downstream process in interconnected stages, starting from harvesting of microalgal biomass, following several productive processes, until alternatives to use the remaining biomass and wastewaters. The aim was improving the overall downstream process by adapting each process step to produce various products of commercial interest: proteins, biodiesel, biogas and methane.

Experimental
Harvesting step
To study harvesting methodology by using $FeCl_3$, the effects of both $FeCl_3$ dosages and culture initial pH were analyzed. Culture from *Nannochloropsis gaditana* was used in the study and was characterized by a biomass concentration of 0.34 gVSS/L and pH 8.37. The first experiment to study $FeCl_3$ dosage effect was carried out by preparing a $FeCl_3$ solution of known concentration (2 g/L). $FeCl_3$ solution volumes between 10 and 60 mL were added to flasks containing 400 mL of culture to obtain final concentration between 16.9 to 90.6 (mg FeCl3/g biomass dry basis). After coagulant addition, the samples were stirred 2 minutes in magnetic stirrer at 150 rpm, and were then stirred at 20 rpm during 5 minutes to obtain the flocs. Subsequently, the mixed samples were allowed to settle down during 20 minutes.

Protein extraction step
The process used for obtaining soluble protein after harvesting by avoiding both fatty acids and carbohydrates loss was proved by using microalgal biomass from *Botryococcus braunii*, directly after harvesting with 15 (wt% dry basis).
Response surface methodology (RSM) was used to carry out the study. Variables studied were: pH at 8, 10 and 12, reaction time at 20, 40 and 60 (min) and temperature at 20, 40 and 60 (°C). Responses studied were: protein solubilization yield (Response 1), accumulation or diminishment of fatty acids in feedstock (Response 2) and accumulation or diminishment of carbohydrates in feedstock (Response 3). A central composite matrix with 3 levels was used and 40 runs were carried out in a random order. Each experiment was performed in duplicate.
All reactions were carried out in 50 mL centrifuge tubes containing 45 grams of biomass in a shaker at 100 rpm. pH was previously adjusted by using NaOH 1M and/or HCl 1M. After each reaction, the samples were centrifuged at 4000 rpm x 15 minutes. Upper layer was quantified (volume) and characterized in terms of soluble protein content (Lowry method) and soluble carbohydrate content (Dubois methodology). Fatty acid content in the remaining biomass was measured by gaseous chromatography after lipid extraction by Bligh and Dyer methodology. Carbohydrate content in the remaining biomass was indirectly determined by the difference of 100% - (moisture% + lipids% + proteins% + ash%).

Biodiesel production after protein extraction
Protein extraction was applied to *Scenedesmus sp.* obtained after centrifugation, with a final biomass concentration of 150 g/L and a total protein content of 67%. NaOH was used for protein solubilization. The process consisted of adding NaOH until reaching a pH of 12. The reaction was carried out at 50ºC, for 40 min at 200 rpm. With this process a 64% of the total protein was solubilized. The protein was separated in the supernatant. The remained biomass was used for biodiesel production by Wet Direct Transesterification (WDT) process. The WDT was performed using a methanol-to-biomass ratio of 10 (L/Kg dry basis) and a sulfuric acid biomass ratio of 1.5 (Kg/Kg). The reaction was carried out during 2 h at 75ºC and 200 rpm.

Biogas production from B. braunii produced after WDT
Biochemical methane potential (BMP) of the remaining biomass, produced after WDT, was determined by batch tests. First of all, the remaining biomass needed pH neutralization for anaerobic digestion (as shown in Table 8). Additionally, theoretical methane potential was determined. BMP determination was performed in 117 mL serum bottles, with approximately 60 mL of headspace. Nutrients were added as well as sodium bicarbonate as pH buffer. Methane production was determined by pressure increase in the headspace, coupled with gas composition analyses performed by gas chromatography (GC) with a thermal conductivity detector (TCD). Experiments were conducted at 35°C. Granular anaerobic sludge was used as inoculum at a ratio 1:1 with the remaining biomass. Determinations were performed in triplicates, and a blank assay was included (without remaining biomass) in order to account for endogenous biogas formation. The assays were carried out until biogas production stoppage.

Results and discussion
The new biorefinery process was composed by consecutive interconnected steps, which generate different products and byproducts (FIG. 1). The steps order allows optimal utilization of feedstock compounds: proteins, carbohydrates, lipids and others. Therefore, the global process allows diminishing residue production and biomass losses. The downstream process includes adapting each

process step to improve the next stages of the production process The steps of the invention are the following: Harvesting to produce feasible feedstock to be used in next steps (1), Protein and/or amino-acid production by protein solubilization through a chemical method (2), Biodiesel production by using fatty acids from biomass (3), Biogas production from the remaining liquid and biomass (4) and alternatively, methane production after biogas upgrading (5).
Alternatively, process units can be operated by a single unit, combining some units or, operating all units in a whole process. Wastewater reuse is considered to be carried out after the steps of harvesting, protein production, and by using the liquid digestate generated in biogas production. Biogas upgrading also can be carried out when photosynthesizing microorganism cultures are used.

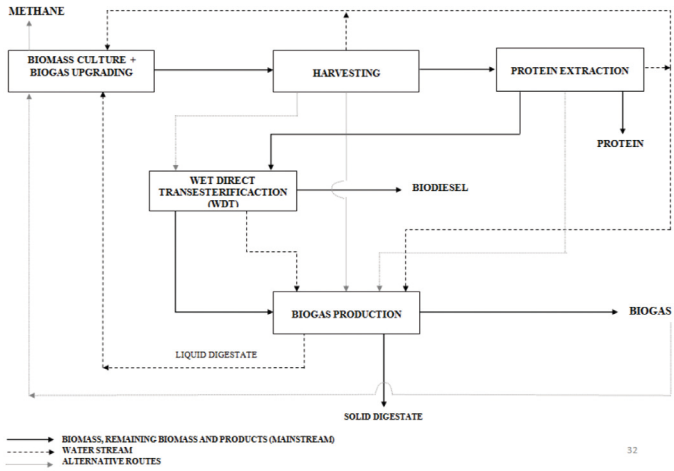

Figure 1: Scheme of the biorefinery process proposed

Harvesting step

Figure 2 shows the results obtained where at 48.5 (mg $FeCl_3$/g biomass $_{dry\ basis}$) was feasible to obtain an optical density in the supernatant of 2.4, at 750 nm. Therefore, doses over this value are recommended to an effective harvesting process.
The aim of the second experiment was to know initial culture pH effect on flocculation effectiveness. HCl 1 M and NaOH 1M were added to flasks containing 400 mL of culture to adjust it pH value between 4 and 9. After that, a dosage selected according to the first experiment was added to the flaks (63 mg $FeCl_3$/g biomass $_{dry\ basis}$). Subsequently, the same procedure described previously was performed until obtaining biomass sedimentation. Figure 2B shows the results obtained where over pH 6.95 optical densities in supernatant were increased.

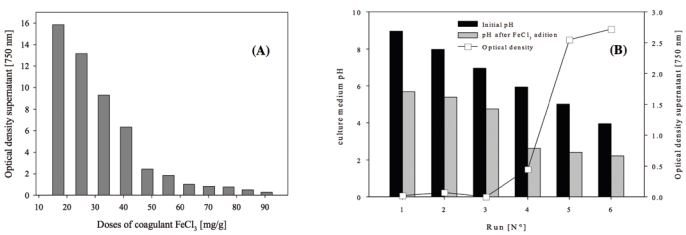

Figure 2: Determination of flocculation conditions by using $FeCl_3$.

Protein extraction step

FIG.3 shows the response obtained according to the generated model by the RSM analysis at constant temperature (40°C). FIG. 3A shows protein solubilization yield obtained at different reaction times and variable pH, where moderate reaction times and high pH favour protein extraction. FIG. 3B shows that moderate pH and moderate reaction times favour fatty acids accumulation in the remaining biomass. Additionally, in FIG. 3C it can be seen that high quantity of carbohydrates can be accumulated at high pH.

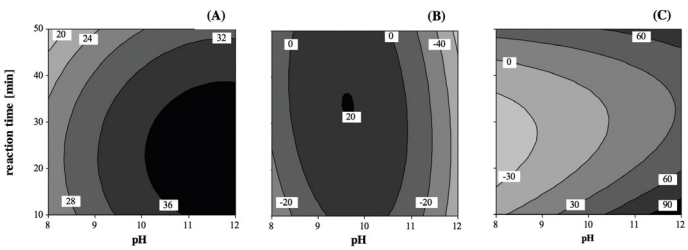

Figure 3: Contour plot at 40°C and 100 rpm of (A) protein solubilization yield, (B) accumulation or diminishing of fatty acids and (C) accumulation or diminishing of carbohydrates.

Biodiesel production after protein extraction and biogas production produced after WDT

The WDT was applied to the remaining biomass after protein extraction. The fatty acid content of the biomass was 8.1%. The biodiesel yield obtained (74%) was 10% higher than biodiesel obtained from biomass after harvesting (FIG. 4). Additionally, biochemical methanogenic potential by using remaining biomass after WDT was evaluated. The results showed that a 43% of theoretical methane potential was produced (FIG. 4B).

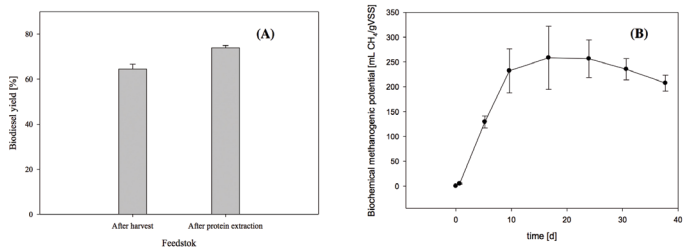

FIG. 4: A) Comparison of biodiesel production yields by using whole microalgal biomass and remaining biomass after protein extraction. B) Biochemical methanogenic potential by using remaining biomass after WDT.

The results described were used to build a pilot plant to biodiesel and biogas production (FIG. 5). The plant was placed in Tocopilla- Chile where is located the microalgae production plant which is feeding with CO_2 from the thermoelectric company E-CL. The start-up of a pilot plant to biodiesel and biogas production was on December 2013. The biofuel obtained in the biodiesel plant is produced by wet direct transesterification by using directly the microalgae biomass after harvesting in the same place. In this process are produced a crude biodiesel along with remaining microalgae biomass. After that, anaerobic digestion to produce biogas is carried out in the plant by using the remaining microalgae biomass generated during biodiesel production.

FIG. 5: Pilot plant of biodiesel and biogas production located in Tocopilla-Chile.

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Aravanis A, Goodall B, Mendez M, Pyle J, Moreno J. Methods and Systems for biofuel production. US 2010/0297749.

Multi-scenario economic evaluation for a biorefinery based on microalgae biomass with application of anaerobic digestion

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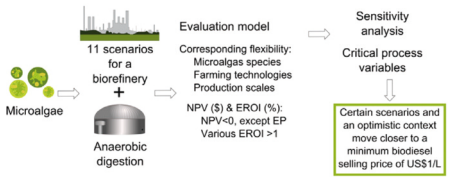
Graphical abstract



Introduction

Global energy consumption has continued to rise in recent years, primarily driven by the economic development and opening up of emerging nations (Brazil, Russia, India and China). Projections from the Energy Information Administration (EIA) postulate that the current energy consumption of non-OECD (Organisation for Economic Co-operation and Development) nations will almost double by 2040, resulting in a global consumption increase from 529 QBTU (QBTU = Quadrillion British Thermal Unit, 10^{15} BTU) (in 2012) to 820 QBTU (projection to 2040) [1]. Accordingly, one of the main challenges of the 21st century will be finding sustainable energy sources capable of sustaining the projected energy scenario, as well as the lifestyle of contemporary society.

Bioenergy is a renewable alternative to consider, of which it is possible to recover a fraction from solar energy and from waste. It is specifically in this field where the motivation of the present study was born. Traditional forms of bioenergy relate to electricity generated from the direct combustion of biomass and/or biogas, stemming from their anaerobic degradation, as well as the use of liquid biofuels which entirely or partially displace those derived from petroleum [2,3]. Among the liquid fuels, ethanol is usually produced via the fermentation of raw material rich in glycosides (or carbohydrates), such as corn and sugar cane [4,5]. Alternatively, biodiesel is obtained through the esterification and transesterification of used oils and oleaginous products obtained from the farming of soybean, rapeseed, palm oil and other different seeds [4,6]. However, there is a less conventional alternative to consider and evaluate within the bioenergy industry: biomass obtained from microalgae.

While research in the field of microalgae has increased over the last decade, it dates back to the mid-19th century, when isolated microalgae were cultured in laboratory conditions [7]. Subsequently, towards the end of the 1970s the US Department of Energy created a division called the Aquatic Species Program (ASP), which remained active until 1996. The aim of this program was to study the economic feasibility, the scaling to pilot, industrial scales and the application of different technologies for the farming and processing of microalgae for bioenergy purposes [8]. Currently, industrial-scale farming is restricted to the production of feed for the aquaculture industry, or as a source of high-value metabolites (proteins, special oils or antioxidant pigments) which are of interest to the pharmaceutical industry [9].

The production of bioenergy from microalgae reached only pilot level, due to its high operational and related capital costs. Diverse research groups have conducted evaluations into the cost of producing biofuel from microalgae, determining that the minimum selling price of biodiesel should be around US$4/L, in order to ensure the sustainable economic development of the industry (see Fig.1) [10–15]. This far exceeds the US$0.94/L of diesel, as per its global average price at the beginning of 2015 [16].

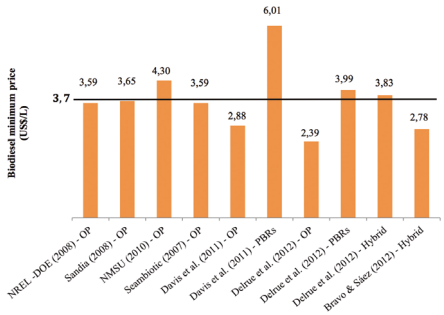


Fig.1 - Estimates of the minimum selling price per liter of microalgae biodiesel
(OP=open ponds; PBRs=photobioreactors; Hybrid=OP+PBRs)

Economic evaluation results of microalgae biodiesel demonstrated that its production on an industrial scale will only become economically viable through the generation of products with a higher commercial value than that of traditional fuels. This provides greater relevance to the biorefinery concept. Biorefineries are chemical plants or factories which integrate the concept of "zero waste", in which all biomass fractions (proteins, glycosides and lipids) are utilized to generate different types of products and energy [6,17,18].

Microalgae biorefineries can use the Anaerobic Digestion (AD) as a final step for energy recovery. However, there should be consideration and some safeguards regarding the process inhibition caused by: 1) composition of the cell wall and specific metabolism of the treated species [17], 2) microalgae protein content (nitrogen) and reactor pH level, which together determine the equilibrium between ammonia (NH_3) and ammonium ion (NH_4^+), being the first toxin for methanogenic microorganisms and inhibitory for the overall process [19,20], and 3) sodium ion (Na^+) present in the moisture of the biomass of marine species [20,21].

Fortunately, there are alternative biomass pre-treatments (physical, chemical, biological, etc.) that combat the inhibitory effects mentioned above [17,20,22–24]. This is why there are different evaluated scenarios in the present study.

Consensus is still lacking as to what is the best pre-treatment to apply to microalgae AD. This area requires further research in order to broaden the knowledge regarding a greater number of microalgae species and technology combinations [25]. However, analysis of techno-economic evaluations, such as the presented one, may help to ensure the early detection of certain alternatives. By means of process engineering and the evaluation of industrial upscaling, feasibility comparisons can be made of the different processing options, prior to the investment of time and resources at the laboratory level in search of new technologies.

Methodology

This research aims to compile sufficient information to generate an economic evaluation model to help with the design of process that consider different species of microalgae and pre-treatment technologies for power generation and by-products, including AD, with main product generation achieved through biomass processing. Furthermore, the aim is to uncover critical variables for establishing the economic profitability of the process by means of conducting a sensitivity analysis, and to present the effective potential of AD applied to a biorefinery.

Given the large number of options and variables of the processes reviewed in the literature, the model begins with the definition of the particular project undergoing evaluation, in which each of the main stages of the process are outlined step-by-step (as shown in Fig.2).

Relevant technical aspects of the model

- *Evaluated microalgae and inoculum preparation (laboratory):* Production of Tetraselmis sp. and Isochrysis sp. with operation of a laboratory for isolated strains culture.
- *Culture medium:* Marine species production, therefore, considered the use of sea water containing added nutrients.
- *Farming stage:* Hybrid system, inoculum production through PBRs and industrial scale farming in raceway ponds (RWPs).
- *Harvest:* Sequential harvest system with four stages (fast flocculation, slow flocculation, sedimentation and centrifugation).
- *Pre-treatments (evaluated scenarios):* 1) Baseline [BL], 2)Direct Anaerobic Digestion [DAD], 3)Protein Extraction [PE], 4)Lipid Extraction [LE], 5)InSitu Transesterification [TiS], 6).Co-digestion [CoD], 7) Cell Disruption [CDisr], 8) CDisr+CoD, 9)PE+CoD, 10)LE+CoD y 11)TiS+CoD.
- *Biomass processing:* Treatment of the diverse biomass fractions to generate end products.
- *AD:* Traditional moisture anaerobic digestion treatment with Combined Heat and Power generation (CHP).

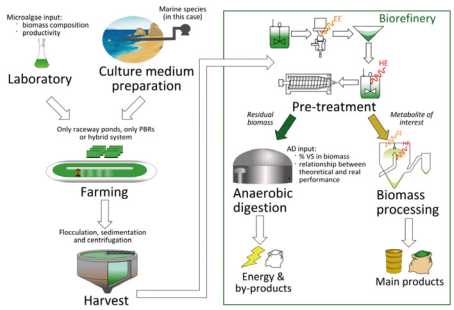


Fig.2 - General diagram of the microalgae production process with a biorefinery evaluated in this study

Relevant aspects of the economic evaluation

A traditional project evaluation was conducted (revenue estimate and capital and operating costs), considering international currencies, costs as state of the art equipment and various moderate assumptions. The main outputs of the evaluation are the economic profitability expressed in terms of the Net Present Value (NPV) and the Energy Return On Investment (EROI).

Principal results and discussion

Below a summary of the main results of the energy-economic evaluation of the eleven scenarios of a microalgae biorefinery is presented.

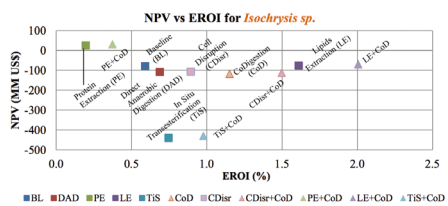


Fig.3 - NPV vs EROI for the eleven scenarios evaluated on the basis of average production scales (MM=millions)

Fig.3 It is a summary of some results (depending on NPV and EROI) and shows only four alternatives that present favourable EROI, none of which are profitable. Only the scenarios in which PE is considered are NPV

positive. However, and counterproductively, these are the worst alternatives from the energy point of view. Fig.3 also demonstrates that the CoD is an alternative that increases the EROI for any pre-treatment to which it is coupled, without significantly altering the NPV. Ideally, scenarios with NPV-EROI combinations would be expected, located on or close to 0 on the y-axis and to the right of one in the x- axis.

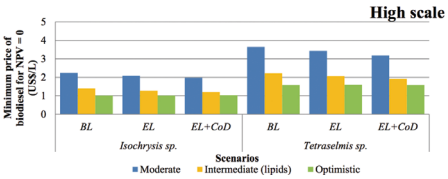


Fig.4 - Minimum price of biodiesel for high scale and scenarios that have positive economic returns, microalgal species and contexts defined by variables sensitized

Tab.1 - Definition of the contexts for comparison, following the sensitivity analysis

Variable	% Lipid	Prod. CT	$CT	%EffE	$EE
Context	Δ%	Δ%	Δ% capital cost	Δ% consumption	US$/ kWh
Moderate	0%	0%	0%	0%	0.1
Intermediate (lipids)	+70%	0%	0%	0%	0.1
Optimistic	+70%	+20%	-15%		0.05

Fig.4 shows result derivatives from the sensitivity analysis and that a change in the lipid percentage (%Lipid) of the biomass, either through an increase (an effect of the intermediate context) or a change of microalgae, reflects an important reduction in the biodiesel minimum selling price. Specifically in the evaluated intermediate context, the microalgae is considered to increase its lipid composition by 70%, which for Isochrysis sp. means achieving 40% lipids in the biomass. However, this is insufficient in approaching competitive prices in terms of fossil diesel (US$1/L). In order to achieve this, a combination of key variables is required, defined according to the optimistic context, in which only large-scale Isochrysis sp. generated a price of US$1.01/L, for both BL and LE.

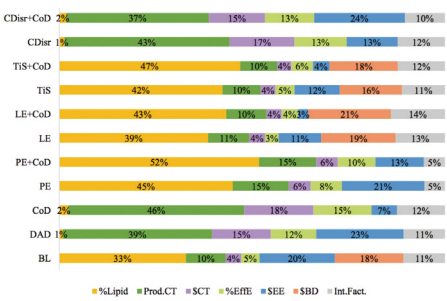


Fig.5 - Chart of the weight of factors measured on the NPV for the hybrid farming of Isochrysis sp. on the medium scale

Therefore, the effect of all the factors measured on the NPV and EROI was evaluated by means of a statistical and regression analysis, which considered a fixed effects model. Fig.5 summarizes part of the results of this analysis (only for medium scale), demonstrating the average weight of the factors or percentages associated with each of the variables submitted to the sensitivity analysis and that modify the NPV.

Conclusions

Microalgae are still not a sustainable source of bioenergy capable of competing against fossil diesel; including addressing their production by means of a biorefinery able to value all biomass fractions. Considering the use of the aforementioned traditional technologies and moderate values of the most significant variables, none of the scenarios evaluated in this study were capable of producing more energy than it consumed (EROI>1) while simultaneously achieving economic profitability (NPV>0).

As expected, the PE is the most profitable scenario. However, it had the worst energy evaluation. This result produces a warning, which must be heeded by the industry agenda, since it could be economically attractive in terms of entering the pharmaceutical market and the commercialization of proteins, which would mark a departure from the bioenergy approach. However, the interest in studying microalgae is based on a need to find sustainable energy sources. As such, future research and business in the area of microalgae depends on its ability to remain motivated with the issue of energy as its guiding principle.

The sensitivity analysis conducted in this study allows the critical variables to be detected for the entire process of bioenergy production based on microalgae, in addition to certain optimistic scenarios, through which economic profitability can be achieved. Throughout the entire process, reference has been made to the evaluation of stages prior to farming and up to the commercialization of the final products. This allows comparisons to be made of the magnitude of investment and operational costs of the different technologies used throughout the production process. Other economic evaluation studies adopt a “door to door” methodological approach, which limits analysis of the results obtained. This model, however, proposes a “cradle to door” analysis (i.e., from the extraction of the raw material to its positioning on the market), allowing a broader and more holistic view to be generated to the advantage of the industry.

The results of the multi-scenario economic evaluation of a biorefinery, despite being largely negative, allowed the authors of this research to approach the threshold of US$1/L of biodiesel (the price of fossil diesel). This was possible due to the optimistic contexts or considerations applied to the critical variables detected. This allows for continued hope regarding the microalgae bioenergy industry and helps to determine future fields of study in which effort and resources can be placed in Research and Development.

Acknowledgments

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Abbreviations

QBTU Quadrillion British Thermal Unit
AD Anaerobic Digestion
OP Open Pond
RWP Raceway Pond
PBR Photobioreactor
BL Baseline
DAD Direct Anaerobic Digestion
PE Protein Extraction
%Lipid Percentage of change to the composition of biomass lipids
Prod.CT Biomass productivity in farmingtechnologies
$EE Cost of electricity
LE Lipid Extraction
TiS inSitu Transesterification
CoD Codigestion
CDisr Cell Disruption
CHP Combined Heat Power Generation
NPV Net Present Value
EROI Energy Return on Investment
%EffE Percentage of energy efficiency achieved
$CT Cost of investment in farming technologies
$BD Selling price of biodiesel

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High pressure biomass conversion processes for biofuels and chemicals production

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High pressure supercritical alcohol processing of biomass and vegetable oils has been the subject of many research works in the last decades. There are many sources of non-edible vegetable oil that can be a sustainable raw material for chemicals and liquid biofuels production. This technology is versatile and allows treating microalgae, agro-industrial residues, and non-edible vegetable oils. In this work, the non-catalytic supercritical alcoholysis of different substrates at high temperatures (240 °C to 350 °C) and moderated pressures (80 bar to 200 bar) is presented. Bio-oils with high contents of fatty esters and other valuable chemicals were obtained. Moreover, a high pressure technology for the fractionation of fatty acid esters and acylglycerides up to high purity is presented. The integration of the discussed technologies may contribute to pave the way towards oil-based second generation biorefinery development.

Introduction

The emerging high pressure processing techniques are an interesting alternative to transform the concept of chemical biorefinery plants regarding more compact, safe, energy-efficient, environment-friendly sustainable processes. The supercritical alcohol process (SAP), for instance, is an interesting pressure intensified technology to obtain biodiesel and acylglycerol derivatives from non-edible vegetable oil, microalgae, waste cooking oils, and oil by-products.
The SAP has been highly studied in the last years for the production of biodiesel from raw vegetable oils with high contents of free fatty acids and water [1]. The process is carried out at temperatures higher than 325 ºC and pressures higher than 150 bar, using a 40:1 molar ratio of methanol to oil (MeOH/Oil). Under these conditions, it is possible to produce biodiesel in specification (96.5 wt.% FAMEs) in 30 min. of reaction time [1]. Nevertheless, it is also possible to obtain acylglycerides (ACs) if the process is carried out at milder operating conditions. It was determined that, working with a lower molar ratio of 30:1 MeOH/Oil (≈50 g/g%) at 573 K and 130 bar, a complete conversion of triglycerides can be obtained in 20 min. of reaction time, but the reaction product contains a high concentration of monoglycerides (MG) and diglycerides (DG) [2]. Reaction products obtained in the SAP can be fractionated in order to obtain FAMEs, MGs and DGs of higher added value.

Second generation oil-based biorefineries are aimed to enrich any residue with oil content. For example, the great increase of worldwide production of soybean and sunflower oil, impacted not only in the oil market but also in the oil refining by-products (phospholipids sludge and distillates of the deodorizer), which prices are rapidly changing, and because of the high production volume of vegetable oils this by-products are becoming a waste with disposal-associated problems. An alternative sustainable technology for this by-product is the direct alcoholysis of phospholipids and vegetable oil (triglycerides) occluded in the wet gum to produce fatty acid esters and derivatives [3]. The SAP of sunflower wet sludge (lecithin) produced a bio-oil (hexane soluble hydrocarbon compounds obtained from a solid-liquid-liquid extraction of the reaction products with n-hexane and water), with up to 60 g/g% of FE content. This reaction products obtained in the SAP of sunflower wet sludge could be further processed to separate the FE, acylglycerols and chemical derivatives.

Oleaginous microalgae have been proposed as a sustainable alternative to produce biodiesel in order to replace the vegetable oil derived from oilseed crops. Different freshwater microalgae have been recognized as a promising oil feedstock, due to its capacity to accumulate lipids. Particularly, recent studies pointed out the potential of N. oleoabundans, cultured in sea water or in anaerobically digested dairy manure, to produce triglycerides with high content of monounsaturated fatty acids [4]. SAP of this microalgae has been carried out at medium temperature (250–280 ºC), for a fixed ethanol/ dry algae mass ratio of 2.3 g/g (equivalent to 70 g/g% ethanol) [5]. The bio-oil yield (mass of hexane soluble products / dry microalgae) obtained in the supercritical alcoholysis remained almost constant (≈30 g/g % bio-oil/dry microalgae) independently of temperature. Also, FE contents in the microalgae bio-oils exhibits constant values of ≈32 g/g%, fatty esters/bio-oil.

Supercritical CO_2 extraction and fractionation is a green technology that has been widely studied, and some applications, like the extraction of special lipids and the fractionation of tocopherols from deodorizer distillate, have reached commercial scale [6]. The CO_2 fractionation of fatty acid esters, acylglycerides and other chemical also appears as a promising process with direct application in the biorefinery sector. In a previous work [2] it was shown that FAMEs and ACs can be effectively fractionated using CO_2 under liquid or supercritical state. A group contribution thermodynamic model (GCA-EoS), revised for asymmetric mixtures, was implemented to correlate experimental data on the ternary CO_2 + FAMEs + ACs system under liquid-liquid and liquid-vapor equilibria, and simulates an equilibrium stage counter-current column [2].

In this work, the SAP of different substrates (vegetable oils, soy wet gums by-products, and microalgae) is studied. Continuous supercritical alcohol transesterifications of sunflower oil were carried in a bench scale unit to produce biodiesel with high contents of acylglycerides (≈20 g/g%). Then, these oily mixtures (FE and AC) were fractionated by liquid-liquid (L1-L2) crosscurrent extraction using in a modified high pressure Soxhlet apparatus. Issues related to the continuous SAP of other feedstock (phospholipid sludge and microalgae) are analyse. Reaction products obtained in the SAP of different substrates could be further processed using the CO_2 technology in order to isolate the FE and other valuable chemicals.

2. Materials and methods

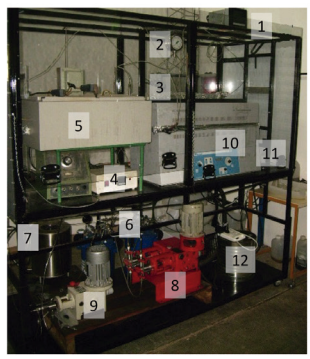
2.1 Materials

High oleic sunflower oil obtained from hybrid seeds (traded by Dow Agrosciences, Bahía Blanca, Argentina) was used to carry out the transesterification reactions. Absolute methanol (99.5%) and pyridine (99,7%) were purchased from Anedra. Hexane (98,5%) was provided from Cicarelli. BSTFA (bis[trimethylsilyl]trifluoroacetamide, 98,6%), TMCS (trimethylchlorosilane, 97%), tetradecane (99%) and methyl heptadecanoate (99%) were purchased from Sigma-Aldrich.
Sunflower wet sludge was provided by OMSA (Bahia Blanca, Argentina) and characterized in our lab as follows: (moisture: 51.0 g/g%, phospholipids: 29.6 g/g%, triacylglycerols: 19.0, non-soluble in hexane: 0.4 g/g%) [3].
Microalgea (*N. oleoabundans*) was cultured in CERZOS (Bahia Blanca, Argentina) and is oil content is estimated in: 19.8 g/g% neutral lipids. It was dried in a convection oven up to 25 g/g% water content.

2.2 Continuous high pressure reaction unit

Figure 1 shows a continuous high pressure bench scale reaction unit build in our laboratory to study the fatty ester production from different substrates at high temperature and pressure. The reactor and experimental methods has been described in detail elsewhere [7].

1 Temperture datalogger
2 Pressure sensor
3 Heat exchanger
4 Temperature controller
5 Pre-heating system
6 Feeding tanks
7 Mixing tank
8 Secondary pump
9 Primary pump
10 Tubular reactor
11 Back pressure regulater
12 Product



Alcohols:
•Methanol
•Ethanol (96.5)

Vegetable oils:
•Coconut
•Sunflower
•Soybean
•Waste cooking oils

T. max.: 400 ºC
P. máx.: 350 bar
Flowrate: 3 a 30 mL/min.

Figure 1. Continuous high pressure bench scale reaction unit

2.3 High pressure liquid-liquid fractionation

Figure 2 shows a schematic diagram of the equipment used in the fractionation of FAME and ACs. It consist of a high pressure vessel (4000 mL capacity, I.D: 100 mm) with a quick clamp closure system and a flat end vessel head assembled with a type B Polypack seal. Tube lines are used to load the solvent and oily mixtures as well as to obtain the purified products. An external electrical heating element in the bottom and an internal cooling system at the top of the recipient allows for an internal recirculation of the solvent. A modified Soxhlet type apparatus was adapted inside the vessel to effectuate liquid-liquid extraction of FAME from oily mixtures of FAME+ACs mixtures with liquid CO_2. The experimental procedure has been described in a previous work [8].

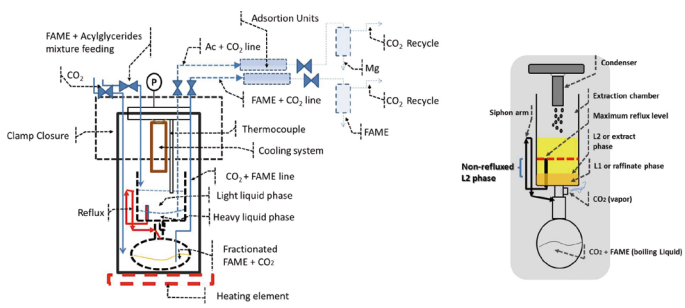


Figure 2. High pressure extraction unit used in CO_2 fractionation experiments.

3. Results and discussion

3.1 Supercritical alcohol process (SAP) of vegetable oils

The operating conditions used in SAP experiments were selected according to previous works on transesterification of vegetable oils with supercritical alcohols

[2,7,8]. Basically, the vegetable oil and alcohol were pumped at 10 g/min. in a given molar ratio (42 g/g % and 59 g/g%, ethanol) to the reactor (360 ml) operated at 180 bar and temperatures between 573 K and 603 K. Fatty acid profile of the vegetable oil used as raw material, consist about 87% of oleic acid (C18:1), 7% of palmitic acid (C16:0) and 6% of linoleic acid (C18:2). High oleic sunflower oil was used in the study to facilitate the GC analysis of the reaction products.

In the SAP of vegetable oils with methanol it was obtained a complete triglycerides conversion at 573 K with a FE content of ≈80 wt.% and a good selectivity towards MG compounds. On the other hand, MG concentrations in the reaction products were very low at 603K at the operating conditions studied in this work. Moreover, evidence of FE degradation was observed at this temperature in the chromatographic analyses, which was more noticeable for the lower methanol concentration. Based on this results obtained it was decided to use the oily mixture obtained at 573 K to study the fractionation of FE and ACs by liquid CO_2. Results shown in table 1 for SAP of vegetable oils are in agreement with previous works published in the literature about supercritical methanolysis of vegetable oils [2,7,8].

Table 1. Analysis of the reaction products obtained in the SAP of sunflower oil

T, K	Alcohol wt.%	FE, wt. %	MG, wt. %	DG, wt. %	FAs, wt. %	N.D*
Vegetable oils: biodiesel composition						
573	42	78.1	14.2	6.0	1.8	0
	59	83.5	11.5	3.0	2.0	0
603	42	90.2	4.0	0.0	1.0	4.8
	59	94.0	2.5	0.0	0.2	3.3

FAEE: fatty acid ethyl esters; MG: monoglycerides; DG: Diglycerides; FA: free fatty acids; N.D: non-detectable compounds via GC analysis.

3.2 SAP of sunflower wet sludge and microalgae

Sunflower wet sludge (lecithin) and microalgae SAP products obtained in batch mode reactions have a dark brown aspect, with solids in suspension. A solid-liquid-liquid extraction of the SAP products with hexane and water produced a bio-oil rich in hydrocarbons and other valuable compounds. In the case of sunflower wet sludge, it is obtained a bio-oil yield of ≈75 g/g% (bio-oil/reaction products). This bio-oil, depending on temperature (553-593 K), alcohol concentration (50 to 80 g/g%), and substrate pretreatment (water content) has up to ≈60 g/g% of FE content [3]. SAP of microalgae for similar operating conditions (523–553 K, 70 g/g% ethanol) produced oily mixtures with considerable quantity of solids and it is obtained bio-oil yields of up to ≈30 g/g% (bio-oil/dry microalgae) with FE contents of ≈32 g/g% [5]. High concentrations of FFA were also detected in SAP products of both substrates due to the high initial water content [3,5]. A drying process of the raw materials reduces notably the presents of these compounds in the reaction products [3].

Results obtained in the different studies are pointing out a bio-oil production higher than the initial quantity of neutral lipid (triglycerides) present in the substrates. However, the operating conditions have to be carefully controlled to maximize the FE content in the bio-oil because a reaction temperature higher than 523 K by prolonged reaction times could degrade the fatty esters. Also, technical problems related to the partial miscibility between the alcohol and the substrates at the reactor inlet as well as to the solids present at the reactor outlet are an important problem that needs to be solved for a continuous processing of alternative materials.

The reaction products obtained in the SAP of sunflower wet sludge, microalgae, and other substrates could also fractionated by CO_2. The technology presented below for the fractionation of biodiesel oily mixtures with liquid CO_2 could be perfectly adapted to the processing of raw products obtained in the SAP of biomass where several chemicals products are obtained.

3.3 Fractionation of SAP products by liquid CO_2

Experimental measurements of CO_2 + oily samples (FE + ACs) under liquid-liquid-vapour equilibrium reported in previous works [3,8] show that it is possible to fractionate the FE and ACs components using liquid-liquid extraction. The ACs solubility in the light liquid phase of the three phase system remains very low in comparison with their FE concentration. Figure 3 shows as an example, a reaction product with 80 g/g% FE exhibits two liquid phases when it is put in contact with liquid CO_2. A light liquid phase rich in solvent with ~6 g/g% of FE and ACs contents lower than 0.01 g/g%. On the other hand, the raffinate or oily liquid phase has 30 g/g% of ACs in a solvent-free basis, and it can be re-extracted with fresh CO_2 up to obtain a raffinate liquid phase concentrated in ACs components [3].

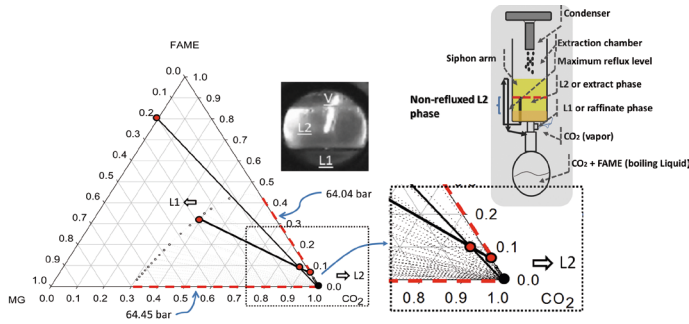


Figure 3. Liquid-liquid-vapour (L1-L2-V) equilibria at 283 K of the system CO2+FE+AC. Symbols: experimental data measured in this work for CO_2 + oily samples. Pointed lines are liquid-liquid tie lines predicted by the GCA-EOS of CO_2 + methyl oleate + monoolein at L1-L2-V equilibria Red dashed lines are liquid-liquid tie lines of the binary systems [3,8].

The modified high Soxhlet apparatus permits to carry out several liquid-liquid contact stages. In a typical experiment a given amount of oily sample (20 to 50 g) was first loaded in the extraction chamber of the modified Soxhlet apparatus and it was introduce inside the vessel. Then, after the high pressure vessel was closed, a gentle CO2 gas stream was flushed inside the recipient through an internal line to purge the air. A given amount of CO_2 (≈750 g) was loaded to pressurized the vessel. Afterwards, liquid CO_2 (≈300 g) was loaded to the recipient (outside the modified Soxhlet apparatus) to start the separation. The process start-up consisted in operating the cooling and heating systems to reflux the solvent. The boiling CO_2 vapor rises up through the vessel to the cooling system. The condensed drops of solvent fall into the extraction chamber producing two liquid phases L1-L2 and dissolving out the FAME from the oily mixture in the L2 phase. When the side-arm fills to overflowing, it initiates a siphoning action and the CO_2+FAME liquid phase is siphoned into the boiler through an extraction tube designed to avoid the reflux of "L1" heavy liquid phase (rich in ACs). Boiling solvent vapor from the flask continue rising up to the condenser and fresh solvent drops continue to fall into the extraction chamber to repeat the cycle. A thermocouple located in the extraction chamber at the siphon-arm level allows determining the number of cycles that take place in the process because of the slightly temperature difference (~1 K) in the L2 and the vapor phase rising up from the boiler.

Cross-current experimental extractions of ~50 g oily sample (FE: 78.1 g/g% and ACs: 23 g/g%) were carried out operating the high-pressure vessel at 323 K at the bottom and 278 K at the top. It was possible to obtain in the extract phase up to 95 g/g% of the initial FE present in the sample after 20 extraction stages and the raffinate phase was concentrated in ACs from 23 g/g% up to 80 g/g%.

4. Conclusions

In this work the SAP of vegetable oils was carried out to obtain FE and ACs mixtures. It was possible to obtain complete triglycerides conversion with up to 78 g/g% of FE and 20.2 g/g% of AC (≈70 g/g% MG). Liquid-liquid extractions were carried out with CO_2 in a modified high pressure Soxhlet apparatus to study the fractionation of FAE and ACs mixtures. The results show that it is technically feasible to perform the fractionation to purify FE from a biodiesel mixture, obtaining also a raffinate phase enriched in ACs compounds. Most FE present in the system can be recovery using 20 stages (solvent mass ratio of 30 g CO_2/ g initial FE). The extract phase exhibit a high FE concentration without pigments and minor components normally observed in biodiesel samples. The GCA-EOS model was a useful tool to study the liquid-liquid-vapor equilibria of the ternary system and to evaluate the liquid-liquid separation process through a simple mass transfer model. The high pressure CO_2 fractionation of the reaction products obtained from the SAP of different substrates could also be carried out in order obtain pure fatty acid esters and other valuable chemicals.

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Energy recovery through the anaerobic digestion of the residual microalgae biomass from a biodiesel production process

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Abstract

Nowadays, biodiesel from microalgae seems as an important step for the development of biofuels that could replace actual fossil fuels. However, the low energetic yield of the process is an important issue that must be overcome. Anaerobic digestion (AD) of the spent biomass appears as an alternative to solve this problem, due to the production of biogas which allows valorization of this waste. This work analyses the energetic potential that the anaerobic digestion of the spent biomass has in an integrated process scheme with the biodiesel production, using microalgae *Botryococcus braunii* and *Nannochloropsis gaditana*. The results shows that bio-methane potencial reaches values of 449 and 484 mL CH_4 / g VS for spent microalgae *B.braunii* and *N.gaditana*, respectively. Calculation computed for energy balance in total microalgae revealed that less than 20% of total energy in whole microalgae can be achieved by biodiesel production and 40 and 44% of total energy is recovered when spent microalgae is anaerobically degraded for *B.braunii* and *N.gaditana*, respectively. A hypothetical process for biodiesel and biogas production from microalgae was analyzed considering both electrical and thermal demands and contribution of biogas produced through energy obtained in biogas co-generation. Results showed that for electrical demands less than 50% of energy supply for biogas co-generation was reached. For thermal demands close to 75% may be supplied considering thermal energy in co-generation process. Thus, anaerobic digestion of spent microalgae represents a process able to increase global energetic yield in global biodiesel process.

Keywords

Anaerobic digestion; microalgae; *Botryococcus braunii, Nannochloropsis gaditana.*

Introduction

Currently, most of the efforts to take advantage of microalgae as a source of bio-energy have been directed to biodiesel production, but despite the advantages above mentioned, there is concern related to a potentially low energetic yield in the biodiesel-from-microalgae production process using current technologies. Indeed, some authors had calculated a negative energetic balance, with the largest production costs associated to harvesting and drying steps. In this scenario, different strategies had been proposed in order to improve the energetic yield of the process. The anaerobic digestion of the residual biomass seems to be one of the most promising strategies, due to the energy recovery in the form of biogas, the potential re-use of the released nutrients in the microalgae culture and to the fact that anaerobic digestion can be used to stabilize the waste biomass and avoid other costs related to its disposal and management. The aim of this work was to determine the experimental energy recovery through the anaerobic digestion of the lipid extracted biomass of a microalgae specie with potential for biodiesel production, using *B.braunii* and *N.gaditana* Bio-methane potential tests (BMP) were carried-out in order to evaluate potential energy recovery through produced biogas, and nutrients release. Spent microalgae *B. braunii* and *N. gaditana* were used as substrate.BMP was determined in 600mL vials, containing 400 mL of media. Assays were done in triplicate and performed at 35 °C. An initial substrate concentration of 5 g/L of volatile solids (VS) was applied. Anaerobic granular biomass from a full scale UASB reactor treating brewery wastewater was used as inoculum. Anaerobic biomass/substrate ratio was 1:1, expressed as VS. Medium was supplemented with yeast extract (200mg/L), sodium bicarbonate (5 g/L) and macronutrients: NH_4Cl (65 mg/L), KH_2PO_4 (18.5mg/L), $CaCl_2{\cdot}2H_2O$ (4mg/L), $MgSO_4{\cdot}7H_2O$ (5.7mg/L). Methane production was determined based on the evolution of pressure and composition of the gas contained in the headspace.

Experimental

Bio-methBMP was computed considering produced methane and VS content of spent microalgae. Total ammonium nitrogen and phosphate were determined in the liquid phase by the end of the BMP assays. Endogenous biogas production and release of nutrients from anaerobic biomass was determined by blank assays containing only inoculum. The biogas pressure and methane composition were measured through pressure transducer (Cole-Parmer) and gas chromatography (GC-TCD), respectively.

Results and discusion

BMP values for lipid extracted *B.braunii* and *N.gaditana* were 449 and 484 mL CH4 gVS^{-1}, respectively (Fig. 1 and Fig. 2).These values are high considering literature reported BMP values around 90 – 450 mL CH_4 / g VS [1-4]. In terms of biodegradability, *B.braunii* and *N.gaditana* reached values of 74 and 80%, respectively. These percentages are calculated comparing theoretical BMP (based on COD composition) and experimental BMP obtained in this research (Fig. 1, 2). For *N.gaditana*, lower biodegradability than in this research have been reported, reaching values of 44 % for spent microalgae [3]. Differences between reported values of other researches and this work are most likely associated with microalgae composition, which likewise is influenced by growth conditions [2].

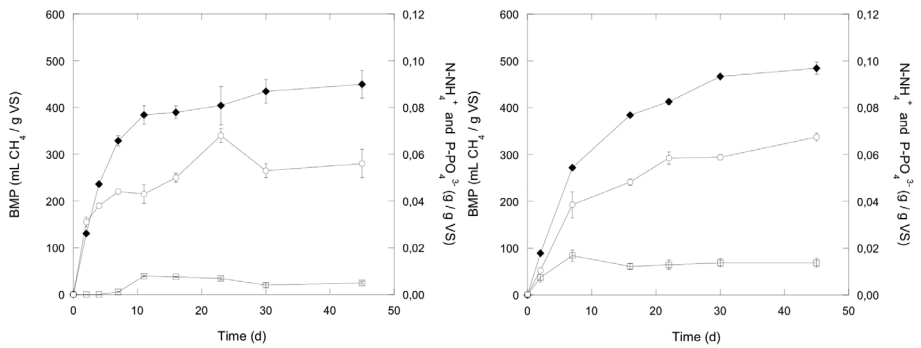

Figure 1: Bio-methane potential (BMP) and nutrients release of spent microalgae *B.braunii* (left) and *N.gaditana*.(right) (Bars indicate standard deviation)

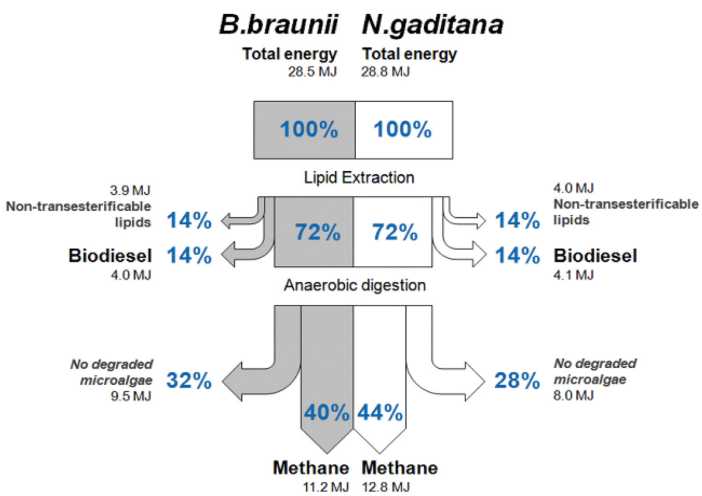

Figure 2. Energetic distribution of *B.braunii* and *N.gaditana* through biodiesel and anaerobic digestion processes. Results were calculated considering 1kg VS calculation basis.

Figure 2 shows energetic distribution of microalgae through biodiesel and biogas production in *B.braunii* and *N.gaditana*. Energy values were computed considering 1kg VS calculation basis. The no recovered energy was associated with two fractions, first a lipid fraction not convertible into biodiesel, which has been indicated as neutral lipids no containing fatty acids, such as hydrocarbons, sterols and pigments [5] and a second fraction of organic matter (spent microalgae) which has not been anaerobically degraded into methane. Biodiesel production was computed considering lipid content measured close to 24 and 25% respectively and extraction efficiency of 88 and 89%, respectively. Under this scenario, biodiesel contribution is low (<20%) considering total energy contained in whole microalgae. 50% of easily trans-esterificable neutral lipids (fatty acids) for both microalgae was used in order to calculated biodiesel production [5]. Anaerobic digestion is able to recover approximately half of total energy contained into microalgae, which represents an important energy input to process. Theoretical calculations [2,6] have showed that 50-75% of energy in a global process for producing both biodiesel and biogas from microalgae is recovered as methane.

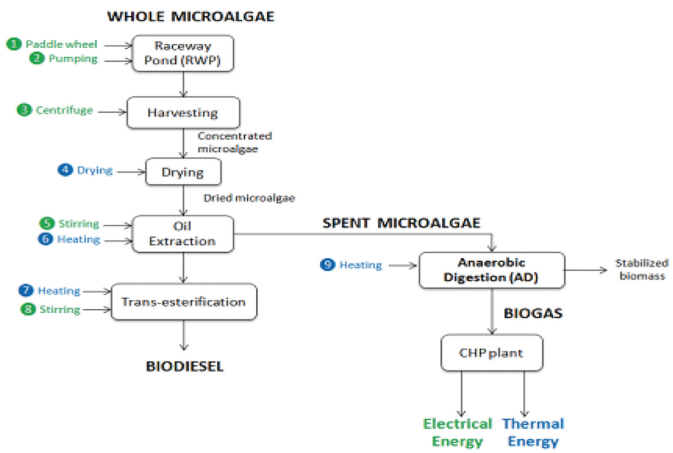

Figure 3. Scheme of process used for calculation of energy production and demands of microalgae *B. braunii* and *N. gaditana* growing in raceway pond.

Biogas production from spent microalgae could be then considered as a source of energy supporting biodiesel requirements, whether indeed biodiesel is considered as the main economical product. In order to test this hypothesis, an energy balance was set, considering what could be referred to as a classical biodiesel production scheme from microalgae. Then, the potential contribution of biogas was evaluated, comparing methane productivity and energy requirements of the biodiesel production process. Calculations were computed considering a 200 m^3 raceway pond and volumetric productivities for *B.braunii* and *N.gaditana* of 0.1 and 0.13 kg/m^3 ·d, respectively. All parameters of those calculation are described in [7]. Electrical demands in process showed in Figure 3 are related to stirring and pumping, and thermal demands are related to heating.

Figure 4 shows that neither electrical nor thermal energy produced by biogas co-generation could fully supply energy demands of the considered microalgae-biodiesel production process. This is the result of the high energy demand of the decanter used for microalgae harvest, which actually would consume close to 85% of electrical power demands. Co-generation using biogas would produce 33 and 45% of the overall electricity needs for *B.braunii* and *N.gaditana*, respectively. It is clear that biofuel production from microalgae could only be feasible if low energy harvesting methods are developed, as has also been stated by other authors [8,9]. Recovered thermal energy would potentially account for close to 75% of total heat requirements. However, the analyzed scenario does not consider any thermal recovery actions, so it represents a better case scenario. Most of the thermal energy demand is related with biomass drying. Process enabling other types of drying may be then interesting, or the use of wet biomass for direct trans-esterification.

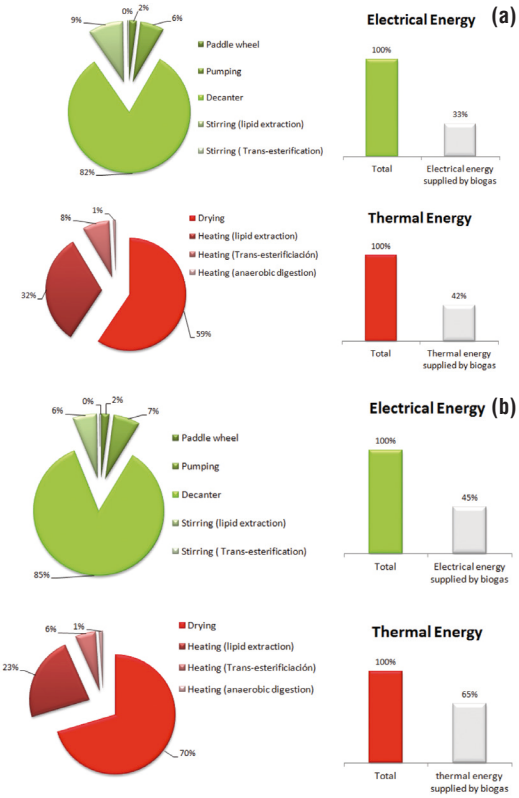

Figure 4. Electrical and Thermal demands in process (Figure 3) and energetic contribution from anaerobic digestion of spent microalgae for *B.braunii* (a) and *N.gaditana* (b).

Conclusion

Thus, anaerobic digestion of spent microalgae can be considered a key process in biodiesel production from microalgae from an energetic point of view decreasing the gap between input and output energy.

Acknowledgements

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Evaluation of technical feasibility of biogas upgrading using microalgae

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Abstract

Biogas is produced from anaerobic digestion of organic matter and it is composted for a gas mixture, principally CH_4 (55-75%) and CO2 (25-45%) with smaller amounts of H_2S. Biogas can be used with minimal or moderate levels of purification for heat and electricity production, but many applications such as vehicle and household use require CO_2 removal. The use of photosynthetic microorganisms, such as microalgae culture, has been proposed as a new alternative to biogas upgrading. The aim of this work was to evaluate the technical feasibility of biogas upgrading using microalgae. An open-photobioreactor connected to a bubble column was used to control the CO_2 and O_2 content in the biogas. In this way, the CO_2 absorption was physically separated from the O_2 desorption, in a continuous two stages process. Using this system the effect of day/night photoperiod on the biogas quality and the capacity of H_2S removal from biogas by microalgae were evaluated. The most important operational parameters were the recirculation flow between the photobioreactor and column, biogas flow, ratio between photobioreactor volume and column volume and dilution rate.

Introduction

Many applications of biogas, such as vehicle and grid injection, require the removal of CO_2 in order to produce a gas of equivalent characteristics as that of natural gas. There are regulations that establish the technical specifications of biogas for injection in gas grid or for use of biogas as vehicle fuel. For example, European standards establish a maximal CO_2 concentration in biogas between 2 and 6% [1]. An alternative way for biogas upgrading is the use of microalgae culture. Microalgae are able to capture solar energy, have high growth rates and can be adapted to different environmental conditions. When using microalgae, CO_2 is not only removed, but it is also transformed to biomass. The generated biomass is not a waste, but it can be used as feedstock for biofuel production, such as biodiesel, bioethanol and/or biogas [2]. To evaluate the technical feasibility of biogas upgrading using microalgae, the study of the following key topics is needed: effect of CH_4 and CO_2 on microalgae growth, control of O_2 content in the upgraded biogas, effect of photoperiod on the operation of a photosynthetic biogas upgrading system and capacity of H_2S removal from biogas by microalgae.

Methodology

Effect of CH_4 and CO_2 on microalgae growth

Batch photobioreactors of 500 mL were set-up. Potential CH4 inhibition was tested using three gas mixtures: 0, 50 and 100% CH_4, balanced with N_2. $NaHCO_3$ was used as carbon source. Potential CO_2 inhibition was tested using six gas mixtures: 0.3, 3, 6, 9, 15 and 30% CO_2, balanced with N_2.

Control of O_2 content in the upgraded biogas

Microalgae perform oxygenic photosynthesis. Therefore, to control de O_2 content in the upgraded biogas, the physical separation of the CO_2 absorption from the O_2 desorption in a continuous two stages was proposed (Figure 1). A 75 L continuous open-photobioreactor was implemented. The photobioreactor was connected to a bubbling column operated in counter-flow mode. The system was operated injecting real biogas continuously in the bottom of the column, while microalgae culture was continuously circulated between the photobioreactor and the column, by means of a peristaltic pump.

Effect of photoperiod on the operation of a photosynthetic biogas upgrading system

An automatic on/off of lighting was programmed, simulating a 12:12 light/dark cycle, in the system showed in Figure 1. Biogas was injected continuously into the system. Two flows of biogas were used: 72 and 50 L d-1. Gas samples were taken from sampling point located at the entrance and exit of the column for gas composition determination. Samples were taken from the microalgae culture to measure dissolved inorganic carbon concentration and biomass concentration. The CO_2 concentration in the upgraded biogas, pH in the photobioreactor and pH in the output of column were measured on line.

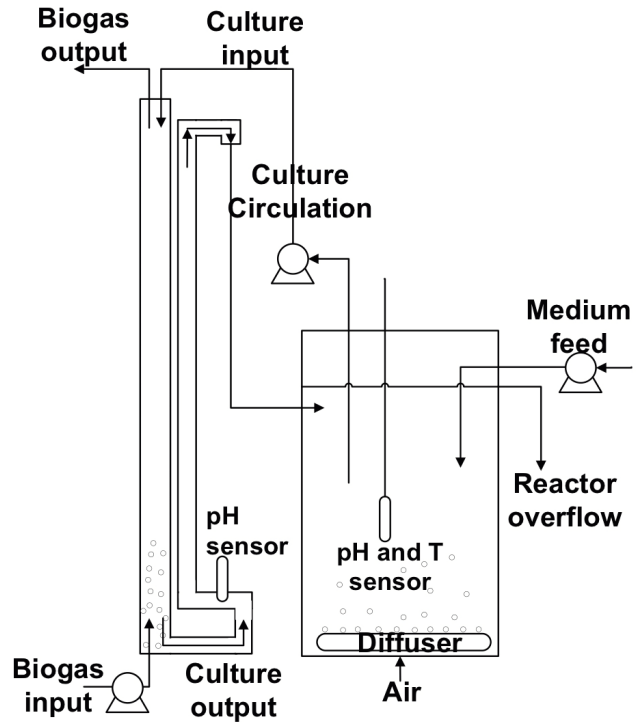

Figure 1. Two-stage process for biogas upgrading by microalgae.

H_2S removal from biogas by microalgae

Two continuous photobioreactors of 2 L were operated (Figure 1): system 1 (biogas without H_2S) and system 2 (biogas with H2S). A recycle flow of microalgae culture between the photobioreactor and the column of 0.4 L d-1 was applied. Samples were taken from the culture medium to determine dissolved inorganic carbon, sulphate, thiosulfate, sulfide and microalgae biomass concentration. Gas composition (O_2, CO_2, CH_4 y H_2S) at the inlet and outlet of the columns was measured.

Results and discussion

Effect of CH_4 and CO_2 on microalgae growth

Figure 2A presents growth curves measured with atmospheres containing different levels of CH_4 (0, 50 and 100%). Biomass growth was similar under all conditions, a specific growth rate of 0.1 d-1 can be calculated. It is then inferred then that an atmosphere containing CH_4 did not produced any significant effect over the growth of microalgae. Figure 2B presents the results of the batch cultures performed at different CO_2 levels. Results show that atmospheres containing CO_2 at 0.3, 3 and 6 %, generated a similar response. At these conditions, a specific growth rate of 0.16 d-1 was determined. When CO_2 level was 15% or higher, clear signs of inhibition were observed. Growth inhibition may have been at high extent the result of pH, which was less than 5.0 for the highest tested CO_2 levels.

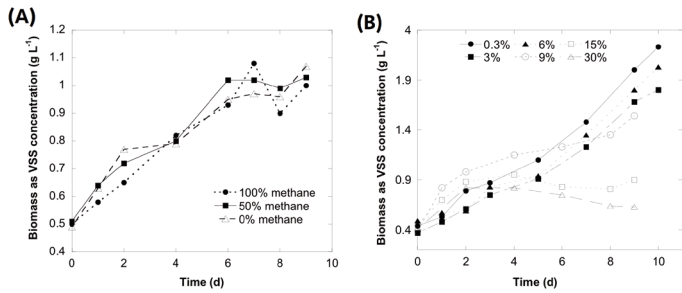

Figure 2. Microalgae growth curves A) under different levels of CH4 in the gas phase; B) under different levels of CO2 in the gas phase.

Control of O_2 content in the upgraded biogas

Figure 3 shows the operation of photobioreactor connected to a mass transfer unit. Four different levels of liquid phase circulation between the photobioreactor and the mass transfer unit were applied: 14.4, 41.8, 72.0 and 115.2 L d-1.

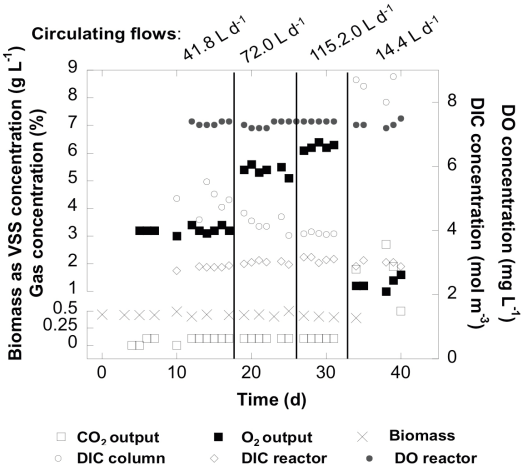

Figure 3. Operation continuous photobioreactor connected to bubbling column. (Biogas composition: 72 ± 2% CH4; 28 ± 2% CO_2, DIC column refers to the condition in the liquid effluent of the column).

Operation of the two-stage system showed to be able to promote lower levels of oxygen in the effluent gas. At a circulating flow of 14.4 L d^{-1}, CO_2 and O_2 concentrations of 1.9 ± 0.6% and 1.2 ± 0.1 % were achieved in upgraded biogas. Liquid flow between the photobioreactor and the column proved to be a relevant factor determining the quality of the upgraded biogas. As the circulating flow gets lower, the dissolved oxygen that is transferred to the mass transfer unit (dissolved in the liquid) is lower, promoting a lower O_2 concentration in the upgraded biogas. However, a lower circulating flow reduces the mass transfer capacity, causing a rise in the concentration of CO_2 in upgraded gas.

Effect of photoperiod on the operation of a photosynthetic biogas upgrading system

Figure 4 presents the operation of the column-photobioreactor system using a day/night photoperiod of 12:12 h and a continuous biogas flow of 72 L d^{-1}. A circulation flow between photobioreactor and column of 43 L d^{-1} was applied.

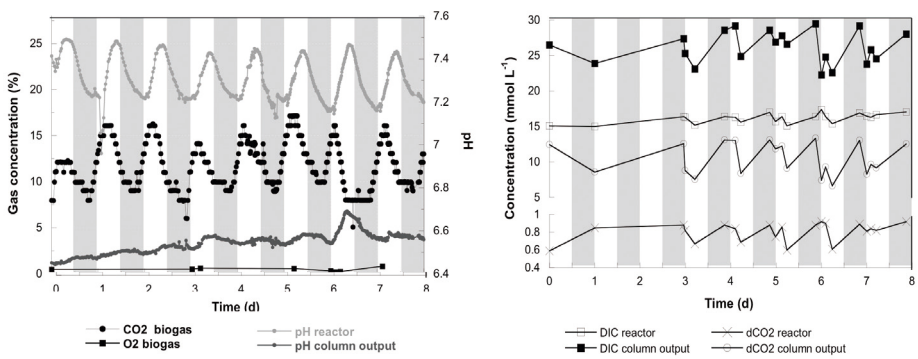

Figure 4. Operation of column-photobioreactor system using a biogas flow of 72 L d^{-1} and 12:12 light/dark photoperiod (White color: light period; grey color: dark period; dCO_2 is dissolved CO_2).

When light was turned on, microalgae captured CO_2 through photosynthesis causing an increase of pH and a reduction of dissolved inorganic carbon (DIC) concentration in the photobioreactor. On the contrary, during the period of darkness, pH decreased and DIC concentration increased because photosynthesis was stopped and the biogas injection was continuous. A decrease in the CO_2 transfer rate and in the CO_2 removal efficiency was expected during the night, as consequence of pH reduction and DIC rise in the column. However, the effect of light/dark photoperiod on the mass transfer was not instantaneous and a delay in the response was observed. Since CO_2 concentration in the treated biogas did not fulfill biomethane standards, when a biogas flow of 72 L d^{-1} was applied, the biogas flow was reduced to 50 L d^{-1}. When a biogas flow of 50 L d^{-1} was used, CO_2 concentrations fluctuated between 5.6 and 0.8%, fulfilling most of the standards.

H_2S removal from biogas by microalgae.

The system photobioreactor-bubble column could remove 100% of the H_2S content in the biogas (Figure 5). The high dissolved O_2 concentration in the microalgae culture (9 mg L^{-1}) allowed a fast oxidation from H_2S to sulphate. A percentage of this produced sulphate could have been used by microalgae as source of sulfur. Additionally, upgraded biogas with a composition of 0.5% CO_2 and 2% O_2 could be obtained.

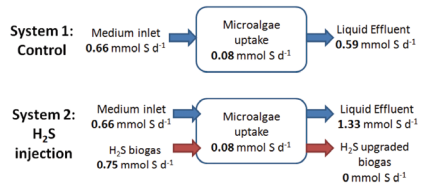

Figure 5. Sulfur mass balance of control system (injection of biogas without H_2S) and system with injection of biogas with H_2S.

Conclusions

Biogas upgrading using microalgae is a feasible alternative. An upgraded biogas with 1.9% CO_2 and 1.2% O_2 was obtained using a 75 L open-photobioreactor connected to a 0.7 L bubble column by continuous recirculation of microalgae culture. Biogas could be upgraded continuously during day/night photoperiod using microalgae culture, without necessity of biogas storage during darkness. The H_2S and CO_2 could be simultaneously removed from biogas using microalgae culture.

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Production of PHB production from glycerol waste by *B. xenovorans* LB400

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Abstract

Glycerol is accumulated in large quantities as a by-product during biodiesel and bioethanol production. Hence, glycerol utilization in the production of bio-based materials is an increasing interest for the biorefinery development [1]. Polyhydroxyalkanoates (PHAs) are biobased thermoplastic polyesters that exhibit thermal and mechanical properties similar to polypropylene. PHAs are produced as intracellular carbon and energy storage polymer by diverse bacteria [2]. *Burkholderia xenovorans* LB400 is a model bacterium for the degradation of polychlorobiphenyls and aromatic compounds [3]. Strain LB400 grown on glucose and xylose can synthesize poly(3-hydroxybutyrate) [(PHB)] [2]. The aim of this study is to determine if strain LB400 is able to produce PHB from glycerol waste. Strain LB400 was grown in M9 medium under nitrogen limiting conditions with either raw or pure glycerol (20 g L^{-1} glycerol and 1 g L^{-1} NH_4Cl). Cells were freeze-dried and subjected to propanolysis. The propylesters were extracted with organic solvents and analyzed by gas chromatography coupled with a flame ionization detector (GC-FID). GC-FID indicated the production of PHB by LB400 cells grown on glycerol waste and pure glycerol as sole carbon source under nitrogen-limiting conditions. PHB production by *B. xenovorans* LB400 was quantified. These polymers were extracted using chloroform. PHB was accumulated in LB400 cells up to 30% in both conditions. The production of PHB was enhanced from 1.1 g L^{-1} with xylose as carbon source to 1.5 g L^{-1} with glycerol waste as carbon source. PHB production by *B. xenovorans* LB400 from glycerol waste may be attractive for biorefinery.

Introduction

From the year 1999 to 2013 biodiesel production increased from 500.000 gallons to 1800 millions of gallons, increasing glycerol waste as well [4]. Glycerol is approximately 10% of the final weight of biodiesel. Increasing for biodiesel production has led to an increase in the availability of glycerol, lowering its price [5]. Glycerol is of increasing concern in the biodiesel industry. Polyhydroxyalkanoates (PHA) are polyesters synthesized as carbon and energy storage compounds by several bacteria under unbalanced nutritional conditions [2, 6, 7]. These polymers represent a class of compounds with physical and chemical features similar to petroleum-derived plastics such as polypropylene, polyethylene and polystyrene, but are environmentally compatible and completely biodegradable to carbon dioxide and water [5, 8]. *B. xenovorans* LB400 is a model strain for the degradation of polychlorobiphenyls and aromatic compounds [3, 9]. LB400 genome (9.7 Mb) is one of the largest bacterial genomes reported [3, 9]. Diverse carbon sources including glucose, mannitol, xylose, gluconate and valeric acid have been used for short chain length-PHA production by strain LB400 [2]. Nevertheless, the high cost of PHA production, mainly due to the cost of substrates, has forced to search for novel carbon sources as by-products from other industrial processes. In this aspect, glycerol is a promising raw material, and growth of strain LB400 on this carbon source under limiting nitrogen conditions was evaluated, along with the production of PHA [1, 2, 5 and 8].

Experimental

Bacterial strain and growth conditions

B. xenovorans strain LB400 was cultivated in LB broth medium (tryptone, 10 g L^{-1}; yeast extract 5 g L^{-1} and NaCl 5 g L^{-1}) with agitation (150 rpm) during 24 h at 30ºC. Strain LB400 was cultivated in mineral medium M9 with trace solutions [3 and 9] and pure glycerol, glycerol waste (20 g L^{-1}) and xylose (16 g L^{-1}) as sole carbon sources. Cells were cultivated in these conditions for 48 h for the accumulation PHB in shake flasks.

Total cell dry weight

To determine the total cell dry weight, a known volume of the fermentation medium was centrifuged at 4863g for 15 min and the supernatant was separated. The bacterial pellet was lyophilized for 24 h and weighed.

PHA accumulation and composition

About 10 mg of freeze-dried cells or purified PHA were subjected to propanolysis [10]. The propylesters were analyzed with a GC-FID (Agilent 7890A GC System) equipped with a HP5 capillary column after sample split (1:25). Helium (0-8 mL min^{-1}) was used as carrier gas. Injector and FID temperatures were 250 and 300 °C, respectively. The oven was programmed at 100 °C for 1 min, increasing temperature at a rate of 8 °C min^{-1} up to 210 °C, which was maintained for 15 min. Benzoic acid was used as the internal standard. External standards were PHB and PHBV (Sigma-Aldrich, Germany).

Results and discussion

Strain LB400 was studied for PHB production using sugars (glucose, xylose, mannitol and gluconate) as carbon sources, and for PHBV production using a mix of a sugar with valeric acid (10:1) (unpublished data). Due to the high cost of these substrates, glycerol was studied as carbon source. Strain LB400 was able to grow on pure glycerol as sole carbon source. In addition, strain LB400 was able to use glycerol waste from a biodiesel plant associated to flour mill as sole carbon source. The biomass and production of PHB were compared. Moreover, PHB production from both pure and waste glycerol with PHB produced from xylose were compared (Figure 1). Significant differences in PHB production from pure or waste glycerol were not observed, suggesting that glycerol waste might be suitable carbon source for larger scale production of PHB. The biomass achieved from both glicerol was 4.7 and 4.9 g L^{-1}, in contrast to 3.8 g L^{-1} from xylose. PHB accumulated from the three carbon sources were around 27-30% of biomass. PHB accumulated (1.5 g L^{-1}) from glycerol waste as carbon source was slightly higher.

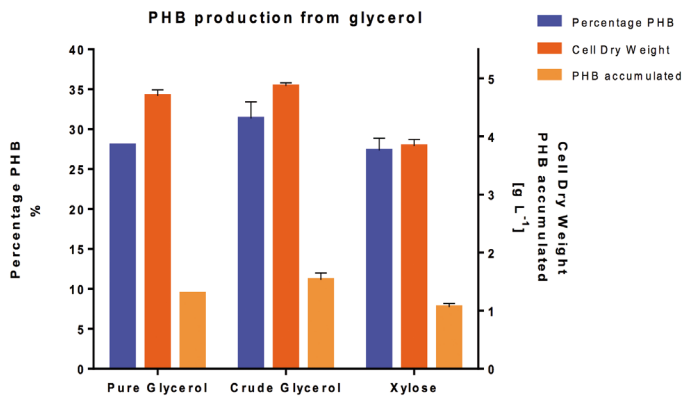

Figure 1. PHB production by B. xenovorans LB400 from pure glycerol, crude glycerol or xylose as only carbon source under unbalanced nutritional conditions.

The production of PHB strain LB400 is similar to PHB production from different co-products as carbon sources previously reported [11].This results suggest that the production of PHB using glycerol is attractive to scale up to a bioreactor. It has been reported that PHB from glycerol has major molecular weight and better mechanical strength than PHB from other sugars being an attractive alternative for biorefinery development [6].

The copolymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) was produced by strain LB400 grown on glycerol in presence of valeric acid. The polymer was purified, obtaining a film that is shown in Figure 2. It has been reported that PHBV has better mechanical properties than the homopolymer PHB. Therefore, the accumulated biomass should be measured in further studies [1, 2, 6 and 7].

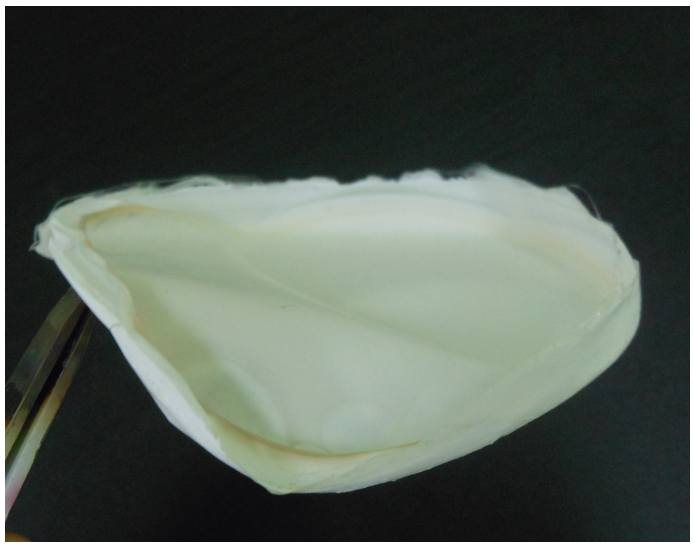

Figure 2. PHBV film extracted from *B. xenovorans* LB400 culture grown on glycerol waste and valeric acid.

Conclusions

The strain LB400 is an interesting bacterium for the PHB and PHBV production from glycerol waste associated a biorefinery model. The production of PHAs by strain LB400 from novel raw products should be studied. This is an attractive scenario to associate industrial waste and biosynthesis of biodegradable compounds.

Acknowledgements

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Medium chain length production by *Pseudomonas fluorescens* and unrelated carbon source

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Abstract
This work performed the medium chain length Polyhydroxyalkanoates PHA_{MCL} employing *Pseudomonas fluorescens* and sugar cane molasses as a substrate, the fermentation culture medium condition was evaluated in shake flasks, 150 rpm, initial pH 5,5 and the carbon/Nitrogen relation (C/N) was setting up from modification of molasses concentration and different chemical nature of nitrogen sources urea and ammonium sulfate. Molasses as a substrate have a favorable nutrients composition to microorganism growth and promote the PHA_{MCL} production, even though this is an unrelated substrate with length chain of the polymer produced. However, molasses concentrations around 60 g/L can inhibit microorganism growth. Urea as a nitrogen source has appropriate metabolism *Pseudomonas fluorescens* conditions, it permits a 40% increase in microorganism growth compared to fermentations using ammonium sulfate.

Afterwards, the evaluation of PHA_{MCL} production give up as result that to 26°C, 50,7 g/L of molasses and 168 h of time fermentation was obtained a yield of 253,27 mg PHA/g biomas and biopolymer with Mw 998 kDa, polydispersity index of 3.62 and chain lengths of between 8-18 carbons.

Keywords: Biopolymer, Polyhydroxyalkanoates, Medium chain length Polyhydroxyalkanoates, *Pseudomonas fluorescens*; sugar cane molasses.

Introduction
PHA are aliphatic polyesters integrated by hydroxy acid monomers and accumulated into bacteria cytoplasm as a material reserve [1], [2]; are biocompatible, biodegradable, thermoplastic, have high polymerization degree, high crystallinity, optically active and isotactic piezoelectric and water insoluble [3].
Pseudomonas sp., synthesize a wide variety of monomers (R) -hydroxy [4], and containing at least two different polymerizing enzymes with different substrate specificity and allows them to synthesize 3-hydroxybutyrate monomer and medium chain from different carbon sources. Some agro-industrial residues as substrate for production have been used like alternatives to decrease cost of PHA production [3].

In this study the polyhydroxyalkanoates medium chain length production PHA_{MCL} was evaluated from the control variables such as carbon source concentration and nitrogen source, and finally the biopolymer chemical structure obtained was established.

Methods
Operations conditions and culture medium
Pseudomonas fluorescens migula ATCC 49838 was used and the culture medium was selected using sugar cane molasses and glucose by differents carbon source concentrations. Subsequently, urea and ammonium sulphate as a nitrogen source was evaluated by means of factorial design 2^3. Temperature fermentation, carbon and nitrogen sources concentrations was evaluated by response surface design with star, and orthogonal rotatable (RMS). Finally, the effect of fermentation times of 96 and 168 h in the structure of the biopolymer and the fermentation performance was evaluated.

Medium composition used is as follows: 1 g/L $(NH_4)SO_4$, 1,5 g/L KH_2PO4, 1 mL/L sln 20% (p/v) $MgSO_4.7H_2O$, 1 mL/L sln 1% (p/v) $CaCl_2.2H_2O$ y 1 mL/L of micronutrients solution [5]–[7]. Biomass concentration to a fermentation time of 60 h was evaluated as response variable. The tests were conducted in Erlenmeyer flasks of 250 mL, 120 rpm, pH initial 5,5 and 30 °C.

Analitycal methods
Biomass quantification
Biomass concentration was performed by absorbing radiation in a spectrophotometer UV/VIS (Shimadzu, UV 1606 PC).

Biopolymer extraction
It was performed by disruption membrane cell by alkaline digestion with a solution of sodium hypochlorite 1% (v / v) for 1 h with constant shaking 120 rpm and temperature 24 ° C, subsequently centrifuged at 9000 rpm for 20 min. Then, 25 mL of chloroform were added to 1 g of biomass was allowed to stir for 12 h temperature 24 ° C, then centrifuged (BOECO centrifuges, U-320R) for 30 min at 9000 rpm and the organic phase was concentrated by evaporating to 1/5 of initial volume, precipitated at -20 ° C in a proportion 1/10 methanol and finally dried at 40 ° C for 24 h and the amount of biopolymer obtained was quantified [8].

Biopolymer characterization
Infrared Fourier Transform Spectroscopy (FTIR): Nicolet serie 6700 with ATR [9].
Gas chromatography–mass spectrometry (GC-MS): GC-MS analyzes were performed on a mass spectrophotometer Thermalaux 2 coupled to a gas chromatograph with a FID detector and a capillary column Agilent HP 19091J-113-5 to 5% Phenyl Methyl Siloxane. Helium as carrier gas was used at a flow of 1 mL / min. The temperature conditions used were: initial temperature 100 ° C - 1 min, gradient of 5 ° C / min to 200 ° C - 3 min, 250 ° C - 330 ° C and 4 min - 5.33 min.
Molecular Weight Analysis: It was established by gel permeation chromatography (GPC) coupled with light scattering detector (LS) column temperature 40 ° C. Tetrahydrofuran (THF) was used as eluent at a flow rate of 0.5 mL / min. The calibration curve was determined with polystyrene low polydispersity (Mw / Mn <1.1) with molecular weight: 9100, 18100, 37900, 96400, 190000 and 355000 Da [9], [10].

Results and discussion
Evaluation of operating conditions and culture medium
Pseudomonas fluorescens showed a decrease in the growth inhibition caused by microorganism molasses concentrations higher than 60 g/L, a maximum concentration of dry biomass is achieved 1,0435 g/L for medium molasses concentration of 40 g/L. This inhibition is caused by components presence such as phenols originating from the fibrous part of the rod and are derived from para-hydroxybenzoic acids and hidroxinámico, as reported Fajardo, Erika, Sarmiento, 2007 and Serrano, 2013 inhibit the microorganisms growth at concentrations above 0.5 g / L, damaging the membrane and denature bacterial proteins act as microbicidal or bacteriostatic.

Factorial design showed an increase in growth of the microorganism 0.72 g / L with respect to change in the chemical nature of the nitrogen source, urea biomass concentration was 1,76 ± 0,001 g/L and PHA production 37,35 ± 2,40 mg PHA/ g dry biomass, while for the ammonium sulfate was 1,04±0,030 g/L with PHA production 17,13 ± 0,58 mg PHA/ g dry biomass. Haba et al., 2007 report on their investigation that when urea is used against other inorganic nitrogen source such as sodium nitrate increased PHA accumulation of around 37% was obtained, confirming the results obtained in the design of experiments performed.

Subsecuently, surface design showed in the optimal point a yield biopolymer production for 96 h of 29,59 ± 2,1 mg PHA/ g biomass, while for a fermentation time of 168 h it was obtained a yield greater than 7 times 253,27 ± 26,26 mg PHA/ g biomass, This increase in the amount of biopolymer not due to increased concentration of biomass as it decreased by about 7%.

Additionally, a comparison of the spectra of the biopolymer obtained 96 h 168 h was observed which are different spectra and the structural modification of the biopolymer is significant to increase the time of fermentation was done, sharper peaks and higher intensities corresponding to the carbonyl group vibrations were obtained 1726 cm^{-1}, 1278 cm^{-1} and 977 cm^{-1} which is the band trans configuration of the double bond of the fatty acids. Vibrations for the straight chain alkyl group C – H between 2962 – 2853 cm^{-1} also have changes at 2917 cm^{-1} which shows a slight shift 2923 cm^{-1}, vibration to 2954 cm^{-1} disappears and and identifies new vibration to 2954 cm^{-1}, the vibration corresponding to the double bond C=C around to 1640 cm^{-1} It is overlapped by the vibration of the carbonyl group in 1726 cm^{-1} and additionally a typical double bond peak was observed around 980 cm^{-1}.

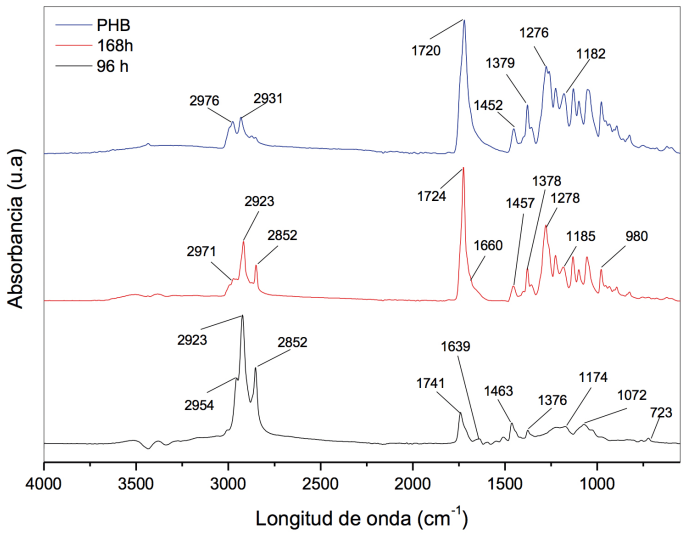

Figure 1. Infrared spectra of the biopolymer to different fermentation times and commercial PHB

Biopolymer obtained to 168 h showed the greatest similarity to the commercial PHB biopolymer, however, it is important differences around 2923 cm^{-1} as showed Hong et al., 1999 PHAMCL exhibit a strong band, as observed in the Figure 1. Comparing the spectra for 96 and 168 h the band showed a decrease by doubling the fermentation time and comparing it with commercial PHB is higher indicating that the biopolymer obtained for both times is a PHAMCL or a mixture of PHB y PHAMCL by the spectra similarity.

Biopolymer structure by GC-MS presented as most abundant monomer C15:0 (27,3 %) and C18:1 (18,22 %) with lesser amounts of C14:0 (3,68 %) and C8:0 (7,64 %). From monomeric structures it is concluded that the biopolymer is medium chain (MCL) or extended chain (LCL) [14]. Additionally, the biopolymer showed a Mw of 998 kDa and Mn 276 kDa with a polydispersity of 3.62. Haba et al., 2007 reports that typically PHA molecular weight is in a range between 1 – 10^3 kDa, and specifically Mw del PHB is of 522 kDa and a polydispersity of 1,96 [10].

Conclusions

Molasses has favorable nutrient for growth of the microorganism Pseudomonas fluorescens and promotes the production of PHA_{MCL}, despite being a substrate unrelated to the length of the chains produced in the biopolymer. However, concentrations of about molasses 60 g/L can inhibit the growth of the microorganism. Urea has appropriate conditions for metabolism of *Pseudomonas fluorescens*, and allows a 40% growth with respect to ammonium sulfate.

Polyhydroxyalkanoates production from Pseudomonas fluorescens and molasses to 168 h, 26 °C and 0,9 g/L of urea are determining parameters in the fermentation yield and structure of the biopolymer, with a production of 253,27 mg PHA/ g biomass, PHA_{MCL} with a Mw 998 kDa and polydispersity of 3,62.

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Life cycle assessment of biomethane from waste water algae: The All-Gas approach

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Abstract
This paper presents an extract of an LCA which was prepared for an algae biorefinery currently under development within the EU-funded project All-Gas (FP7-268208). For this assessment extensive process data was collected from a real algae biorefinery which uses municipal waste water as a culture medium for microalgae.
The results show that the algae biorefinery has proved to be an attractive alternative to conventional waste water treatment which contributes not only to the conservation of fossil energy carriers but also to climate protection.

Introduction
Several life cycle assessments (LCAs) have been published focussing on energy balances and greenhouse gas (GHG) emissions of microalgae biofuels such as biodiesel, bioethanol, biohydrogen or biomethane (Murthy 2011). They show that the provision of fresh water and nutrients has a significant influence on the environmental impacts of algal biofuels. The use of waste water for the cultivation of algae seems to have advantages above conventional algae production since freshwater and nutrients required for algae growth can be saved thus lowering potential environmental impacts (Zhou et al. 2014). Moreover, waste water consumed in algae cultivation reduces energy intensive purification of waste water in conventional waste water treatment plants.
The EU-funded project All-Gas wants to demonstrate on an industrial scale the feasibility of the sustainable production of algal biofuels based on low cost microalgae cultures grown with municipal wastewater. The full chain of processes from algae ponds, downstream processing for biomethane production, as well as the demonstration of vehicle use, is being implemented on a 10 ha facility in Chiclana de la Frontera. In order to reduce the environmental impacts, the investigated algae biorefinery seeks to lower environmental impacts by replacing energy required for conventional waste water treatment, avoiding extra fertiliser, avoiding the use of fresh water for algae cultivation and using flue gas as CO_2 source from a nearby biomass boiler.

Material and methods
The method applied for assessing the environmental performance of the investigated All-Gas approach is a full life cycle assessment as described by (Bradley et al. 2015).
For the selection of the impact categories, the integrative concept of sustainable development (Kopfmüller et al. 2001) is taken as a normative framework for identifying the sustainability criteria appropriate for microalgae production. The integrative concept of sustainable development is a multidimensional approach that tries to operationalize sustainability by referring to constitutive elements of sustainable development and not by addressing sustainability dimensions directly. Based on this concept and addressing the sustainable principles "protection of human health", "sustainable use of non-renewable and renewable resources", and "sustainable use of the environment as a sink" the following midpoints and endpoints (hierarchist approach) from ReCiPe (Goedkoop et al. 2013) are included.

Table 1: Summary of the criteria and indicators applied to the sustainability assessment of the investigated algae biorefinery

Principle of sustainability	Sustainability criteria	Life cycle impact assessment indicator	Unit
Protection of human health	Human health	Disability adjusted life-years (DALY)	Years
Sustainable use of non-renewable resources	Savings of non-renewable energy	Fossil energy savings (FES), alternatively EROI	kg oil-eq., -
	Depletion of minerals (nutrients) and metals	Abiotic depletion potential	kg Sb-eq.
Sustainable use of renewable resources	Area efficiency	Urban land occupation (ULO)	m^2*yr.
	Water deprivation	Water scarcity footprint	m^3
	Impact on biodiversity and ecosystems	Loss of species during a year	Species*yr.
Sustainable use of the environment as a sink	Further emissions into the air	Base saturation, PM_{10} intake, Stratospheric ozone concentration, Photochemical ozone concentration	kg SO_2-eq., kg PM_{10}-eq., kg CFC-11-eq., kg NMVOC-eq.
	Climate protection	Infra-red forcing	kg CO_2-eq.
	Water contamination	Phosphorus concentration Nitrogen concentration Hazard-weighted concentration	kg P-eq., kg N-eq., kg 1.4 DCB-eq.

Two indicators are not taken from the ReCiPe method. The indicator water deprivation refers to water scarcity footprint (WSF) which is calculated according to equation 1 and is based on the blue water consumption and the regional specific water stress index (WSI) as defined by (Pfister et al. 2009).

$$WSF = \sum_i \frac{CWU_i * WSI_i}{WSI_{global}} \quad (1)$$

CWU_i = consumption of blue water in region i
WSI_i = regional water stress index in region i
WSI_{global} = global average water stress index (value 0.602)

In order to address the depletion of minerals (nutrients) and metals, characterisation factors for "abiotic depletion potential" developed within the CML 2002 method (Guinée et al. 2002) are used. Its choice is recommended by the Joint Research Centre of the European Union (JRC 2010) and, in contrast to the ReCiPe indicator for mineral resource depletion, it covers phosphate. For this reason the CML indicator is selected.
The LCA follows the idea of life cycle thinking choosing a "cradle-to-grave" approach. As presented in Figure 1 the system boundaries encompass (1) anaerobic waste water pre-treatment, (2) cultivation of microalgae in primary treated waste water, (3) harvesting of algae, (4) biogas production from algal biomass, (5) biogas upgrading and provision at a service station, (6) application of fermentation residues on the field, and (7) CO2 and energy generation in a biomass boiler.

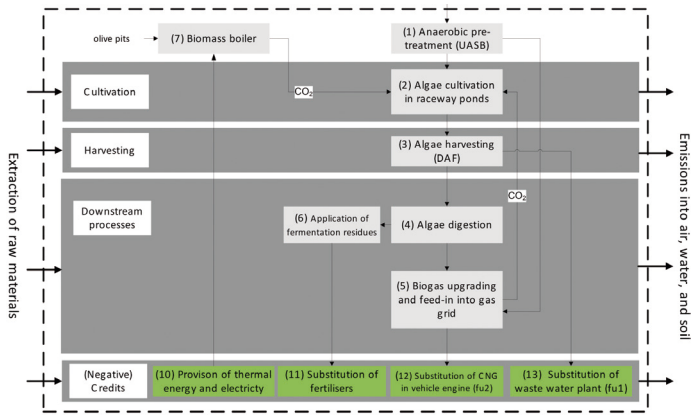

Figure 1: System boundaries of the algae biorefinery which purifies waste water and produces biomethane, fertiliser, and thermal energy

In order to determine potential environmental impacts caused by the algae biorefinery, the functional unit "1 m^3 of treated waste water" is selected. The amount of treated waste water directly refers to the amount of microalgae cultivated, harvested, and further processed into multiple products.
In order to analyse the environmental performance of biomethane produced within the algae biorefinery and used as automotive biofuel, the functional unit and reference flow respectively "1 MJ CNG (LHV) used in a gas engine" (fu 2), is selected. This functional unit was used in other studies for algal biofuels, too (Lardon et al. 2009; Sander, Murthy 2010), and allows a comparison between the algae cluster projects (Bradley et al. 2015).

Most available LCA studies on algal biofuels are based on theoretical primary data including (Batan et al. 2010), (Campbell et al. 2010), (Lardon et al. 2009), (Sander, Murthy 2010), and (Stephenson et al. 2010). Only very few contain real data such as (Passell et al. 2013) and (Beal et al. 2012). Nevertheless, the life cycle inventory data of this LCA comes from pilot scale facilities.

Results and discussion
An energy analysis was performed that includes direct energy flows such as the electricity and fuel consumption of the algae biorefinery and upstream energy consumptions (indirect energy flows). The main direct energy flows are presented in Figure 2. All values presented in Figure 2 refer to the operating of the algae biorefinery for one day.

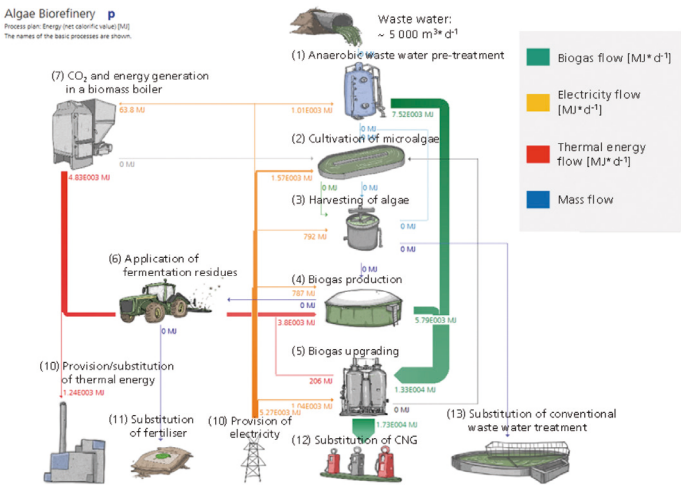

Figure 2: Direct energy flows of the algae biorefinery per day of operation, base line scenario, own illustration in LCA software GaBi

The electricity consumption of the algae biorefinery makes up 50 % of the total primary energy demand which is approximately 30 000 MJ per day of operation with a throughput of 5 000 m³ of waste water. The distribution of the primary energy consumption by process area (UP: unit process) is shown in figure 3.

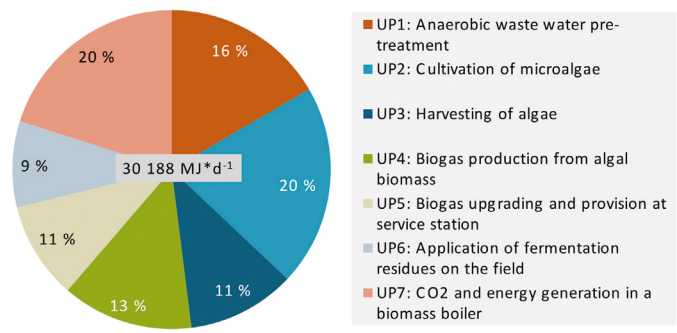

Figure 3: Distribution of total primary energy consumption by process area also considering indirect energy flows (European energy mix)

As shown in Figure 4 the net greenhouse gas emissions per m³ of waste water treated by the investigated algae biorefinery (AB) are 0.28 kg CO_2-eq. and those of conventional waste water treatment (cWWT) are 0.47 kg CO_2-eq. This means the AB allows greenhouse gas reductions of about 47 % compared to cWWT.

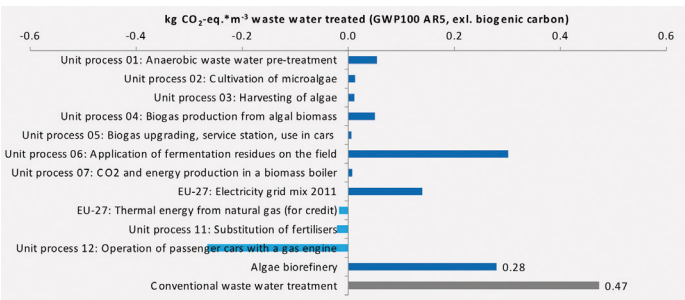

Figure 4: Comparison of GHG emissions caused by the treatment of one m3 of waste water

The main benefit (credit) results from the substitution of the automotive fuel CNG by biomethane which is produced by the AB. On the other hand the application of fermentation residues causes significant greenhouse gases, in particular nitrous oxide emissions.
In summary it can be stated that the AB reveals clear benefits with regard to the protection of the climate, protection of fossil resources, ozone depletion, and probably also due to depletion of minerals and metals. However, independent of the selected functional unit negative impacts are calculated with regard to impacts on human health, occupation of land, emissions of particulate matter, photochemical oxidant formation, acidification, water deprivation, eutrophication, and impacts on biodiversity. Since data for calculating fresh water ecotoxicity is not reliable, no clear statements can be made.

Conclusions

An algae biorefinery-specific indicator system was developed based on the integrative concept of sustainability. Several tools were selected and further developed in order to assess the identified sustainability indicators.
Although the LCA uses real pilot scale data, there still remains the challenge to get data with a high quality in particular for algae cultivation which requires a consistent data acquisition over several years. Such long-term measurements are necessary for example because of changing weather conditions in the seasons, changing waste water composition, changing composition of algae species or the presence of natural predators.
Although there are still technical and environmental drawbacks associated with the algae biorefinery under investigation, the positive aspects already present at this time make it look worthwhile to further develop this technological approach.

Acknowledgments

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Cellulose fibers and microfibers

Kraft pulps from *Eucalyptus* and *Pinus radiata* - raw materials for nanocellulose production and novel bio-applications

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Abstract

Nanocellulose research has been intensified during the last decade. Advances have been made with respect to e.g. improving the production efficiency, tailor-making nanocellulose qualities and demonstrating novel applications within various industrial sectors. Additionally, the characterization of nanocellulose has been an important research area. Nanocellulose is a complex material, which may be composed of a series of structural components, having also specific surface chemistry. Proper and objective characterization of nanocellulose is thus demanding and requires a comprehensive set of tools, from the micro- to the nano-scale, including also chemical and biological aspects. In this paper, research that has been performed during the last years on kraft pulps from Eucalyptus and Pinus radiata as raw material for nanocellulose production is revised, focusing specially on various applications within paper, bionanocomposites and biomedical devices.

Introduction

Nanocellulose can be produced from various sources, including wood pulp, annual crops, and agro-forestry residues (Wågberg et al., 2008; Saito et al., 2009; Syverud et al., 2010; Klemm et al., 2011; Jonoobi et al., 2012; Alila et al., 2013) and following specific pre-treatments (Saito et al., 2006; Wågberg et al., 2008; Liimatainen et al., 2012; Chinga-Carrasco and Syverud, 2014).

In this presentation the production, characterization and application of various types of nanocelluloses will be reviewed. Although, there is an exponential amount of studies being published every year about nanocellulose, this work focuses exclusively on research performed during the last 8 years on kraft pulp from Eucalyptus and P. radiata from Chilean plantations.

Results and discussion

In addition to having large differences with respect to the fibre morphology, the fibre wall nano-characteristics of the two assessed pulp fibres differed considerably (Fig. 1). The P. Radiata pulp fibres revealed a more open fibrillar structure compared to the Eucalyptus pulp fibres (Chinga-Carrasco et al., 2011). The more open structure of P. radiata pulp fibres apparently facilitated the fibrillation of the fibres into cellulose nanofibrils (Chinga-Carrasco, 2011).

Several pre-treatments have been applied in order to tailor-make nanocellulose materials with defined morphology and surface chemistry. The tested pre-treatments included TEMPO-mediated oxidation, carboxymethylation and periodate oxidation, followed by mechanical fibrillation (Syverud et al., 2010; Chinga-Carrasco and Syverud, 2014). Some characteristics of typical nanocelluloses are given in Table 1.

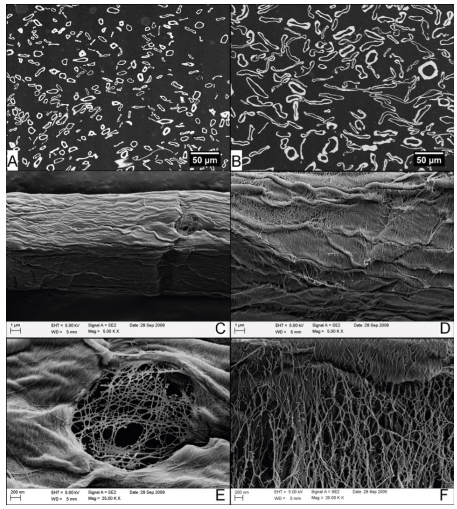

Fig. 1 Cross-sectional (A and B) and surface images (C, D, E and F) of Eucalyptus and P. radiata, respectively. The cross-sectional images were acquired in SEM-BEI mode. The surface images were acquired in FE-SEM-SEI mode. Note the more open microfibril structure on the fibre surface of *P. radiata* (F), compared to the *Eucalyptus* fibres (E). *Reproduced from Chinga-Carrasco et al.* (2011).

Table 1 Some characteristic details of nanocelluloses produced from *P. radiata* pulp fibres. For details see Syverud et al. (2011), Chinga-Carrasco et al. (2011), Chinga-Carrasco and Syverud (2014), Rees et al. (2015).

Pre-treatment	Aldehyde content (µmol/g)	Carboxyl content (µmol/g)	Nanofibril morphology	
			Width (nm)	Length (µm)
Mechanical			<100	>1
TEMPO mediated oxidation	71	855	<20	>1
Carboxymethylation	12	440	<20	>1
Carboxymethylation + periodate oxidation	1202	393	<30	<0.5

The produced nanocelluloses have been comprehensively characterised by laser profilometry, field-emission scanning electron microscopy (FESEM), UV-vis, atomic force microscopy (AFM), transmission electron microscopy (TEM), X-ray microtomography and advanced nanorobotics, covering scales from the micrometre to the nanometre level (Chinga-Carrasco et al, 2011; 2012; 2013; 2014; Mikczinski et al., 2013; Miettinen et al, 2015).

Due to the nano-dimensions, nanocelluloses offer a wide range of opportunities for various applications. Paper and packaging are two areas where cellulose nanofibrils have a major potential to e.g. improve the mechanical and oxygen barrier properties, respectively. Hii et al. (2012) demonstrated the potential of cellulose nanofibrils from *P. radiata* for reinforcing paper structures filled with $CaCO_3$ (Fig. 2). Using the appropriate filler and nanocellulose content the strength and optical properties can be improved, without affecting the drainage of the pulp considerably. This is most important as an increment of the filler content can contribute to increasing the solids content after wet pressing and thus reduce the energy consumption during drying of paper (Hii et al., 2012).

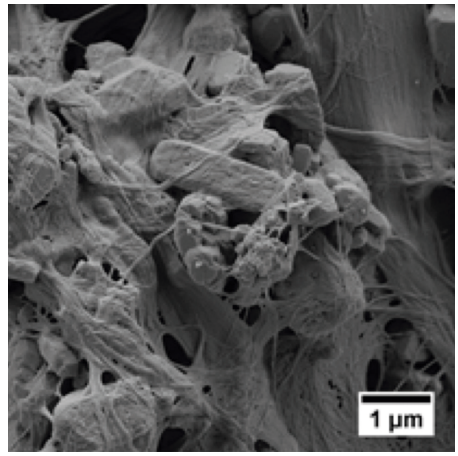

Fig. 2 Cellulose nanofibrils from P. radiata, used as reinforcement in newsprints filled with $CaCO_3$. *Reproduced from Hii et al. (2012).*

Cellulose nanofibrils can form translucent, dense, smooth and strong films (Chinga-Carrasco et al., 2012; Josefsson et al., 2015) with high oxygen barrier properties (Chinga-Carrasco and Syverud, 2012; Fig. 3). However, due to the hygroscopic nature of nanocellulose, the films are affected by humidity, which causes swelling and thus an increment of the oxygen transmission rate (Miettinen et al., 2014).

Interestingly, the hygroscopic properties of nanocellulose can be exploited in biomedical applications, e.g. wound dressings and scaffolds for tissue regenerations (Syverud et al., 2011; Chinga-Carrasco and Syverud, 2014; Rees et al., 2014). Alexandrescu et al. (2013) demonstrated that nanocellulose from *Eucalyptus* and *P. radiata* is not cytotoxic against a 3T3 fibroblast cell lines. Additionally, Nordli et al. (2015) produced for the first time ultrapure nanocellulose that demonstrated to be non-cytotoxic against human fibroblast and keratinocyte cells, thus confirming the suitability of the material for biomedical applications. Importantly, nanocellulose films and hydrogels can hold a significant amount of water, roughly between 2000 and 16000% (Chinga-Carrasco and Syverud, 2014; Fig. 4). This is a major advantage for wound healing management where a moist environment is beneficial to facilitate the healing process (Powell et al., 2013; Powell et al., 2015).

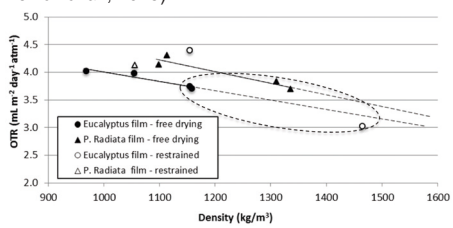

Fig. 3 Oxygen transmission rate (OTR) as a function of density for films with a grammage of 20 g/m2, measured at 50 % relative humidity. The measurements corresponding to the TEMPO pre-treated samples have been encircled. *Reproduced from Chinga-Carrasco and Syverud (2012).*

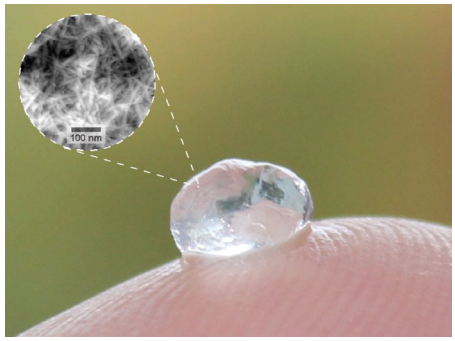

Fig. 4 TEMPO-mediated oxidized nanocellulose hydrogel from *P. radiata*. Inset: AFM image exemplifying the nanofibrillar structure. *Photo: Gary Chinga-Carrasco, PFI.*

Conclusions

Nanocellulose can be produced from a variety of biomass resources. In this work the production of various types of nanocelluloses based on Eucalyptus and *P. radiata* from Chilean plantations has been revised. The nanocelluloses have been extensively characterized from a chemical, structural and biological point of view. Additionally, various potential applications have been explored, including the reinforcement in paper and bionanocomposites, as oxygen barrier for packaging and as a biomaterial for biomedical applications.

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Comparative analysis of commercial cellulases cocktails for the production of nanocrystalline cellulose

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Abstract

Nanocellulose is a biobased nanomaterial, isolated from cellulosic (or lignocellulosic) biomasses, in which one of the dimensions is less than 100 nm. The conventional methods for nanocellulose production from cellulosic pulps involve intensive mechanical treatments, for cellulose nanofibrillated (CNF), or severe acid hydrolysis, for cellulose nanocrystalline (CNC). During the isolation of CNC, the less organized regions of the cellulose are hydrolysed, leaving the highly organized crystalline cellulose. Although it is a relatively simple concept, the strong acid hydrolysis has some disadvantages, i.e. low yield and high amount of water neded to remove the residual acid from the crystals, which imposes challenges to process scale up. As an alternative to this conventional process, a more environmentally friendly, enzyme-catalysed hydrolysis has been recently proposed. The enzymatic-mediated nanocellulose production relies on the higher specificity of endoglucanases to the less organized regions in cellulose fibers, enriching the crystalline fraction. In this work, we compared the action of three commercial cellulases cocktails, Celluclast 1.5L, Cellubrix and Cellic CTec2 with different industrial applications (food industry and biofuels) for CNC production from *Eucaliptus* kraft pulp. Results from reaction kinetics and crystallinity changes during the reaction revealed very different behaviour for each enzyme preparation, and after comparison, it was possible to identify the enzyme preparation that seems most suitable for CNC isolation.

Introduction

Plant cell wall is known to be a strong load-bearing structure, allowing plants to grow vertically, up to several meters. This impressive capacity is a result of the combination of the three main components of cell wall matrix, namely cellulose, hemicelluloses and lignin, and their organization in a hierarchical structure. Of these components, cellulose represents almost 50% of the lignocellulosic materials (on dry weight) and, although the others are essential to maintain both the structure and function of the cell wall, it is the most important strengthening agent (1).

The isolation of cellulose from wood or other lignocellulosic materials, which generally involves chemithermomechanical treatments, followed by a top-down deconstructing strategy, chemical or mechanical, results in the release of nanocellulose, a fibrillar organization of cellulose molecules, in which the width is less than 100 nm (2–5). The interest in nanocelluloses has been increasing over the past few years due to their wide potential applications, including reinforcement agents in composites, drug delivery, cosmetic industry, photonic films and others (6). Basically, two types of nanocellulose can be isolated from lignocellulosic biomass: cellulose nanofibrillated (CNF) and cellulose nanocrystalline (CNC). The differences between them start with the isolation methods, which results in nanomaterials with different physicochemical properties (3).

The production of cellulose nanocrystalline (CNC) involves the breakage of the glycosidic ether bonds of cellulose, achieved with a hydrolysis step (5). During the hydrolysis, the amorphous regions are preferentially hydrolyzed, as the less organized cellulose molecules in these domains leads to higher accessibility to the catalyst (7). As a result, the highly organized crystalline regions are released, material known as cellulose nanocrystalline, or cellulose whiskers. Since cellulosic fibrils are cut transversally during hydrolysis, CNC is shorter in length than NFC (8). Also, due to the severe hydrolysis conditions normally applied, hemicellulosic polymers are solubilized, leading to a high purity nanomaterial (9).

The conventional method for CNC production is through severe hydrolysis with concentrated sulfuric acid (concentration above 60%) (10). However, the search for more cost-effective and environmentally friendly treatments has been the goal of many researchers, and among these treatments, enzymatic hydrolysis is an important possible alternative (11–13). Here we assessed the effect of three different enzyme preparations, Cellubrix, Celluclast and Cellic CTec2 (Novozymes), in the hydrolysis of bleached eucalyptus kraft pulp for production of CNC. We also discuss the properties of the residual solids after the hydrolysis (chemical composition and crystallinity), in order to identify the characteristics of an enzyme mixture suitable for CNC production.

Material and methods

Chemical composition of the pulp

The chemical composition of the bleached eucalyptus kraft pulp was conducted according to NREL analytical procedure (14).

Determination of Endoglucanase activity

The endoglucanase activity in the cellulase mixtures was determined according to Ghose (15).

Enzymatic hydrolysis

The pulp was hydrolyzed with the commercial enzyme mixtures Cellubrix, Celluclast and Cellic CTec2 (Novozymes), keeping the endoglucanase loading fixed (200 EGU / g of pulp). The hydrolysis were conducted in erlenmeyer flasks at 2% solids, 50 ºC, pH 4.8 (50 mM acetate buffer), 180 rpm. After previously determined time points (1 – 72 h), aliquots were withdrawn, heated to 100 ºC for 15 min and centrifuged (4500 x g, 15 min) to separate the solid and liquid fractions. The soluble sugars (glucose, cellobiose and xylose) were analyzed in a Waters HPLC system, as previously described (16).

Crystallinity measurements

Changes in the crystallinity in the residual hydrolysis solids were monitored in a Shimadzu XRD - 6000 diffractometer, at room temperature, with CuKα radiation and graphite monochromator. The measurement conditions were: $10 < 2\theta < 40$; 2θ step: 0.02º, 30s per step. The crystallinity index was calculated according to the Segal method (17).

Results and discussion

An expressive amount of xylan was observed in the pulp (Table 1). This was expected as eucalyptus hemicelluloses are mainly xylans, considerable stable in kraft pulping conditions (18). The lignin content was very low, as kraft pulping aims lignin solubilization.

Table 1: Chemical composition of the pulp

	Pulp components (% on pulp basis)		
	Cellulose	Xylan	Lignin
Bleached Eucalyptus Kraft Pulp	78.6 ± 0.6	14.6 ± 0.1	2.8 ± 0.1

Although the endoglucanase loading was 200 EGU / g of pulp for all the enzyme mixtures tested, the rate (table 2) and yields (table 3) of hydrolysis of cellulose and xylan were very different. As CNCs are high-purity cellulosic products, an ideal scenario involves a more efficient xylan hydrolysis, compared to cellulose. Comparing the hydrolysis rates and yields, Cellubrix seems to be more adequate, as both parameters are higher for xylan hydrolysis. With Celluclast, both the rate and yields are similar, and for Cellic CTec2, cellulose hydrolysis rate is even higher.

Initial hydrolysis rate (%.h^{-1})		
	Cellulose	Xylan
Cellubrix	5.0	6.6
Celluclast	7.1	7.3
CTec 2	10.3	7.6

Table 2: Initial (4 h) hydrolysis rates of cellulose and xylan treating the pulp with the enzyme mixtures

72 h hydrolysis yield (%)		
	Cellulose	Xylan
Cellubrix	59.7	68.9
Celluclast	81.4	83.4
CTec 2	100.0	100.0

Table 3: Cellulose and xylan hydrolysis yields after 72 h enzyme treatment.

The kinetics of hydrolysis was also analyzed (Figure 1). An efficient hydrolysis of the polysaccharides requires more than just endoglucanases. Complex combination of cellulases and accessory enzymes results in a complete depolymerization of cellulose (and hemicelluloses), as observed when hydrolyzing the pulp with Cellic CTec2 (19,20). This efficient depolymerization is the goal of processes that aim monomeric sugar production. Nevertheless, to release CNC at high yield, ideally only the amorphous regions should be hydrolyzed, leaving the nanocrystals intact. Given that approximately 50% of the wood cellulose is crystalline (3), an ideal CNC yield would be close to this value. Considering this, Cellubrix and Celluclast might be enzyme mixtures more suitable to hydrolyze more selectively the amorphous regions (55% and 35% of the cellulose preserved in the solid after 24h hydrolysis, respectively). However, higher yields must be followed by an increase in cellulose crystallinity, indicating this selective hydrolysis.

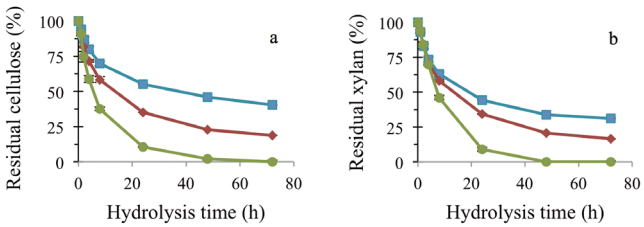

Figure 1: Decrease of cellulose (a) and xylan (b) content in the solid fraction during the enzymatic hydrolysis of the bleached eucalyptus kraft pulp. Hydrolysis conducted at 2% solids, and 200 EGU / g of pulp, using the enzyme mixtures Cellubrix (■); Celluclast (◆); Cellic CTec2 (●).

As mentioned above, efficient hydrolysis of the cellulose is not desired considering the isolation of CNC. Instead, modifications of the physical properties of the residual solid (crystallinity, fiber size) are more important. Increase in crystallinity associated with low hydrolysis yields would be an interesting modification caused by enzymes in the production of nanocrystals. In order to access the most suitable enzyme mixture tested, the amount of cellulose preserved in the solid fraction at maximum crystalline index was compared between the enzyme preparations (Table 4). The maximum CrI value observed in all the conditions tested was 71%, after 4h hydrolysis (increase of 7% in the CrI, comparing with the initial pulp). The percentage of cellulose preserved in the solid fraction at this given CrI was higher after hydrolysis with Cellubrix (80.1%) and lower for Cellic CTec2 (58.7%). It is worth noticing that this value of crystallinity is already in the range of CNCs reported in the literature (21), although nanocelluloses were still not isolated. These results indicate that, under the conditions studied, Cellubrix might be more adequate as catalyst for cellulose nanocrystalline isolation.

Table 4: Percentage of celulose preserved in the solid fraction at maximum crystallinity index after hydrolysis with the enzyme mixtures.

	Maximum CrI (%)	Residual cellulose (%)
Cellubrix	71	80.1
Celluclast	71	71.6
CTec2	71	58.7

Conclusions

Although endoglucanases are the most important enzymes for CNC production, other components also are important, as they act synergistically. However, an ideal proportion should be achieved, as complete hydrolysis is not desired in this process. From the hydrolysis data, it was possible to observe that the kinetics are very different between the enzyme mixtures, even keeping the endoglucanase loading the same, as a reflection of the presence of other enzymes in distinct proportions. Measurement of the crystallinity index indicated that treatment of the pulp with Cellubrix resulted in increase of crystallinity, with minimum cellulose hydrolysis, compared with other enzyme mixtures. Other physical characterization data (fiber size and scanning electron microscopy) will provide more information about the modifications in the fiber structures.

Acknowledgement

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Biocomposites from microfibrillated cellulose and biodegradable polymers

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Abstract
Interest in waste sources arises from the biomass left behind which tends to be an environmental problem as it has strong fibers which breakdown very slowly under natural conditions. For this reason fibers from crops and especially from agricultural byproducts, are likely to be more suitable for preparation of polymeric composites. In order to enhance reinforcing effect of these fibers, higher aspect ratio could be promoted by means of applying an intensive mechanical process to cellulose isolated from vegetable fibers in order to produce microfibrillated cellulose (MFC).
This work studies the feasibility of the use of agricultural byproducts as source for microfibrillatedcellulose production by meansof applying an intensive mechanical process as microfluidization. Husks from different sources were grinded and chemically treated in order to isolate cellulose from non-cellulosic components prior to microfluidization process.
Formulations of MFC with different plasticizers were prepared in order to obtain composite materials using biopolymer matrixes. Polyethylenglycol (PEG 400) and acetyl tributyl citrate (ATBC) were tested as plasticizers. PEG 400 was able to preserve cellulose fibrils free-associated, whereas ATBC yield poor MFC fibrillated structures. Composites films from PHB and MFC, were formulated using PEG 400. MFC-PEG 400 produced an increase in Young modulus and a increase in water vapour barrier properties.

Keywords: Agricultural byproducts, Microfibrillated Cellulose, MFC, biocomposites.

Introduction

Cellulose is the most abundant biopolymer material produced by nature, as is widely distributed in higher plants. In general, cellulose is a fibrous, tough, water-insoluble substance that plays an essential role in maintaining the structure of plant cell walls. Cellulose does not occur as an isolated individual molecule, but it is found as assemblies of individual cellulose. These structure formed by approximately 36 molecules[1] are known as elementary fibrils, which in turn are packed into larger units called microfibrils.The cellulose microfibrils are associated via hemicellulose, another polysaccharide, to form a cellulose-hemicellulose network, which is embedded in a pectin matrix. However, diverse cellulose packing may occur depending on its source.Cellulose microfibrils, with high aspect ratio structure, exhibit all the qualities required to be ideal candidates as reinforcements in polymeric composites.. Fibers from crops andespecially from agricultural byproducts, are likely to be more suitable for preparation ofmore environmental friendly polymer composites.

In order to obtain these cellulose microfibrils, first stage consists on isolating cellulose from its lignin and hemicellulose matrix from the vegetable fibers. To achieve this, a pulping process as used on paper industry must be carriedout. The aim of pulping is to break down the bulk structure of the fiber source into the constituent fibers. There are different pulping technologies available. The chemical and thermal treatments reduce the amount of energy subsequently required by the mechanical treatment, and also reduce the amount of strength loss suffered by the fibers providing long, strong and stable fibers [2].

On chemical pulping, sodium chlorite is frequently used to delignify wood as an initial step in the isolation of cellulose. However, environmental concerns associated with chlorinating agents have led to the increased use of more environmentally benign agents for delignification such as hydrogen peroxide or an acetic acid–nitric acid mixture in both elemental chlorine-free (ECF) and totally chlorine-free (TCF) isolation sequences [2,3].

The cellulose isolated from vegetable fibers requires intensive mechanical treatment to produce microfibrillated cellulose (MFC).The aim of applying fibrillation process onto isolated cellulose fibers is to increase fibers aspect ratio to enhance its reinforcing effect on a polymeric matrix. Methods for producing MFC were first reported by Herrick [4] and Turbak [5].The term "microfibrillated cellulose" (MFC), should not be confused with the term "microfibril" since MFC usually consists of aggregates of cellulose microfibrils. Thus, MFC features a lower aspect ratio L/d (100-150) than microfibrils (>1000) [6].

This work studies the feasibility of the use of an agricultural byproduct, like rice husks, as a source for microfibrillated cellulose production, and its use in biodegradable polymeric composites. In order to preserve the microfibrillated structure achieved, water employed in microfluidization process was exchanged for a plasticizer suitable for traditional polymer composite processing. Different plasticizers were tested.

Methodology

Materials

Rice husk were kindly provided by EWAR Argentina S.A, La Plata, Argentina. α-cellulose was purchased from Sigma (C8002). Poly(hydroxybutyrate) (PHB) was provided by TianAn (PHB ENMAT Y1000) All chemicals employed were reagent grade.

Isolation of Cellulose from Rice Husks

Rice husks were grinded and sieved to mesh 40. In order to remove non cellulosic components from rice husks,an alkaline treatment was applied (5 % sodium hydroxide, 50oC), after which an oxidizing treatment was performed (2% hydrogen peroxide, pH 11, 50oC). Then isolated cellulose was grinded and sieved.

Preparation of Microfibrillated Cellulose

Cellulose isolated from rice husks were processed as 1% in weight dispersion in water using a Microfluidizer M- 110P(Microfluidics Corp) at 1500 bar. For comparison purposes pure α-cellulose was microfibrillated in the same conditions.

Fibers Characterization

After each treatment, fibers were characterized by:

Compositional Analysis. Sequential method of neutral detergent fiber (NDF), acid detergent (ADF), lignin (ADL) according to Goering and Van Soest[7] was performed. Each cell wall component is digested at a different stage of treatment protocol. Components are determined as:

- Insoluble Fiber in ash free neutral detergent with alpha amylase (NDF) (*) Insoluble Fiber in ash free acid detergent (ADF) (**)
- Lignin in ash free acid detergent (ADL) (*)
- Hemicellulose (NDF-ADF) (**)
- Cellulose (ADF-ADL) (**)

(*) Directly measured
(**) Indirectly determined (by difference)

Scanning Electronic Microscopy (SEM). SEM micrographs of untreated and treated fibers were taken using a scanning electron microscope Field Emission Gun (FEI Quanta 250 FEG).

*Atomic force microscopy(AFM)*Topographyc images were taken using a Surface Imaging System AFM,in non contact mode, with silicon tips coated with Pt/Ir. MFC water suspension (1/100 diluted from 1% MFC dispersion) were placed in mica covers and dried at 60°C, 20 min .

Preparation of Poly(hydroxybutyrate) Composites

A fixed proportion of 10% of polyethylene glycol 400 (PEG400) was used to introduce 1% of MFC into PHB. Compounding was produced in a batch mixer (BrabenderPlasticorder), at 175 °C, for 10 min at 50 rpm. Then films of PHB with 10 % in weight of PEG400 were modified with 1% of MFC from rice husks. For comparison purposes, films with 1 % of MFC form α-cellulose were also prepared.

Composites Characterization

Prior to any characterization samples were conditioned 7 days to PHB composites at 25oC and 53% RH. Mechanical tensile properties of the films were evaluated using a universal testing machine INSTRON 5569A following the guidelines of ASTM D638. The water vapor permeability (WVP) was determined according to guidelines of ASTM E96 (Method A).

Results and discussion

Isolation of Cellulose

Results of fiber composition by Comparative Sequential method for each step of the chemical treatmentare shown in Figure 2for rice husks. Cellulose content increasedfrom about 40 to above 85 % as the purification process proceeded.

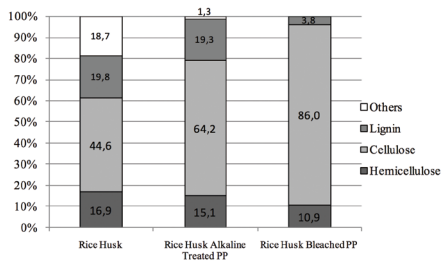

Figure 1.Comparative composition of rice husksfibers after each chemical treatment step

Microfibrillation Process

Microfibrillationof each type of fiber was noticeable as an increase in suspension viscosity, milk-like slurry aspect and no noticeable flocculation during storage. SEM micrographs of MFC from rice husks (Figure 2) and α- cellulose (Figure 3) showed the microfibrillatedstructure achieved. Allmicrofibrillatedproductshad wider aspect ratio distribution than isolated cellulose mainly due to much smallerdiameterachieved,ranging from 20 to 100 nm and several micrometers in length.

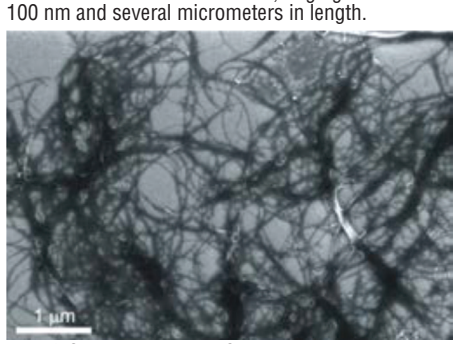

Figure 2. SEM micrographs of MFC fromrice husk

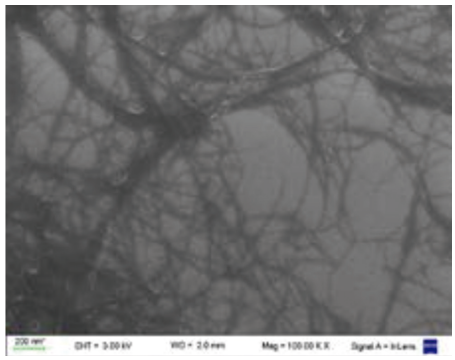

Figure 3. SEM micrographs of MFC from α-cellulose

Incorporation of MFC into Polymeric Matrix

In order to incorporate MFC to PHB during processing, MFC water suspensions undergo simple solvent evaporation and freeze drying treatment. In both cases (Figure 4) cellulose fibrils free-associate, losing the high length/diameter ratio achieved during the microfibrillation process. Inter and intra molecular interactions among cellulose fibrils are strong (hydrogen bonding and dipolar interactions) which cannot be overcome during composite processing by the molten viscous PHB matrix.

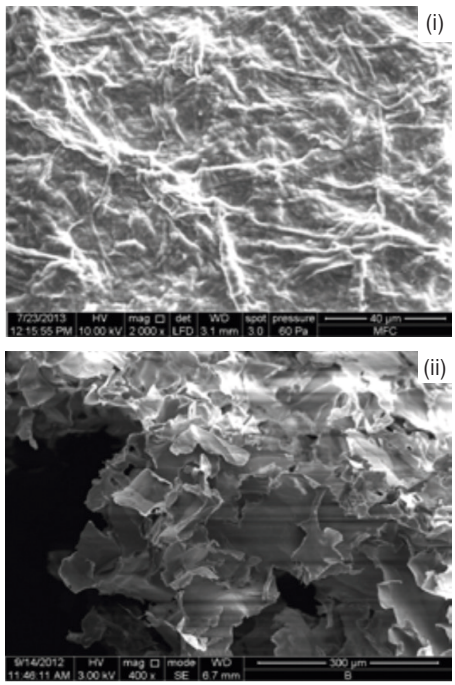

Figure 4. (i) MFC dried by evaporation at 40oC and (ii) MFC dried by freeze-drying

As an alternative to formulate PHB-MFC composite materials, solvent exchange strategy was evaluated. Exchange-solvent requires eliminating water from the system preserving MFC structure. Water could be replaced with another compound which must keep the MFC structure and be compatible with PHB formulation and processing; also MFC concentration could be raised. PHB plasticizers appeared as a good option. Polyethylenglycol (PEG 400) and Acetyl Tributyl Citrate (ATBC) were tested as exchange compounds. After solvent exchange, MFC-PEG gels (8.4 and 1.3 % w/w MFC in PEG) were obtained, but in ATCB no good MFC dispersion was found, as shown in Figure 5 and 6. MFC-PEG gels (Figure 6)were evaluated by SEM in order to observe if the fibers preserved the microfibrillated structure (Figure 7).

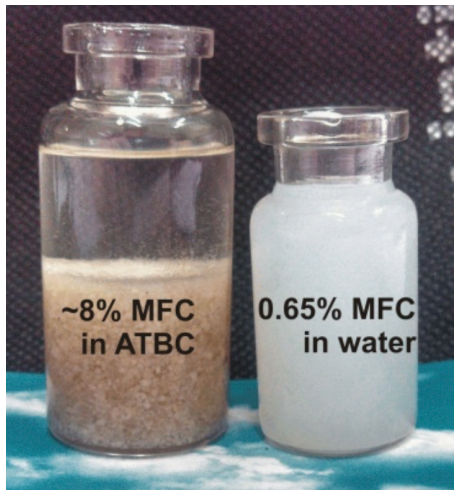

Figure 5.Microfibrillated Cellulose in ATBC and Water

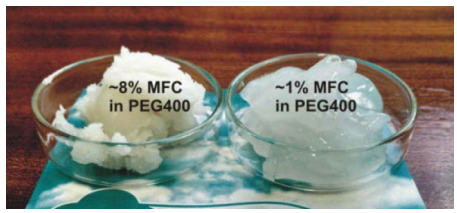

Figure 6.Microfibrillated cellulose in PEG400

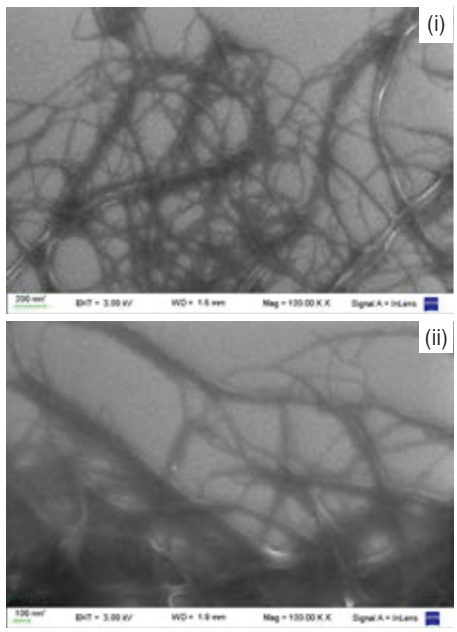

Figure 7.Microfibrillated Cellulose (i) 1.3% and (ii) 8.4% in PEG400

Composites

Once preservation of the microfibrillated structure of the fiber on the PEG400-MFC was certain, PHB-MFC/PEG 400 composites were formulated. Figure 8 and 9show the influence of MFC, on PHB composite films on water vapor permeability and mechanical properties, respectively.

Composite properties are visibly affected by the incorporation of 1% of microfibrillated cellulose, acting not only as a reinforcement agent strengthening the PHB/PEG400 matrix, but also reducing its water vapor permeability.

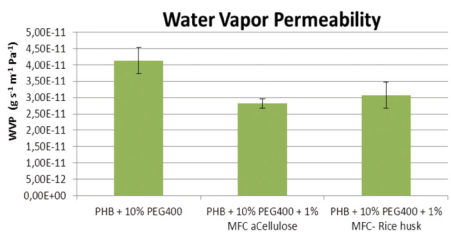

Figure 8.WaterVaporPermeability for PHB-PEG/ MFC composite

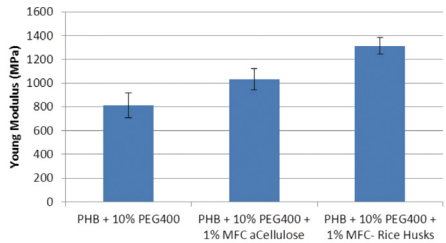

Figure 9.Modulus for PHB-PEG/ MFC composite films

Conclusions

We succeeded in obtain microfibrillated cellulose (MFC) from an agricultural byproductas rice husks, after chemical treatments. Chemical treatments promoted adrastically reduction of non cellulosic components of fibers, tear individual fibers from fiber bundle, and increase surface roughness of fibers. Cellulose yield after chemical treatment procedure was high, above 85%.

Microfibrillated structure was preserved after MFC water suspensions were solvent exchange to PEG 400. ATBC, a plasticizer also used in PHB formulations, seemed not polar enough to maintain high aspect ratio structure in MFC. Solvent exchange appears as a suitable alternative to produce composites using MFC without significant loss of the high aspect ratio structure.

Composites films from PHB and MFC obtained by solvent exchange procedure, using PEG 400, a common PHB plasticizer, showed an increase in water vapor barrier properties and increase in Young modulus, when compare with PHB-PEG 400 films, obtained by thermocompressionmoulding

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Cellulose nanofibrils from agro-industrial waste: Production and characterization

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Abstract

Agro-industrial waste (AIW) represents an important underutilized resource, which has potential to be converted into high-value materials. Cellulose nanofibrils (CNF) is a biobased material usually derived from wood sources. However, the production of CNF from non-wood sources, such as AIW, has been gaining attention in recent years. The aim of this study was thus to assess the suitability of five AIWs (corn husks, corn stover, olive stone, vine shoots, oat husks) as a source for the production of CNF. Firstly, a comprehensive characterization was performed, including TAPPI methodology and experimental procedures of Wise and Rowell, to quantify the content of lignin, hollocelulose, α-cellulose, hemicelluloses, ashes, extractives and moisture. The characterization of the AIW showed differences between the assessed materials with respect to the carbohydrate composition. The AIW with the highest content of cellulose was corn husks. In addition, corn husk had a low content of extractives and ashes, which facilitated the processing of this pulp. Secondly, based on the characterization and local availability it was found that corn husks were most appropriate for production of CNF. Chemical and mechanical procedures for production of CNF from corn husks were thus performed. The chemical pre-treatment was a 2,2,6,6-tetramethylpiperidinyl-1-oxyl (TEMPO)-mediated oxidation. The mechanical treatment was performed with a high-pressure homogenizer. Finally, CNF films were manufactured and a comprehensive characterization was performed to reveal the morphology of the fibrillated material. The applied characterization techniques included mechanical testing of films, laser profilometry and scanning electron microscopy (SEM). The results from this study reveal a potential of corn husk as an underutilized resource for the production of CNF, which have a range of valuable applications.

Introduction

Wood is the most important industrial source of CNF production. However, the search of new sources of fibers such as non-wood sources are likely to be of increasing interest: they offer environmental benefits owing to their annual renewable nature and their low energy consumption in production [1]. The CNF from non-wood sources are relatively easy to separate from the primary wall [2], hence demanding less energy during the fibrillation of this pulp. As a matter of fact, compared with wood, non-wood sources have generally lower lignin contents, shorter growing cycles with moderate irrigation requirements, annual renewability and a high annual yield of cellulose.

Non-wood sources are mainly produced as waste from agro-industrial activity, which are either burned, or used for low-value products as animal feed. Generally, for every ton of cereal production worldwide, about 1,5 tons of waste could be obtained as a by-product [3]. World production of cereals exceeds 1,000 million tons per annum, which means about 1,500 million tons of cereal straw are produced every year that could be used as raw material for the development of high value products [3]. As an example, the production of corn was 1,437,560 ton per year (2011-INE) in Chile, which is comparable with wheat production [4]. Therefore, waste from corn harvesting (cobs, stems and leaves) could be an interesting alternative as raw material for the production of high value-added products as CNF.

The CNF are a novel material concept derived from plant resources. Besides, CNF are highly crystalline, which provides extremely high mechanical properties. Having a great aspect ratio, a large specific surface area with reactive OH-groups, the CNF are proposed as interesting material for several applications nanocomposite applications, reinforcement in biomaterials, as emulsifiers, encapsulation [5,6,7], emulsion stabilizer of food, paint and cosmetics [8,9]. Recently, applications as strength enhancer in paper and composite materials have been explored [10,11,12]. The CNF seem to be the most satisfactory for barrier applications in novel packaging concepts [13,14,15]. In addition, novel applications have been foreseen within medicine, where CNF may be applied as scaffolds for tissue or bone [16], and as adsorbents for different contaminants in water and air.

Agro-industrial waste shows great potential as an alternative to wood sources for the production of high value-added products. The chemical modification of CNF from agro-industrial waste is a quite attractive research area since it has been little explored presenting interesting challenges in terms of developing and optimizing efficient methodologies, in order to convert these sources into a real alternative to wood for the production of high added value products. Therefore, the main objective of this study is to develop CO_2 adsorbent material from agro-industrial waste CNF.

Experimental

Activity 1.1 Agro-industrial waste screening

The agro-industrials waste under study were supplied by both the Center for Advanced Polymers (CIPA) located in Coronel, Chile and Processed Food Center(CEAP) , located in Talca, Chile. The agro-industrial waste studied were :corn husks, olive stone, grape branches, alperujo and oat husks.

Activity 1.2. Agro-industrial waste characterization

The agro-industrials waste were characterized through standardized procedures (Tappi standards) and instrumental techniques to determine moisture, removable, ashes, lignin, cellulose and hemicelluloses.

The CNF isolation

After the chemical characterization of raw material by Tappi standars was tested and chose chemical fractionation processes that allowed the production of a high quality cellulose pulp. Alkaline-acid hydrolysis (2.1), basic (2.2) and organosolved (2.3) treatment were tested. Experiments were performed in triplicate. It was selected alkaline-acid hydrolysis treatment to get cellulose from the selected agro-industrials waste.

Activity 2.1 alkaline-acid hydrolysis to obtain a purified cellulose from agro-industrial waste

The Selected agricultural waste were treated with a solution 0.1 N NaOH under mechanical agitation at 30 ° C for 18 h. The residue obtained was treated with 0.1 N HNO_3 at 85° C for 1 h to remove mineral traces. Subsequently, the insoluble residue was treated with a solution 3% H_2O_2 at 70° C for 1 h in order to remove the lignin. At the end of each stage, the waste was filtered and washed with distilled water for several hours until reaching a neutral pH. Experiments were performed in triplicate.

Several treatments of TEMPO-mediated oxidation for cellulose obtained at the stage of chemical fractionation were tested, parameters such as temperature, reaction time, reagents dose, pH, reagent concentration, were modified. It was selected TEMPO-mediated oxidation is described below.

Activity 2.4 2,2,6,6-tetramethylpiperidinyl-1-oxyl (TEMPO 3)-mediated oxidation of cellulose

The never-dried cellulose (5 g) was suspended in water (300 mL) containing TEMPO (0.050 g) and sodium bromide (0.50 g). The TEMPO-mediated oxidation of the cellulose slurry was started by adding various amounts of 14% NaClO and conducted at room temperature under gentle agitation. The pH was maintained at 10.5 by adding 0.5 M NaOH. When no longer pH decrease was observed, we considered that the reaction was finished and adjusted pH 7 by adding 0.5 M HCl. The TEMPO-oxidized product was thoroughly washed with water by filtration and stored at 4 °C before further treatment or analysis.

As cellulose fibrillation requires intensive mechanical treatment, after pretreatment, we used homogenizer, sonicator and electrospinning to break down the fibers of cellulose into nanofibrils. Protocols reported in the literature are used.

Activity 2.5 Mechanical treatments to obtain CNF by Homogenizer

The mechanical treatments to obtain the CNF were performed as follows: Fibers of different chemical treatments, are dispersed in distilled water to 1-2% concentration of and were homogenized, using the following protocols:

HPH 1: High pressure Homogenizer, 5 steps at 300 bar and 10 steps at 400 bar.

HPH 2: High pressure Homogenizer, 1 step at 1000 bar.

HPH 3: High pressure Homogenizer, 5 steps at 500 bar and 7 steps at 1000 bar.

Activity 2.6 The CNF films preparation

Petri dishes (d=8.4 cm)
Film diameter: 8,4 cm (or 0.084 m)
Film area: $A = \pi \times r^2$
Basis weight: $G = 20$ g/m²
Dry matter-content: DC = 1%

After this quick calculation and when the suspensions are ready, we are able to make films.

Add the right sample weight according to the targeted basis weight and the area of the film. The important point is to avoid bubbles inside the CNF solution and also ensure a good mixing of the suspension. The CNF suspension is delicately poured into the Petri dish. Drying takes approx. 3-5 days at room temperature (without the petri dish cover).

Activity 2.7 The CNF Characterization

The CNF films were manufactured and a comprehensive characterization was performed to reveal the morphology of the fibrillated material.

The applied characterization techniques included: particle size analysis (nano-zetasizer), scanning confocal microscopy (CLSM), and the CNF morphology was examined by scanning electron microscopy (VP-SEM,SU 3500 Hitachi direct analysis of sample under a microscope).
At a further characterization stage, mechanical testing of films, laser profilometry and analysis of CNF morphology will be performed trough PFI cooperation.

Results and discussion

Table 1 presents the results obtained through characterization of raw materials by using Tappi methodology of five agro-industrial waste: corn husks, olive stone, alperujo, grape branches and oat husks. For raw materials characterization were evaluated their content of lignin, hollocelulose, α-cellulose, hemicelluloses, ashes, removable and moisture. In this Table, raw materials with the highest content of cellulose , therefore, selected for the next stage, focused on obtaining CNF are corn husks. In addition, this raw material had a low content of both removable and lignin, indicating that it is an interesting raw material. However, oat husks are also shaped up as a good candidate to obtain cellulose.

Table 1: Agro-industrial waste characterization.

	Raw Materials				
	Corn husks	Alperujo	Olive stone	Grape branches	Oat husks
Moisture [%]	8.73 +/- 0.26	5.27 +/- 0.40	10.72 +/- 0.07	5.21 +/- 0.50	7.32 +/- 0.35
Ashes [%]	6.40 +/- 0.01	4.49+/- 0.32	5.89 +/- 0.07	3.09 +/- 0.10	6.39 +/- 0.03
Removable [%]	16.50 +/- 1.94	32.24 +/- 1.80	22.59 +/- 2.32	14.67 +/- 0.67	11.41 +/- 0.45
Lignin [%]	16.50 +/- 1.95	20.09 +/- 2.09	28.65 +/- 1.18	24.17 +/- 1.84	10.11 +/- 1.31
Holocellulose [%]	68.30 +/- 1.24	67.94+/- 1.94	72.09 +/- 2.10	79.01 +/- 2.33	74.07 +/- 0.17
Cellulose [%]	48.68 +/- 1.78	20.85 +/- 1.29	25.09 +/+ 2.01	32.78 +/- 2.78	38.72 +/- 1.83
Hemicelluloses [%]	18.11 +/- 1.99	44.98 +/- 1.84	47.00 +/- 2.19	46.23 +/-2.11	35.35 +/- 1.94

The results of the fractionation stage of selected raw materials are presented in Table 2. The three treatments of chemical fractionation evaluated for selected agro-industrial waste (corn husks and oat husks), consisted of an initial solubilization of hemicelluloses fraction (alkali soluble), followed by a delignification treatment to remove lignin, to finally to obtain a insoluble cellulose fraction until the end of fractionation. Alkaline-acid hydrolysis, soda and organosolved treatments focused on obtaining a purified cellulose pulp were evaluated. **Table 2** shows the results of the determination of the most appropriate operating conditions of chemical fractionation of agro-industrial waste for obtaining a purified cellulose pulp. Both corn husks and oat husks, soda and organosolved treatments yielded between 80 and 70% of cellulose, agreeing with reported in literature. However, the alkaline-acid hydrolysis treatment showed a higher yield, 95 and 83% cellulose, from corn husks and oat husks, respectively. Therefore, this method was more appropriate for the type of studied raw materials. In terms of the purity of the obtained cellulose pulp, the FTIR Spectra showed that both, alkaline-acid hydrolysis and soda treatments, were better than organosolved treatment. The alkaline-acid hydrolysis pretreatment was chosen because of the high yields and purity of the obtained cellulose.

Table 2: Conditions of operation at chemical fractionation stage and pulp yield.

Chemical fractionation method	Temperature [°C]	Reaction time [h]	Reagent amount	Yield [%] [1]	Cellulose purity
Alkaline-acid hydrolysis	30	18	NaOH 0.5 M; NaOH 0.5 M H_2O_2 3%; HCl 2M	CH:95; OH: 83	CH: High OH: High
Soda treatment	Room	14	KOH 5%; $NaClO_2$ 1%; HCl 1%	CH:84; OH: 70	CH: Medium OH: Medium
Organosolved treatment	165	10	NaOH 0.5 M; $NaClO_2$ 1.5% CH_3CH_2OH 60%	CH:81; OH:70	CH: Medium OH :Medium

[1] *based on cellulose content determined according to Tappi methodology.*

CH: corn husks

OH: oat husks

Were performed Within this contex to the study and application of different pre-treatments (to oxidize cellulose) and treatments for obtaining CNF. Chemical pretreatment applied to the cellulose obtained by different methods of chemical fractionation, consisted of an mediated-oxidation with TEMPO (2,2,6,6-tetrametilpiperidinil-1-oxyl) according to the Protocol described in this document. Mechanical treatments used to produce CNF were high pressure (HPH), Sonicator and electrospinning.
Once the chemical and mechanical treatments were applied to obtain CNF, the fibrils obtained were characterized using the following instrumental techniques: structural analysis of fibrils using confocal scanning microscopy (CLSM) and scanning electron microscopy (SEM).
The results obtained by applying the oxidation and mechanical treatments to cellulose obtained in previous stages, as well as the Structural characterization of the CNF, are shown below in **Table 3**.

The analysis of the studied cases indicates the following:
Cases 1,2 and 3: Applied TEMPO protocols failed to oxidize cellulose (absence of the band stretching feature around 1600 cm-1 Carbonyl group). The applied homogenization treatments failed to obtain CNF (inner diameter equal to or less than 100 nm), as evidenced by the results obtained by CLSM or SEM. According to the inner diameter measured by both techniques, these fibrils would correspond to residual fibrils, which are also components of a given CNF quality.

Case 4: The TEMPO 3 Protocol was able to oxidize cellulose from CH of treatment 1 (presence of the band stretching feature around 1600 cm-1 Carbonyl group). With the homogenization treatment HPH 3, but cannot get CNF yet, the results indicate that there is a real approach to their production, as evidenced by the characterizations of them by SEM.

Cases 5,6,7 and 8: Applied TEMPO protocols failed to oxidize cellulose. The applied homogenization treatments failed to obtain CNF, as evidenced by the results obtained by CLSM or SEM. According to the inner diameter measured by both techniques, these fibrils would correspond to residual fibrils, which are also components of a given CNF quality.

Case 9: The TEMPO 3 Protocol was able to oxidize cellulose from OH of treatment 1 (presence of the band stretching feature around 1600 cm-1 Carbonyl group). The homogenization treatment HPH 3, but cannot get CNF yet, the results indicate that there is a real approach to their production, as evidenced by the characterizations of them by SEM.

Cases 10 y 11: Applied TEMPO protocols failed to oxidize cellulose. The applied homogenization treatments failed to obtain CNF, as evidenced by the results obtained by CLSM or SEM. According to the inner diameter measured by both techniques, these fibrils would correspond to residual fibrils, which are also components of a given CNF quality.
On the other hand, only oxidation of cellulose was achieved with the TEMPO 3 Protocol, as it checked for cases 4 and 9. The oxidation of cellulose is necessary because, the oxidation of the hydroxyl groups of cellulose to carboxylate groups generate interfibrilar repulsive forces between these groups, due to their bulk (steric hindrance) which facilitates the fibrillation of cellulose when it is subjected to mechanical treatment in the Homogenizer.
Corn husk cellulose fibrils (CH) from treatment 2.1, oxidized according to Protocol 3 TEMPO and were subjected to mechanical treatment in HPH 3, showed an inner diameter between 300 and 1000 nm, according to results obtained by scanning electron microscopy (SEM). These dimensions are near those reported in the literature (Φi MFC≤100 nm).

Table 3: Cellulose fibrils Structural characterization

Case	RM	Fractionation method (yield)	Chemical pre-treatment	Mechanical treatment	Φi (nm) / CLSM	Φi (nm) / SEM	Φi (nm) / SEM
1	CH	2.1 (89%)	TEMPO 1	HPH 1	10000	5000-10000	
2	CH	2.1 (89%)	TEMPO 2	Sonicator 1	10000	10000	
3	CH	2.1 (89%)	TEMPO 2	Sonicator 2			
4	CH	2.1 (89%)	TEMPO 3	HPH 3			300-1000
5	CH	2.2 (80%)	TEMPO 2	HPH 1	10000	5000	
6	CH	2.3 (77%)	TEMPO 2	HPH 2	10000	10000	
7	OH	2.1 (82%)	TEMPO 1	HPH 1	10000	5000-10000	
8	OH	2.1 (82%)	TEMPO 2	Sonicator 3	10000		
9	OH	2.1 (82%)	TEMPO 3	HPH 3		1000	
10	OH	2.2 (79%)	TEMPO 2	HPH 2	10000	15000	
11	OH	2.3 (74%)	TEMPO 2	HPH 1	10000	10000	

Conclusions

1. Cellulose was obtained from agro-industrial waste by the selection of the Alkaline-acid hydrolysis a fractionation method, which was the most suitable in terms of yield, purity of obtained cellulose. Also compared with the others, it was the more easy to implement in terms of time spent.

2. Oxidation treatment of cellulose was implemented with TEMPO 3 protocol. This treatment was the only one who was able to oxidize hydroxyl groups of cellulose to carboxylate groups, therefore the mechanical treatment used later, managed to get CNF with steps and moderate pressures.

3. The mechanical treatment was implemented to obtain CNF by High pressure Homogenizer, using steps and moderate pressures. Sonication treatment was not able by it self to produce CNF. Electrospinning treatment was not successful to produce CNF, due to the low viscosity of oxidized cellulose suspension.

4. The fibrils characterization performed , would indicate theirs would correspond to residual fibers that are also components of a given quality of CNF.

5. It is necessary to submit CNF a comprehensive characterization (in developing to PFI, Norway), since probably we are observing only residual fibers in SEM, and by lack of resolution cannot see CNF.

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Biomass valorisation by heterogeneous catalysis: Ethylene glycol production via hydrogenolysis of cellulose using Pd-WXC/C catalyst

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Abstract

Cellulose has a great potential to be converted into biofuels and value-added chemicals using heterogeneous catalysts. This chemical route presents economic advantages such as the design and control of the physical and chemical properties of the catalyst, as well as the reaction parameters to maximize conversion and selectivity to a desired chemical product. Tungsten carbide catalysts are active in the hydrogenation and hydrogenolysis reactions of cellulose by breaking C-C bonds and decreasing the degree of oxidation. Thus, this work investigated the catalytic properties of a catalyst based on tungsten carbide promoted with 2 wt.% of Pd and supported on activated carbon into cellulose (Avicel) conversion reactions. The reactions were conducted in a 300 mL batch reactor with 800 psi of H_2 pressure, 1000 rpm, time varying from 30 to 120 min and temperature from 190 to 250ºC. After 120 min of reaction, 25% of cellulose was converted at 190ºC, 77% at 220ºC, and 100% at 250ºC. Ethylene glycol yield and selectivity reached 40% and 52%, respectively, at 220ºC. It is worth noticing that at 250ºC, 100% of cellulose was converted. On the other hand, the selectivity to ethylene glycol decreased, leading to several by-products such as levulinic acid from the dehydration of glucose. Acetol and 1,2 – propylene glycol were produced by parallel reactions.

Introduction

The production of fuels and chemicals from sustainable and renewable sources is one of the great challenges facing science over the coming decades due to the environmental damage and risks that are inherent to fossil fuel exploration, refining and processing [1-3]. Thus, biomass has the potential to make a positive contribution to supplying future energy and chemical demands in an environmentally friendly and sustainable manner. Cellulose is the major component of lignocellulosic biomass and the most abundant biopolymer on earth, comprising long-chains of D-glucopyranose linked by β-1,4-glycosidic bonds. This polysaccharide has a great potential to be converted into biofuels and value-added chemicals using heterogeneous catalysts, which is one of the most promising and challenging chemical methods of producing biofuels and chemicals today [4]. Moreover, it has important advantages such as easiness in separation between products from catalyst, control of the physical and chemical properties of the catalyst, as well as the reaction parameters to maximize conversion and selectivity of a desired chemical product.

The first step of cellulose conversion is the hydrolysis of the polysaccharide in order to release the glucose units. Thereafter, the monosaccharide can be transformed in different chemical routes, which depends on the catalyst, reaction media and catalytic reaction parameters [1, 4-6]. Once glucose is formed through cellulose hydrolysis, it can be readily transformed into sugar alcohols by the reduction of glucose carbonyl group with H_2 on the metal active site of the catalyst, leading to formation of hydrogenated products such as sorbitol [7, 8]. The production of sugar alcohols pass through the hydrogenation/hydrogenolysis of the glucose monomer, and is commonly catalysed by supported metallic catalysts, such as Ru/C and Pt/C, which are able to produce C_6 and C_4 sugar alcohols. But, the high cost of these noble metals is a barrier to industrial applications.

Tungsten carbide catalysts are active in the hydrogenation and hydrogenolysis reactions of cellulose, breaking C-C bonds and decreasing the degree of oxidation of the glucose monomers in a called one-pot reaction [9]. Therefore, this compound comes to be a cheaper alternative to replace the noble metals catalysts in this kind of reactions. Thus, this work aims investigate the catalytic properties of catalyst based on tungsten carbide promoted with 2 wt.% of Pd and supported on activated carbon into cellulose conversion reactions.

Experimental

Catalyst Preparation

The tungsten precursor impregnation on activated carbon and after the $PdCl_2$ (Sigma-Aldrich) impregnation were carried out according to a procedure reported at literature [10]. Due to the low solubility of the $PdCl_2$, first it was dissolved in hot HCl (A.R. grade – 600C) under magnetic stirring. Second, the solution was evaporated and water was added, stirred for 5min and also evaporated to remove the chlorine excess. Then, the solution was prepared at room temperature and impregnated into the W/C sample to obtain 2 wt.% of Pd in the final composition.

The carburization process were carried under a total flow of 100 mL.min^{-1} of a mixture of CH_4 (10% v/v) and H (90% v/v) from room temperature up to 850ºC with a heating rate of the 8ºC min^{-1} and kept in the final temperature for 40 min. The methane flow was stopped after 20 min of the isothermal step but the samples remained under H_2 flow during the final 20 min and cooled down to room temperature. The catalysts were then passivated under a 1% (v/v) O_2/He flow (50 mL min^{-1}) overnight before exposure to the atmosphere. The gas phase composition at the exhaust of the reactor was continuously monitored using a quadrupole mass spectrometer (Pfeifer GDS 320). The samples were coded as WXC/C (BET surface area: 709 m2 g^{-1}), with no Pd, and Pd-WXC/C (BET surface area: 366 m^2 g^{-1}), promoted with 2wt.% of Pd.

Cellulose Conversion Reactions

The cellulose (avicel) conversion reactions were conducted in a 300 mL Parr batch reactor under 800 psi of H_2 pressure, 1000 rpm, time varying from 0 to 120 min and temperature from 190ºC to 250ºC. The products were analysed by GC and HPLC and the cellulose conversion were determined by TGA. The products yield was calculated based on the amount of carbon in products and in initial cellulose. For cellulose conversion calculation, the residua mass of cellulose mixed with the solid catalyst after catalytic reaction was determined through thermogravimetric analysis under oxidising atmosphere [11].

Results and Discussion

Catalytic Reactions

WxC/C

Firstly, the catalytic performance of the catalyst in absence of palladium (WxC/C) was conducted as a function of time at 220ºC and 800 psi of H2 pressure. Figure 1 shows that the conversion increased from ≈60% to 93% when the reaction time increased from 30 to 150 min. The major identified product was acetol, achieving a yield close to 23%. Ethylene glycol (EG) yield was almost neglected.

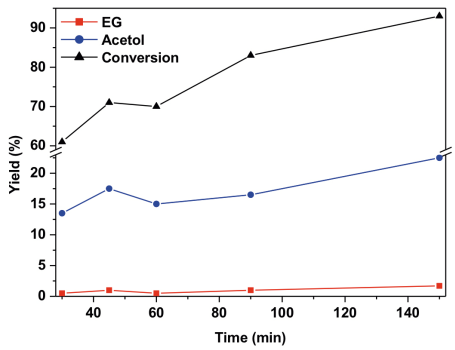

Figure 1 - Catalytic performance of WXC/C in cellulose conversion at 220oC.

Pd-WxC/C – Temperature and Time Effects on EG Production

The Pd-promoted catalyst (Pd-WxC/C) was applied in the cellulose hydrogenolysis reactions at 190ºC, 220ºC and 250ºC. Figure 2 shows the results of the cellulose conversion (right axis) and products yield (vertical bars) of the major identified products (EG, acetol and 1,2 – propylene glycol).

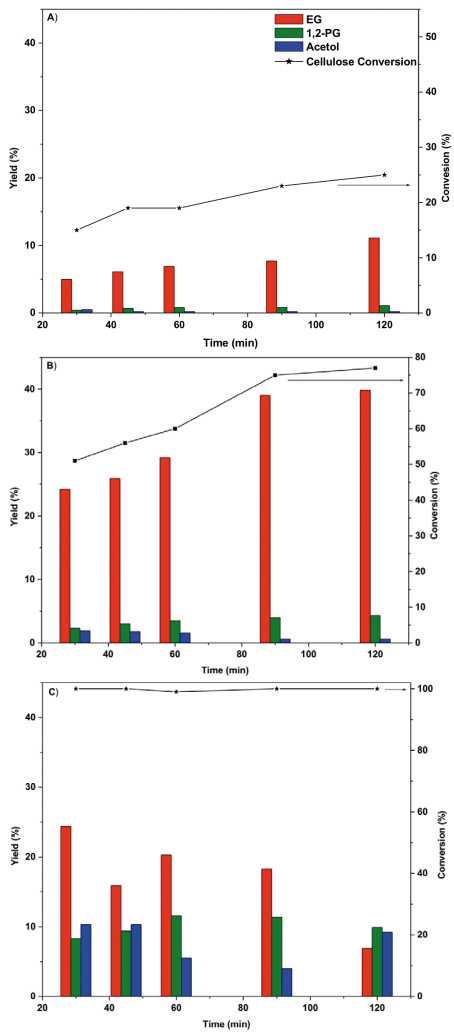

Figure 2 - Ethylene glycol (EG), 1,2 – propylene glycol (1,2-PG) and acetol yields and selectivity and cellulose conversion from catalytic reactions using Pd-WxC/C at A) 190ºC; B) 220ºC; and C) 250ºC.

It is noticeable that the cellulose conversion is strongly affected by the reaction temperature, increasing with temperature. Conversion of 15% was obtained at 190ºC in 30 min. On the other hand, 100% of cellulose was converted at 250ºC at the same reaction time. At 190ºC and 220ºC, the conversion increased as the time reaction increased.

As shown in Fig. 1-A, at 190ºC was obtained 11% of EG after 120 min of reaction. It is important to highlight that 2% of sorbitol was produced in this condition. It could be due to Pd ability in hydrogenation of π-bonds, converting glucose to sorbitol. It seems that sorbitol is an intermediated product, which can be transformed at longer reaction time and/or at higher temperature, because it was not identified in any other conditions.

Ethylene glycol and acetol yields provided for the Pd-promoted catalyst were significantly different from the non-promoted catalyst (Fig. 1 and 2-B) at 220ºC. Pd-WxC/C catalyst was selective to EG, exhibiting 40% of EG after 120 min. However, the non-promoted catalyst produced ≈ 30% of acetol at 220ºC after 150 min and a neglected quantity of EG. The 1,2-PG yield increases until 120min of reaction at this temperature (Fig. 2-B). According to Ooms et al. [9], the 1,2-PG is formed from the retro-aldol reaction of fructose to form acetol, and then the acetol can be hydrogenated to produce 1,2-PG. Some sub products, like methanol and 1-propanol from hydrogenation/hydrogenolysis and levulinic acid from dehydration, were also produced, representing at around 10% of total products yield.

At 250oC (Fig. 2-C), 100% of cellulose was converted by the Pd-promoted catalyst. However, the EG yield have decreased, leading to several by-products. Acetol and 1,2 – PG were produced by parallel reactions. At that higher temperature, the catalytic reaction seems to have enough thermal energy to allow C-C and C-O cleavage, which led to a greater variety of by-products. Moreover, the dehydration process was also increased at 250ºC, which increases the formation of levulinic acid (≈10%). The production of 1,2-PG also increased (≈10%), along with 1-propanol (≈4%) due to the hydrogenolysis of acetol C=O bond [12]. The partial pressure of H_2 in the catalytic reaction at 250ºC is lower than the partial H_2 pressure at lower temperatures (190 and 220º) due to the water vapor pressure contribution. Remind that the hydrogen pressure was applied and fixed at 800 psi after the mixture of catalyst, cellulose and water had achieved the reaction temperature. The product distribution indicates that the lower hydrogen availability in the reactor atmosphere at 250ºC might limit the hydrogenation steps, which favored acetol production instead of EG.

Our catalytic results are in agreement with the reaction steps proposed by Ooms et al. [9] (Fig. 3). It is important to note that the isomerization of glucose to fructose, after the cellulose hydrolysis, is the key step for C_3 sugar alcohols formation.
Pd-promoted tungsten carbide catalyst exhibited high EG yield showing that the interaction Pd-tungsten carbide was responsible for the retro-aldol reaction of glucose to obtain the intermediates to produce EG. On the other hand, the non-promoted tungsten carbide catalyst favored the glucose isomerization to form fructose, which was an intermediated to acetol and 1,2-PG formation.

Conclusions

Tungsten carbide promoted with Pd is an effective catalyst to produce ethylene glycol through hydrogenolysis of cellulose. The optimal conditions were 220ºC and 120 min, in which it was obtained 77% of cellulose conversion and 40% yield of ethylene glycol. The catalyst without palladium produced acetol as major product. After cellulose hydrolysis to obtain glucose, the interaction between Pd and tungsten carbide seems to act in the glucose retro-aldol reactions. However, non-promoted tungsten carbide catalyst promoted the glucose isomerization to fructose, which is transformed into acetol through retro-aldol reaction.

Acknowledgements

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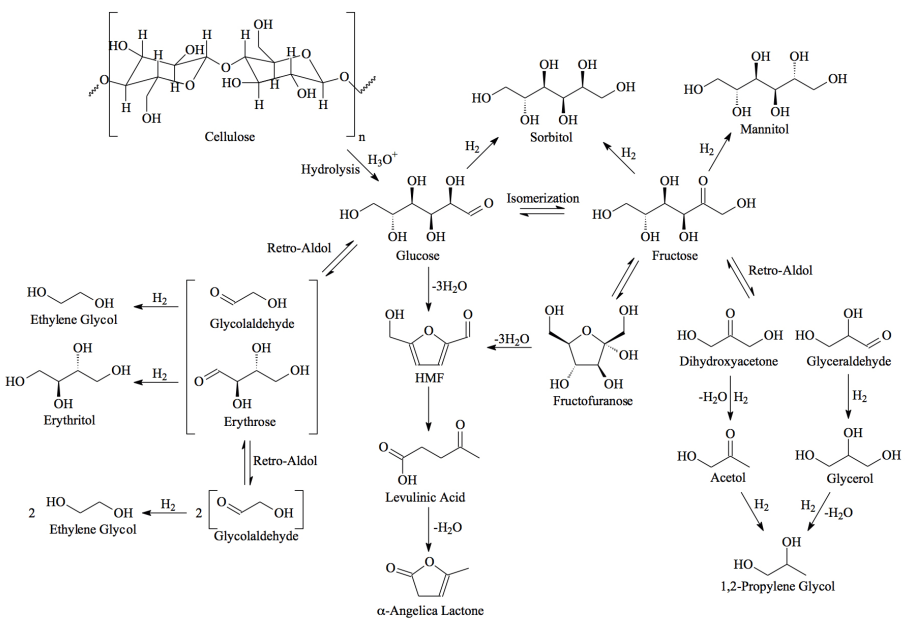

Figure 3 - Reactions network of cellulose conversion using Pd-WxC/C catalyst.

Affibody functionalized bacterial cellulose tubes for bioseparation applications

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Abstract

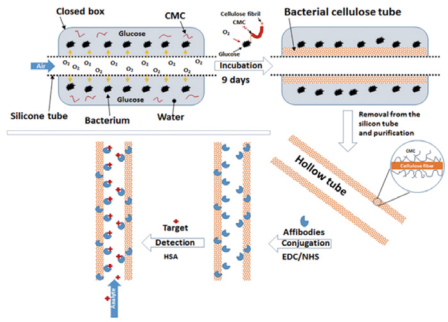
A development of a tubular nanocellulosic biofilter containing affibodies to selectively catch target proteins is demonstrated. Tubular nanocellulosic matrices were obtained from Gluconacetobacter medellinensis (G. Medellinensis) grown in a culture medium in the presence of dissolved carboxymethyl cellulose (CMC) which allowed the in-situ modification of the bacterial cellulose (BC) fibrils. The properties of BC tubes were characterized with scanning electron microscope (SEM), water retention value (WRV), conductometric titration and X-ray photoelectron microscopy (XPS) measurements. The biofiltration concept was demonstrated with anti-human serum albumin (anti-HSA) affibodies, which specifically bind human serum albumin (HSA). Affibodies were covalently conjugated onto CMC-modified BC tubes via EDC/NHS coupling chemistry. Successful conjugation of affibodies was confirmed by using surface plasmon resonance (SPR) on thin cellulose films prepared by Langmuir-Schaeffer method. The specific binding of HSA on the affibody functionalized BC tubes was demonstrated with fluorescence stained HSA. Moreover, it was observed that the presence of CMC in the culture medium significantly alters the WRVs of never-dried and air-dried BC tubes.

Introduction

Some bacteria genus, including *Gluconacetobacter, Agrobacterium, Pseudomonas, Rhizobium,* and *Sarcina*, have the capability to synthesize cellulose (bacterial cellulose, BC) in the presence of glucose, phosphate, and oxygen [1]. BC has a ribbon-like shape and high crystallinity index [2]. Due to the replication of bacteria, nanocellulose fibrils in the BC pellicle form a branched tangled structure, which has good mechanical properties even in the wet state [3]. BC produced from *Gluconacetobacter* strains has been used as a food supplement, in electronics and in several medical applications. Moreover, since the BC growth takes place only in the presence of oxygen, their assembly can be directed to form different shapes, depending on the air-water interface used, for example in closed vessels, tubes (BC-tubes), sheets, sacks, cylindrical balloons, etc.[4]. The non-toxicity and high stability of BC makes it an ideal material in medical applications such as artificial blood vessels [5] and skin wound healing materials [6].

Synthesized BC tubes have shown the potential to significantly resist the internal pressure [4], which is a requirement in biofiltration. It has also been shown that small molecules (molecular mass of the order of 20 kDa) can diffuse through BC pellicles. In addition, immunological cells like globulins (ca. 66 KDa) can be filtered out from solution [7]. Therefore, BC tubes could potentially be utilized in biofiltration assays, such as in separation of immunological proteins like antibodies (ca. 150 KDa). Moreover, the incorporation of antibodies, peptide ligands, protein A, etc. onto the inner walls of BC tubes [8] opens new possibilities in biofiltration for detection and separation of specific target proteins.

In this communication the concept of *in-situ* modification of BC tubes with CMC (CMC-BC tubes) for selective biofiltration is demonstrated. Affibodies, i.e., engineered proteins that mimic the antigen binding regions of native antibodies, were used as active molecules mainly because of their wide availability. They have similar sensitivity and affinity



Scheme 1. Schematic illustration of the synthesis of CMC-modified bacterial cellulose tubes (BC tubes) and their subsequent functionalization with affibodies for targeted bioseparation.

properties when compared to native antibodies [9]. CMC-BC tubes were obtained from *Gluconacetobacter medellinensis* grown in a culture medium in the presence of dissolved CMC. The selection of CMC was based on its non-toxicity for human cells [10], availability, and suitability for anchoring antibodies onto cellulose [11]. Scheme 1 offers an illustration of the developed materials and concepts noting that affibody modification makes them generic for use in the detection and separation of diverse antigens and plasma proteins.

Experimental

Materials. *Gluconacetobacter medellinensis* (*G. Medellinensis*) was provided by the School of Engineering, Universidad Pontificia Bolivariana, Colombia and the properties of the strain are described elsewhere [12]. CMC (DS of 0.7, Mw of 250 kDa,), D-(+)-glucose, yeast extract, sodium phosphate dibasic (Na_2HPO_4), bacteriological peptone, NHS, EDC and HSA were obtained from Sigma-Aldrich. Anti-HSA affibody dimer molecules (Mw 14 kDa) were obtained from Abcam plc. All other chemicals used in this study were analytical grade and used without any purification steps. The water used in all experiments was deionized and further purified with a Millipore Synergy UV unit (MilliQ-water).

Methods. *Synthesis of CMC-modified BC membranes and tubes.* BC was synthesized from *G. Medellinensis* [12] in a standard Hestrin-Schramm (HS) medium [13]. CMC (0 - 5 g/l) was dissolved in the culture medium, and the medium was sterilized with an autoclave (120 °C for 20 min). *G. Medellinensis* was statically incubated at 28 °C for 9 days.

Conjugation of anti-HSA affibodies onto BC tubes. The *anti-HSA* affibody molecules were covalently conjugated onto the CMC-modified (2 g/l CMC in the culture medium) BC-tubes by using EDC/NHS-mediated conjugation [14].

Conjugation of anti-HSA onto CMC-modified cellulose thin films. The conjugation of *anti-HSA* on CMC-modified cellulose and the subsequent binding of HSA on the prepared *anti-HSA-CMC* biointerface were examined by using a surface plasmon resonance (SPR) instrument (Model Navi 200, Oy BioNavis Ltd, Tampere, Finland). Langmuir-Schaefer cellulose films were deposited on gold wafers by using the deposition technique as described by Tammelin et al. [15].

Characterization. *Charge determination.* The amount of weak acid groups (carboxyls) of CMC-modified BC-tubes was determined by a conductometric titrator 751 GPD Titrino (Metrohm AG, Herisau, Switzerland) following a standard SCAN-CM 65:02.

Surface analysis by XPS. The surface chemical composition of BC tubes was examined using a Kratos Analytical AXIS Ultra electron spectrometer with a monochromatic Al Kα X-Ray source at 100 W and a neutralizer.

Imaging via SEM. The morphology of the CMC-modified BC tubes was imaged by using a Jeol JSM 5910 LV microscope operated at 20 kV.

HSA-detection with the anti-HSA functionalized BC tubes. The specific detection of HSA with *anti-HSA* functionalized BC tubes was carried out using fluorescence-stained HSA. HSA was labelled with Dansyl chloride (fluorescence dye) as described elsewhere [16]. The amount of dansylated-HSA bound onto the inner surface of anti-HSA functionalized BC tubes was analyzed by a Leica TCS SP2 confocal laser scanning microscope (Leica microsystems CMS GmbH, Manheim, Germany) with an excitation and detection wavelengths of 488 and 500–530 nm, respectively.

Results and discussion

Synthesis and structural characterization of CMC-modified BC tubes. The synthesis of CMC-BC tubes was carried out by using a closed incubation vessel equipped with a supporting silicon tube with constant oxygen flow for bacteria feeding (see Scheme 1). Synthetized CMC-BC tubes were uniform and without visible defects (Figure 1a-c). The presence of CMC in the culture medium increased the wall thickness of BC tubes compared to that produced in its absence (wet wall thicknesses of 0.9 ± 0.1 and 1.8 ± 0.2 mm for BC and CMC-BC tubes, respectively). It was postulated that CMC disrupts the formation of fibrillar bundles (BC fibrils) by incorporating CMC into BC fibrils leaving the crystallization of microfibrils intact. Therefore, BC fibrils that are synthesized in the presence of CMC are more meandering and loosely packed than unmodified BC fibrils.

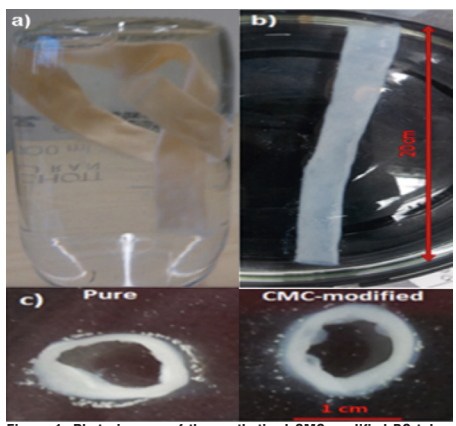


Figure 1. Photo images of the synthetized CMC-modified BC tubes before (a) and after (b) purification. The cross section images of unmodified and CMC-modified BC are also included (c).

The morphology of the synthesized CMC-BC tubes were explored by SEM. Both, the inner surface and cross-section of CMC-BC tubes were analyzed. Figure 2 clearly illustrates that the incorporation of CMC changes the morphology of BC. Fibrils of CMC-BC were more meandered and a slightly thicker when compared to unmodified BC fibrils (Figure 2a,b). These effects are most probably due to the incorporation of CMC into BC fibrils that leads to a looser packing of microfibrils [17].

The effect of CMC incorporation in BC charge and water retention values (WRV) was examined by using the corresponding BC pellicles. It is important to note here that the conductometric titration measures only the weak acid groups (carboxyls), and therefore the measured charge represents the total carboxyl content of the sample. As expected, the charge of unmodified BC was zero (Figure 3) since BC is pure, native cellulose and does not contain any weak acid groups. However, a low amount of CMC (0.1 g/l) in the culture medium raised the charge of CMC-BC to 72 µeq/g. A charge plateu level was reached for CMC concentrations in the culture medium over 2 g/l

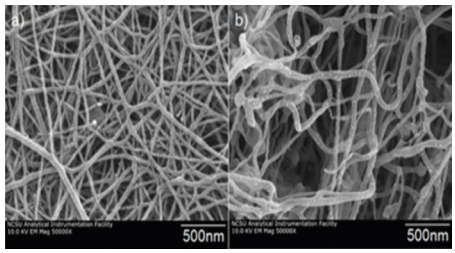


Figure 2. Plane view SEM images of the inner-surfaces of BC (a) and CMC-BC (b) tubes. The magnification used in the images is 50000x.

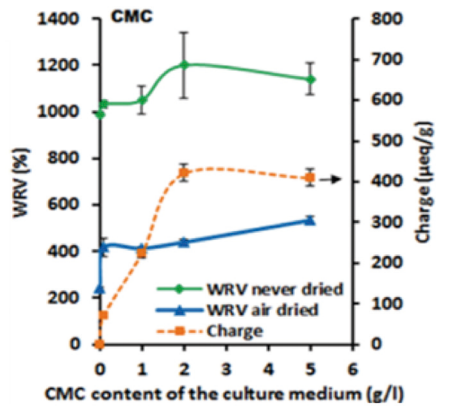


Figure 3. Water retention values (WRV) of never dried (circle symbols) and air-dried BC (triangle symbols) as a function of CMC added in the culture medium (a). The content of the carboxyl groups in BC as a function of CMC added in the culture medium is also shown.

(charge of ~ 400 μeq/g). The highest concentration of CMC used was 5 g/l, which was limited by the resultant viscosity of the solution (this in turn, may influence the formation of BC pellicles). The WRV measurements were performed to demonstrate the effect of CMC on the swelling of fibrillar networks and their water uptake capacity. The high WRV value (990 %) of unmodified BC (Figure 3) was comparable to those previously reported in the literature [5]. The addition of CMC in the culture medium increased the WRV of never-dried CMC-BC, and this increase was clearly associated with the electrostatic charge. The highest WRV values were obtained for samples containing over 2 g/l of CMC, which corresponds to the higher carboxyl content of CMC-BC. The surface chemical composition of CMC-BC tubes was investigated by XPS. No significant differences between the XPS spectra of unmodified BC and a cellulose standard were found (Table 1). However, the O/C ratios of BC samples were slightly lower than that of the cellulose standard. The results indicate the presence of small amounts of impurities, possibly from the incubation process.

Conjugation of affibodies onto CMC-modified cellulose monitored by SPR. Cellulose thin films were utilized to verify the conjugation by using the surface plasmon resonance technique, SPR. First, cellulose films supported on gold sensors were modified by adsorbing CMC from electrolyte solution. As expected, CMC was found to adsorb irreversibly onto cellulose (Figure 4a) [18]. It has been postulated that the prevailing adsorption mechanism is based on the structural similarities of CMC and cellulose.

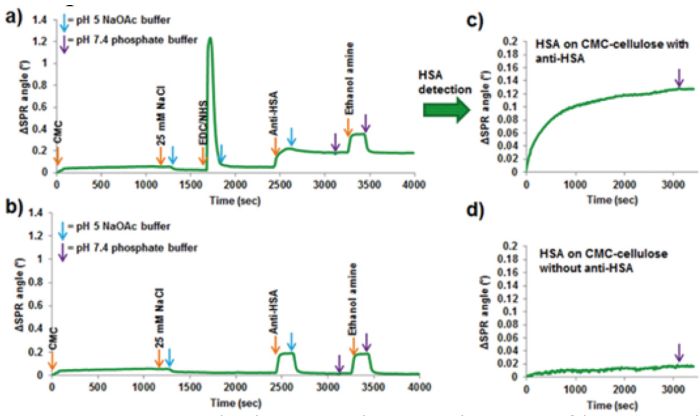


Figure 3. Water retention values (WRV) of never dried (circle symbols) and air-dried BC (triangle symbols) as a function of CMC added in the culture medium (a). The content of the carboxyl groups in BC as a function of CMC added in the culture medium is also shown.

Next, the CMC-modified cellulose was activated by EDC/NHS solution to convert the carboxyl groups to amine-reactive esters. This activation step can be observed as a small increase in the SPR signal. After the EDC/NHS activation, *anti-HSA* affibody molecules were conjugated (via amide bonds) onto the activated CMC-cellulose surface. It should be mentioned, that no adsorption was observed when the conjugation of *anti-HSA* was conducted in the absence EDC/NHS (Figure 4b).

Filtration of HSA with anti-HSA affibody functionalized BC tubes. As a demonstration of the effectiveness of BC carrying affibodies for biofiltration, anti-HSA were immobilized onto the synthesized CMC-BC tubes via EDC/NHS chemistry. It is important to note here that unwanted diffusion of *anti-HSA* through the wall of a BC tube may occur because of the small size of the affibody (14 kDA). Therefore, some of the conjugated affibodies are expected not to be available for binding with the target protein (HSA). In contrast, diffusion is not expected for the relatively large HSA molecules (66 kDa) [7]. Detection studies were carried out by using dansyl-stained HSA via fluorescence microscopy. The fluorescence imaging was conducted on the inner plane view of the BC-tubes noting that the fluorescence of a non-conjugated, *anti-HSA*-free CMC-BC tube was null (Figure 5a). When non-conjugated, *anti-HSA*-free CMC-BC tubes were exposed to dansylated HSA a slight fluorescence was observed (Figure 5b). This is explained by the small non-specific adsorption of HSA. As expected, the fluorescence was significantly increased when HSA was filtrated through the CMC-BC tube containing the conjugated *anti-HSA* affibodies (Figure 5c). This demonstrates that the conjugation of affibodies enhances the affinity and specificity of CMC-BC tubes to capture the target molecules.

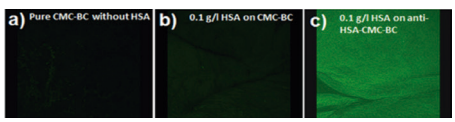


Figure 5. Detection of HSA in BC tubes functionalized with anti-HSA by using fluorescence imaging. Included are the surface of a CMC-BC tube free of anti-HSA before (a) and after (b) exposure to dansylated HSA. The image in (c) corresponds to the surface after exposure to dansylated HSA to CMC-BC tube conjugated with anti-HSA affibodies.

Table 1. XPS elemental data and carbon C1s bonds of unmodified BC and CMC-modified BC (2 g/l CMC added in the culture medium). As a reference, XPS data for pure cellulose is included.

	Element (at. %)					C 1s component (%)			
Sample	**O 1s**	**C 1s**	**N 1s**	**Na 1s**	**C(C-C)**	**C(C-O)**	**C(C=O)**	**C(COO)**	**O/C**
Pure BC	36.0	62.3	1.8	nd.	12.2	67.0	18.8	2.0	0.58
BC with added CMC	36.6	62.5	0.7	0.2	17.8	62.5	17.5	2.3	0.55
Cellulose standard	39.2	60.8	nd.	nd.	5.0	74.5	19.1	1.4	0.64

Conclusions

The synthesis of BC-CMC modified tubes and their functionalization by affibody conjugation is demonstrated. The presence of CMC in the culture medium during BC synthesis has a significant influence in the WRV of never-dried and air-dried BC tubes and reduces irreversible structural changes during drying of BC. In addition, BC activation with CMC improves the removal of protein residues from the synthesized cellulose and facilitates anti-human serum albumin (*anti-HSA*) conjugation by covalently binding the affibody to the carboxyl groups via EDC/NHS coupling. The specific binding of HSA onto anti-HSA ligands supported on the BC interface was demonstrated by SPR. Finally, CMC-modified BC tubes functionalized with *anti-HSA* were used to capture fluorescent HSA from solution. It is expected that the presented generic and robust method for grafting recombinant affibody proteins onto BC materials has a potential to open up new venues in the field of biofiltration, i.e., selective separation and detection of various target molecules from an analyte solution.

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The role of ligno-nanocellulosics in the biorefinery concept

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Abstract
In this contribution, an overview of production of CNF obtained from different raw materials, including different chemical compositions is discussed in terms of properties of suspensions and end-products, depending of morphologic, rheological and structural behaviour. The effect of residual lignin in CNF is discussed and its impact on fibrils size and morphology, as well as interactions of lignin-containing CNF films with water, and their barrier properties are presented. In terms of CNF manufacture, a new enzymatic method to produce high-consistency CNF with no refining stage required is introduced. Additionally, an overview of applications of CNF is presented, with an emphasis in CNF films, functional membranes and emulsion stabilization.

Introduction
Several industrial sectors are constantly seeking alternatives for reducing the world's demand and dependence of petroleum-based products, thus decreasing environmental impact and maximizing the usage of resources. Due to its availability and composition, lignocellulosic biomass is one of the main candidates to be used as raw material for such replacement, as a renewable alternative for the production of bio- chemicals, fuels and products. This does not only add value to- but also decreases the carbon footprint of both, raw lignocellulosics and final materials. Based on the concept of the traditional refining of petroleum, efforts on the development of an integrated biorefinery concept are the main focus of several research groups around the world. With the target of improving to its maximum the profitability of each of the biomass streams, production of nanocellulose is gaining increased attention, since it is a versatile material with superior properties that can increase the application range of lignocellulosic materials.
Obtained by mechanical fibrillation of wood pulps, traditionally by refining, homogenization or a combination of both, the manufacture of cellulose nanofibrils (CNF) usually involves either chemical or enzymatic pre-treatment. Processing method as well as composition of the raw materials, dominate the properties of the CNF suspensions and their effect in the final product. As for the versatility of nanocellulose, they can be produced from a number of lignocellulosic materials, including agroindustrial residues, making a great addition to the value chain of commodities like sugar, ethanol production, among others.
Among its remarkable properties, CNF present high aspect ratio and large surface area with an enhanced hydrogen-bonding capability that allows the formation of strong gels at rather low solid contents (around 2%); while this property can be advantageous in applications such as aerogels, processing and transporting large volumes or water can suppose a major drawback in many cases, limiting the end-use applications of CNF. CNF suspensions also maintain this strong network held after drying forming strong films with high barrier properties against oxygen. In this paper, production of different grades of ligno-nanocellulose from different sources, obtained by means of different methods is presented, as well as a variety of applications are discussed such as nanopapers, membranes and emulsion stabilizers.

Experimental
Nanocellulose from wood and non-wood materials was obtained from bagasse and hemp shives pulps among other alternative raw materials. Both bleached and unbleached pulps were processed at 2 % solids using a wet mill (Masuko MKZA10-15J) equipped with coarse stones for the first pass and with fine stones for the consecutive two passes. Macro structure of fibrillated cellulose was studied using light microscopy while more detailed imaging of spin-coated thin films was done using Scanning Electron Microscope. Transmittance of diluted CNF dispersions was monitored to indirectly estimate particle size of the samples and rheological properties of properly dispersed CNF suspensions were also measured. (1)

Lignin-containing cellulose nanofibrils (LCNF) were produced from SO_2-ethanol-water (SEW) processed Norway spruce fibres. SEW is a very promising fractionation process for lignocellulosic materials within the biorefinery process. By varying process fractionation and temperature, fibres with different content of residual lignin were obtained (3.7 wt.% = 4L and 13.5 wt.% = 14L). (Fully bleached Norway spruce kraft pulp with less than 0.5 % of lignin was used as reference). Fibres were mechanically refined and aqueous suspensions (< 2 wt. %) solids content were processed 6 times in a high-pressure microfluidizer (Microfluidizer M-110 P, Microfluidics Corp., 2010). The effect of residual lignin was investigated in terms of morphology and size of the fibrils, dewatering time of nanopapers production, mechanical and barrier properties of the nanopapers and interaction with water. (2)

Nanopapers were manufactured by filtering LCNF diluted aqueous suspensions (0.8 wt. % solid contents) using over-pressurized filtration. The volume of the filtrate was measured at different intervals of time and dewatering time was monitored. The nanowebs were cold-pressed between blotting papers, followed by hot-pressing resulting in nanopapers with a target dry basis weight of 80 g/m^2.(2)

High consistency suspensions of nanocellulose (HefCel) were obtained from diverse feed stocks such as wood and non-wood pulps, textile waste, and agroindustrial residues, among others. These materials were processed at 20-40% dry matter by mixing the pulp with a cocktail of commercially available tailored cellulases in a horizontal reactor such as sigma mixer. Temperature of the process was carefully controlled in order to control the enzyme activity and to keep at a minimum the soluble fraction of sugars loss.(3, 4)

TEMPO-mediated oxidation of the fibrils was performed on bagasse fibres and in dried bleached softwood pulp (for membranes manufacture) according to Saito et al. (5) Oxidized softwood pulp was fibrillated as such or subjected to further oxidation of aldehyde groups according to Shinoda et al. (6) Partly oxidized pulp was fibrillated using a high pressure homogenizer (Microfluidizer M110-EH) using one pass. In the case of bagasse, the oxidized pulps were fibrillated using the wet mill (Masuko MKZA10-15J). The final consistency of the fibrillated gel was approximately 2 wt.-%. Charge of the pulps, determined by conductometric titration, were 0.935 and 1.12 mmol/g in the case of softwood pulps and 0.8 meq/g pulp for bagasse respectively. (7)

Functional membranes of CNF were obtained by mixing homogeneous suspensions of oxidized softwood CNF (TCNF) with a polyvinyl alcohol in water (10:90 and 25:75 wt.). The membranes were obtained by solvent-casting the suspensions in controlled atmosphere (23 °C/50 % RH) over 5 days. Esterification of the surface carboxyl groups of TCNF-PVA films was done in an acid-activated ethanol bath during 24 hours at room temperature. Posteriorly, esterified TCNF-PVA films were grafted with thermo-responsive poly(*N*-isopropylacrylamide) (PNIPAM) by immersion of the films on PNIPAM solution in ethanol.(8)

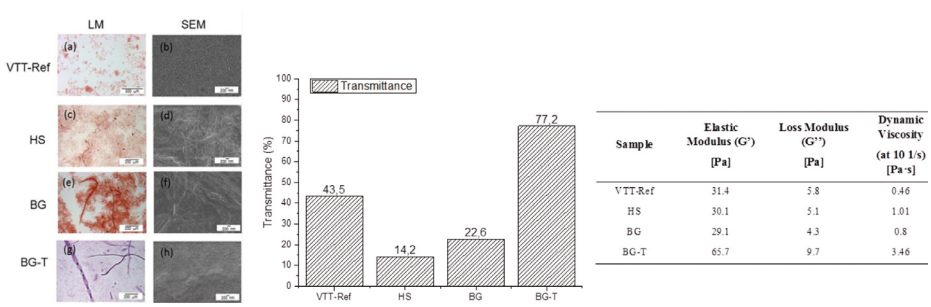
CNF as emulsion stabilizer: water suspensions of native and TEMPO-CNF from never dried bleached birch kraft pulp were used in emulsion formulations with dodecane, by using a high shear mixer according to Table 1. Prior to emulsification CNF and TEMPO-CNF were diluted and mixed in the aqueous phase using the same conditions as during emulsification. pH of the CNF and TEMPO-CNF dispersions was measured (pH=6-8) but not adjusted prior to emulsification. Stability towards flocculation and creaming was evaluated as a function of centrifugation and temperature (up to 80°C). Optical microscopy was used to evaluate the characteristics of the formed emulsions, while droplet size was analyzed using low field NMR method. (9)

Table 1. CNF and TEMPO-CNF concentrations as w/v % of oil phase, dodecane:water ratio is given as v/v percent of total emulsion volume.

CNF [%]	0.1	0.5	1.0	1.5
Do-decane [%]		5	5	5
	20	20	20	20
		35	35	35

Results and discussion
Nanocellulose from different sources
Due to their availability, short growing cycles and overall cost potential, CNF production from agricultural sidestreams is a promising alternative, since it gives the opportunity to convert these materials in more valuable products, at the time of alleviating the pressure of landfills and decreasing airborne contamination due to incineration. (1)

Figure 1 shows optical and SEM of CNF produced from different raw materials (VTT-Ref = bleached kraft birch, HS = hemp shives, BG = bagasse and BG-T = TEMPO-oxidized bagasse). While VTT-Ref CNF suspension is rather homogeneous, CNF obtained from bagasse and hemp shives present a higher amount of fibre fragments and agglomerates



Sample	Elastic Modulus (G') [Pa]	Loss Modulus (G'') [Pa]	Dynamic Viscosity (at 10 1/s) [Pa·s]
VTT-Ref	31.4	5.8	0.46
HS	30.1	5.1	1.01
BG	29.1	4.3	0.8
BG-T	65.7	9.7	3.46

Figure 1. Morphology (optical microscopy and SEM)-left, transmittance –middle and rheological behavior of CNF suspensions-right, obtained from different sources. (1)

of fibrils, most likely as a result of the heterogeneity and different chemical compositions of the starting materials. On the other hand, microscopic images of BG-T suspensions show remaining fibres with a high degree of swelling (Figure 1 g and h). Even though the combination of the oxidation pre-treatment and the mild mechanical treatment is enough to produce a clear, transparent CNF gel, some swollen fibre still remain on the suspension.
Evidence on the heterogeneity of the fibrils size and the overall quality of CNF suspensions is also possible by monitoring transmittance of diluted CNF suspensions, measured at 600 nm wavelength, which is the middle of the visible region, recommended value to be reported for CNF samples (10). (Figure 1) As expected, the transmittance of the BG-T CNF suspension was the highest (77.2%). Additionally, the highest viscosity was observed also in the case of BG-T suspension, also with high elastic and loss modulus, indicating a strong fibrillar and entangled network structure. Smaller aspect ratios of the rest of the samples as a result of more intense mechanical treatment lead to lower viscosity values. (1)

The effect of residual lignin on CNF

Regarding chemical composition, it is well known that the removal of lignin and heteropolysaccharides by chemical treatment of lignocellulosics had proven to yield more flexible fibres. However, the presence of residual lignin in raw materials for the production of nanofibrillated cellulose (CNF) might hold several advantages such as higher yields with reduced costs (lower energy consumption in manufacture process) and potential improvement in barrier properties in the case of films. Also, in composite applications, chemical similarity between lignin and hydrophobic polymeric matrices might be beneficial in terms of compatibilization, thus enhancing thermo-mechanical response of the materials.
The utilization of LCNF showed a significant decrease in the dewatering times for the manufacture of nanopapers when compared to fully bleached CNF suspensions (Figure 2). The presence of lignin had proven to yield stiffer fibrils, less prone to conform to each other while packing over filtration.(2) LCNF present thinner diameters than those from bleached pulps, as observed with AFM. Additionally, remaining lignin that has been detached from the lignocellulosic cell wall during the defibrillation process, penetrates and is distributed among the fibrils during the filtration, to finally melt during the hot-pressing stage, gluing back the fibrils together and decreasing the overall roughness of the nanopaper, mimicking the native composition of the cell wall (see proposed model, Figure 2). In respect to surface energy and wettability, nanopapers with higher content of residual lignin, became less hydrophilic, as evidenced from contact angle increase of more than 2-fold when comparing to bleached CNF and LCNF containing 14% of residual lignin (Figure 2). (2, 11)

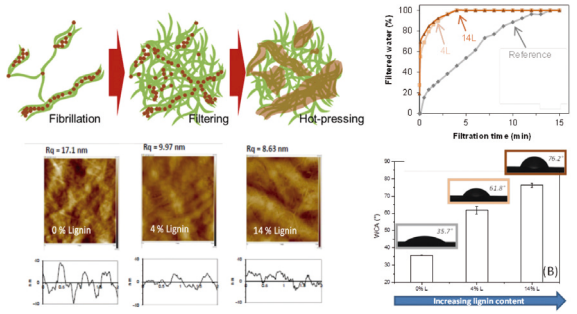


Figure 2. Proposed mode of residual lignin on cellulose nanofibrils during fibrillation and nanopapers formation. Influence of lignin on drainage time of fibrils while forming nanopaper, its morphology (AFM images) and water contact angle as a function of the lignin content (2, 11)

High consistency cellulose nanofibrils (HefCel)

Challenges associated with high energy consumption and difficulties in chemical recovery are a drawback in the industrial production of nanocellulose. In this sense, a revolutionary technology had been developed at VTT, that combines enzymatic high consistency (HC) refining (3) (HefCel method) with mechanical treatment through extrusion process.(4) After treatment, the cellulose fibrils form soft granules and the suspensions formed by these fibrils can either be in the form of a non-sticky, chalky paste, sticky paste or even partially flowing paste, depending on the enzyme dosage and length of the treatment and consistency of the original pulp.
SEM imaging reveals delamination of the fibre cell wall and the formation of nanoscale fibrils, with widths of about 20 nm, which was confirmed with AFM measurements (Figure 3). Strong, flexible and ductile films were obtained with these fibrils by solvent-casting or extrusion methods. Such films proved to be excellent barriers against oxygen and grease.

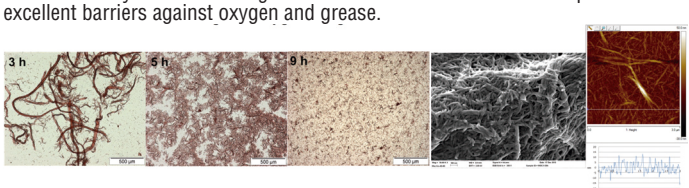


Figure 3. Light microscopy of cellulosic fibres after 2, 5, and 9 hours of treatment, and SEM and AFM images of the HefCel fibrils.

CNF membranes

CNF films are an excellent option to be used in filtration as organic solvent and ion capturing filters, due to their small pore size. (12) However, for such function, these materials need to support high water pressure and often this is a big challenge for nanocellulose-based materials due to their poor performance in wet-state. In this sense, PVA-modified TCNF films exhibited significantly improved wet strength and they were able to capture up to 40% of cationic ions from water. Moreover, by varying the ratio PVA/TCNF, it is possible to further tune the mechanical properties of these materials (Figure 4). Added functionality by PNIPAM surface grafting, further increase the potential of such materials.

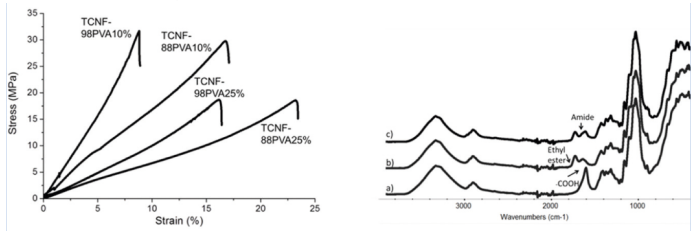


Figure 4. Stress-strain curves of wet TCNF-PVA films with different concentrations and degrees of hydrolysis of PVA and FTIR spectra of unmodoified, esterified and PNIPAM-grafted TCNF-PVA films. (8)

Emulsion stabilizers

CNF and TCNF with dodecanol emulsions were formed with varying ratios (Table 1). Thickness of the creaming layer after centrifugation increased with increasing stabilizer amount or increasing oil ratio. With the exception of 0.1% CNF sample, all emulsions proved stable against centrifugation. CNF strongly flocculated during the first hour after preparation with only minor changes in the creaming layer of emulsions from both fibrils after 24 h, which remained stable for several weeks at room temperature. 1.0% of CNF or TEMPO-CNF and 20% dodecane proved stable to temperature increase, and coalescence did not occur at 80°C (Figure 5).(9)

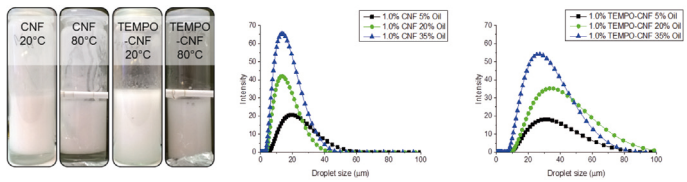


Figure 5. Emulsions from CNF and TEMPO-CNF at 20°C (1h after preparation) and after heating to 80°C (3 days after preparation) and droplet size distribution of CNF and TEMPO-CNF emulsions with increasing oil content.

NMR measurements indicated large differences between droplet size and droplet size distributions measured within the samples. The weighted arithmetic averages of emulsions containing 1.0% CNF or TEMPO-CNF and 20% oil were 14.1 and 36.6 µm respectively. The O/W ratio seems to have a more significant effect on the droplet size, than the amount of stabilizer (Figure 5)

Conclusions

Nanocellulose can be produced from different lignocellulosic materials from abandoned agroindustrial wastes to sidestream of papermaking industry (e.g. lignin-rich fibre fractions). Depending on the targeted final product specifications, it is possible to obtain a wide range of nanocellulose grades, by using different raw materials and processing parameters. In this sense, it is imperative to have a broad understanding of the structure-function relationship of these materials, keeping in mind that different grades will be more suitable for certain applications than others. The versatility of these materials is of great potential in applications, such as packaging, filtration, emulsions and dispersions stabilizers for food and pharmaceutical industry, to exemplify a few.
The inclusion of ligno-nanocellulosic materials in the biorefinary concept, supposes a significant enrichment of the whole biomass value chain.

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Natural polyphenols

Analyzing biorefinery product streams - challenges, requirements and (some) solutions

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Abstract

In many cases, biorefinery product streams are extremely complex mixtures containing biomass-derived biopolymers and their degradation products in different ratios, purity and modifications depending on origin, treatment, and purification level. While technology development has advanced over the years, coming up with processes to fractionate different product classes or modify biomass in a desired way, the analysis of complex biorefinery streams is still lagging behind. Classical laboratory methods developed many years ago are often not fit-for-purpose in modern labs. Traditionally retrieved sum parameters do not yield the desired level of information necessary to describe the chemistry of biorefinery processes in a sufficiently detailed way. Many of those methods rely on laborious techniques, which are both labor intensive and time consuming, far away from the need to analyze larger numbers of samples in a reasonable time frame.

The paper will discuss the specific needs in biorefinery streams and provide approaches to solve some of these problems by modern and also improved classical analytical methods designed to cope with the peculiar features of lignocelluloses. Ways to increase the overall speed of analysis will be discussed as well.

Introduction

Biorefinery approaches usually encompasses separation of lignocellulosic biomass into its fundamental major constituents lignin, cellulose, hemicellulose, and sometimes also minor compounds such as pectin, extractives and inorganics. The processes that can do a sharp separation of all aforementioned products without cross-contamination from other fractions has still to be developed. Hence, no matter what type of separation technology is applied, in most cases a fraction contains impurities from other fractions as well, i.e. the lignin fraction may still contain carbohydrates and inorganics, and the carbohydrate fraction, no matter if still polymer or already degraded, has some lignin in it. In addition, degradation products are present depending on the severity of the destruction process of the biomass. Many processes are multi-step approaches which end up with a considerably load of inorganic impurities. The inorganic load can also complicate subsequent analysis used to chemically describe the mixture. This is even more critical when analytical approaches are not only required to describe the major contents of a mixture but minor components with high accuracy and precision in order to establish reaction mechanisms or the extent of minor side reactions (Figure 1).

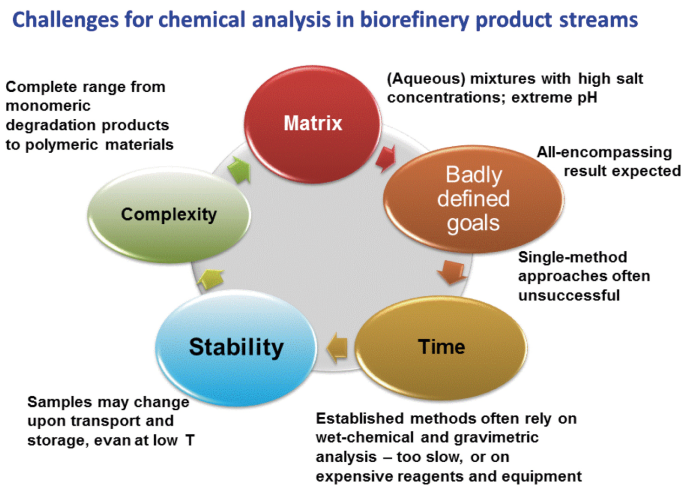

Figure 1: Challenges encountered in the analysis of biorefinery streams

An increasingly important issue in biorefinery research is the efficiency of the analytical methods with regard to time. At a first glance this may not look like a crucial issue, but it will certainly in near future with biorefinery approaches, starting materials and products from renewable resources becoming more and more diverse. Time consuming techniques naturally limit the overall number of measurements, hence statistical data might not be available, variations in different process steps stay invisible and the big picture of a process may remain concealed. First efforts on chemometric approaches in lignin analysis are already available from literature, but they require wet chemical data behind, which are in most cases can only be generated at a rather slow pace. Overall, academia and industry would both benefit from fast methods in biorefinery - and in particular lignin - analysis.

In this paper some examples will be given on how to tackle this task. Analytical methods often work well with standards or standard mixtures, but when it comes to real world samples, strategies to cope with the accompanying complex matrices are required. Three of these strategies will be discussed in this paper, covering in particular effectivity and speed.

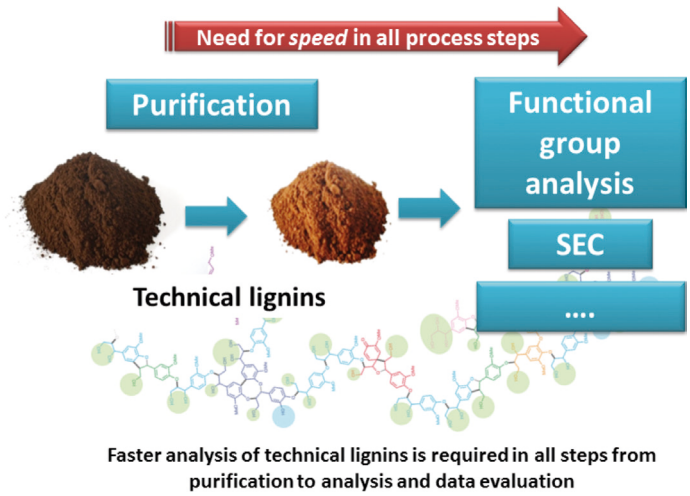

Figure 2: General workflow for lignin analysis

Results and discussion

Example 1: Faster purification of lignosulfonates

Lignosulfonates, independent of the pulping process they originate from, usually contain a high load of low molar mass impurities, both of organic and inorganic origin. Due to the good solubility in water induced by the sulfonic acid groups a precipitation as used for other lignin types is not possible. Hence the lignosulfonates have to be freed from the matrix by ion exchange followed by either an ultrafiltration step or by a selective absorption to resins, washing and subsequent desorption. Both techniques are tedious and also time consuming if done in the traditional way. In order to speed the process for analytical applications we have developed a methods based on solid phase extraction (SPE) which combines ion exchange and adsorption in a single step (Figure 3). The SPE syringe can be used directly for sampling in the industry and shipped for chemical analysis. The amount obtained is sufficient for the most important analytical methods and yields about 30 mg purified lignosulfonate (Sumerskii et al. 2015), sufficient for most subsequent instrumental analyses.

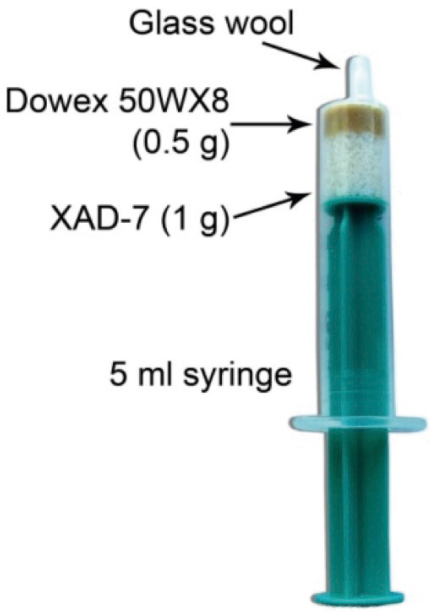

Figure 3: SPE syringe filled with ion exchange resin and adsorption resin for fast purification of lignosulfonates

Example 2: Sugar determination in biorefinery streams

There are numerous analytical methods available for the determination of monomeric carbohydrates, but still sugar analysis in complex matrices is very challenging. The first problem is the stability of any sugar containing sample. Such specimens are usually not stable over time if not immediately frozen after sampling due to contamination with ubiquitous microorganisms which are pleased to utilize the sugars contained. In addition carbohydrates are reactive in alkaline and acid media and may isomerize or even degrade, and hence contain a number of side products depending on the production conditions they have undergone. In order to cope with that heterogeneity and instability of the analyte in addition to a complex matrix, a method is required which can handle a very "rough" mixture without harming expensive equipment by clogging column and capillaries or corroding valves and pumps. We have found High Performance Thin Layer Chromatography (HPTLC) a very good opportunity in this regard - the thin layer plates are only used once. Hence the matrix cannot accumulated as they would in a HPLC column, the plate-setup allows to run up to 16 samples at a time which adds to speediness and to low costs. The remaining questions are twofold – what about resolution, and what about quantification? Figure 4 shows a separation of wood-derived sugars on an HPTLC plate and the chromatogram generated from the density image. The resolution is sufficient and the peak height can be used to quantify with an error of about 5-7%, which is in most cases sufficient for process stream analysis. (Oberlerchner et al. 2014a,b) Those separations are extremely reliable, speedy and cost-efficient compared to all conventional approaches, and most importantly, they are largely independent of matrix and composition effects.

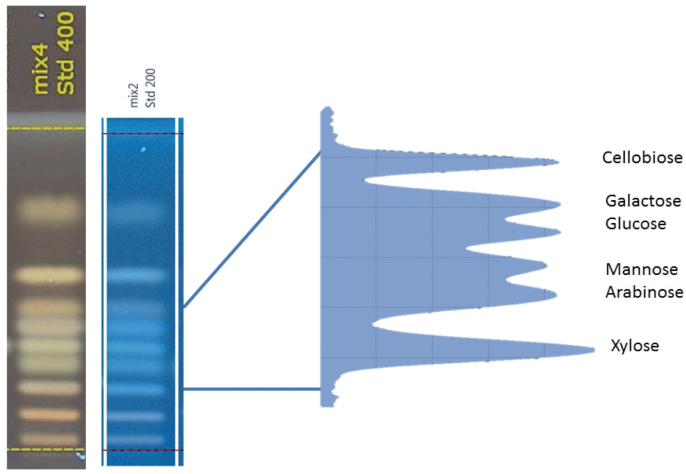

Figure 4: Separation of wood derived carbohydrates by HPTLC.

Example 3: Characterization of polysaccharide degradation products

Often the knowledge of degradation products is important to understand the chemistry behind a process and to optimize selective steps. The analysis of minor compounds is often complicated by high concentrations of other products or as in the present example, inorganics. Hence before the by-product analysis can be performed the compounds have to be quantitatively isolated. The analysis of spinning bath composition in rayon processing is such an example. The bath is very acidic and contains an extremely high load of inorganic salts necessary to adjust the regeneration of cellulose. The approach chosen was an extraction with an organic base (pyridine) and subsequent analysis by GCMS after derivatization (Liftinger et al. 2015). The application of a specific 13C-labelled standard solution obtained from 13C-labelled glucose that was degraded under alkaline conditions served as a special internal standard, which rendered the method rather robust (Figure 5).

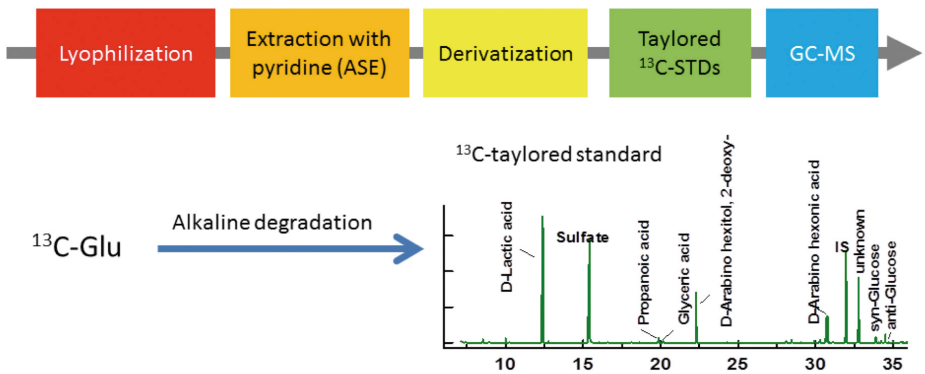

Figure 5. Approach to analyze polysaccharide degradation products in mixtures with extreme salt content.

Conclusions

Modern analytical methods do offer a number of possibilities to overcome problems with complexity and matrix effects in biorefinery product streams. Many allow for a higher throughput, but we are still a far away from a general toolbox for fast and comprehensive characterization of biomass product streams.

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Mild chemical modification of acetosolv lignin from several Chilean sources

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Abstract
Lignin is the most abundant aromatic bio-polymer in nature. However, applications of lignin as building-blocks in material science is affected by several drawbacks. In order to tailor selected properties a mild modification at room temperature (22 °C) was carried out. Acetosolv lignin from *Pinus sp., Triticum sp., and Eucalyptus sp.* were modified with propylene oxide for 24 hours in alkaline media (pH: 12). Physico-, chemical-, and biological-properties were evaluated. Regardless lignin source hydroxypropyl-lignin (HPL) were synthetized in a high yield (87-96%). Strong structure-property relationship was stablished in function of monolignol units. ^{1}H-NMR provide valuable insight regarding reaction efficiency ratio associated to the Syringyl/Guaiacyl ratio. Hydroxypropylation affects solubility, antioxidant activity, and the thermal behaviour. However, changes upon derivatization were related to the specific source and composition. Modified Acetosolv lignin from Chilean sources possess properties that are expected to enhance their role in new polymerization pathways for material engineering.

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Introduction
Lignin are the most abundant phenolic bio-polymer in vascular plants, and comprises a wide group of molecules with a very heterogeneous chemical structure based on three monomer units (H: hydroxyphenyl, G: guaiacyl, S: siringyl).
Lignin exhibit physicochemical limitations for polymer formulation. Drawbacks such as low solubility, limited compatibility in binary and ternary polymer-systems, as well as a challenger recalcitrant behavior are recognized (Kumar *et al.*, 2014). In order to tailor properties chemical modification is an useful strategy. Among several pathways, O-alkylation with propylene oxide (PO) in alkaline medium has been successfully utilized.
Considering the interest to developing new routes for lignin valorization, the aim of this work deals about the mild modification of several Acetosolv lignin feedstocks with PO. Lignins as well as modified derivatives were characterized by several methods and structure-properties relationship was stablished.

Experimental
Lignin from *E. globulus* (hard-wood), *P. radiata* (soft-wood), and *Triticum sp.* (non-wood) were isolated by the Acetosolv process in acid media (Berg *et al.*, 2013).
Monolignol composition. Lignin monomer-(C_9) composition was determine by nitrobenzene oxidation/HPLC technique.
Acetyl-content. Acetate-content was determined by titration (ISI, 1999).
Carboxylic group-content. NIR-FTR spectra were collected using a Perkin Elmer System 2000R spectrometer.
Hydroxypropyl lignin (HPL). Lignin (100 g, ca. 80 mmol) were dissolved in 500 mL aq. 2N NaOH and the pH was adjusted to 12. One-third, each, was combined with different molar equivalents (PO/C_9) in order to establish three degree of substitution (DSHPL: 0.5, 1.0, 1.5). The reaction was carried out for 24 h while stirring at room temperature (~22 °C). Adjusting the pH to 2 using conc. HCl (40%, v/v) produced a precipitate that was centrifuged. The induced precipitate was collected and washed three times with cold distilled water (5 °C) and oven-dried (40 °C, 72 h).
Total phenols content (TP). Lignin and lignin-derivatives were dissolved in methanol (1 gL^{-1}) and 0.5 mL of Folin-Ciocaleu reagent added (Sigma Aldrich).
Antioxidant activity (AA). Free radical scavenging power was assessed by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay (Brand-Williams et al., 1995).
Proton Nuclear Magnetic Resonance (^{1}H-NMR). ^{1}H-NMR spectra were used in order to gain insight regarding chemical structure and substitution patterns. (García et al., 2013).
Solubility testing. The procedure was based on dissolving HPL-samples in solvents at room temperature (21 °C) with vigorous mechanical stirring for 24 h (García et al., 2014).
Thermal stability (TGA). Thermogravimetric analysis was performed on a Pyris 1 TGA instrument (TG 209 F3 Tarsus, Germany). Approximately 6 ±2mg of sample was heated at 10 °Cmin^{-1} to 600 °C under 20 mLmin^{-1} N_2 flow.

Results and Discussion
Characterization of pilot-plant feedstocks
The functional group content of the Acetosolv lignin subjected to chemical modification is provided in Table 1.

Table 1. Chemical composition of Acetosolv lignin subjected to chemical modification.

Variable		Lignin		
		LE	LPT	LPR
Monolignol (%)[1]	H	4 ± 0.1	13± 0.6	3± 0.1
	G	21± 0.8	72± 1.3	65± 1.3
	S	75± 1.2	15± 0.9	33± 1.1
S/G ratio		3.6	0.2	0.50
-$COCH_3$ (%)[2]		8.8± 0.4	3.2± 0.1	7.1± 0.3
-COOH (%)[3]		0.3± 0.01	0.4± 0.01	0.8± 0.02

Average ±standard deviation, [1]monolignol unit (H: p-hydroxyphenyl, S: syringyl, G: guaiacyl) based on nitrobenzene assay/GC-MS, [2]by NaOH tritration (ISI, 1999), [3]by 1FT-IR determination (Kihara *et al.*, 2002).

As expected chemical features in term of monolignol reveals notable differences between procedences. All Acetosolv lignin show low H- content (3-13 %), while G- and S- content was strongly related to the source. LPT exhibited the lowest S/G ratio, LE show the highest S- content, while LPR reveals notable content of carboxylic acid moeties.
Results confirm the differentiated monolignol composition of hard-, soft, and non-wood sources.

Synthesis of HPLs
Lignin modification exhibited yields ranging between 87 and 96 %. Supernatand evaporation revealed low HPT-content.
Lignin derivatization showed high yields regardless the procedence, and the PO-charge. The collected HPL-precipitable fraction seems to be favored by the low solubility of derivatives in aqueous solutions at pH 2.
In addition, the isolation procedure enables an easy recovering of quantitative fractions of modified Acetosolv lignin with a minimun work-up.

Total phenols (TP) content and antioxidant capacity
Hydroxyl-content reveals the availability of -OH groups prone to react in accordance with the monolignol composition (Fig. 2).

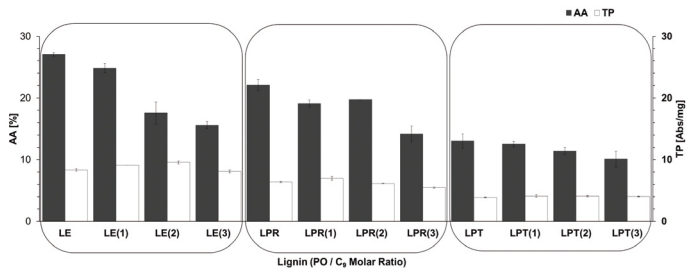

Fig. 2. Polyphenolic content (TP) and antioxidant activity (Antiox. Act.) of lignin, and lignin derivatives.

TP content reveals negligible differences in function to the PO/C9 equivalent. The result surprising because hydroxypropylation replaces aromatic by aliphatic –OH groups. However, the behavior may be explained based on the chemical structure of lignin-monomers in term of hydroxylation pattern.
Lignins and HPLs antioxidant-capacity oscillated between 10 and 29 %. The antiradical power was negative related to the PO-charge (Fig. 2). The range of activity correlated to several reports based on stilbenes, flavonoids, and procyanidins considered as the most active scavenger polyphenols (Stanković *et al.*, 2011).

Reaction efficiency ratio (^{1}H-NMR)
^{1}H-NMR spectroscopy is an accurate technique for detemining structural features in polyphenols. However, peracetylated derivatives spectra with a well-separated baseline acetates signals is required (Glasser et al., 1984). Spectra of native lignin reveals common aliphatic and aromatic signalS, slight differences mainly for aliphatic protons signals in term of multiplicity and intensities between 0.5-1.5 ppm is noticed (data not-shown).
Differences in hydroxypropylation efficiency was noticed in function of the PO/C_9 ratio (Fig. 3, bottom).
Lignin with the highest S/G content (LE, and LPR) show certain chemical resilience to be modified at room temperature. Additionally, LE (S/G: 3.6) exhibited significant content of aromatic –OH even at the highest PO/C_9.

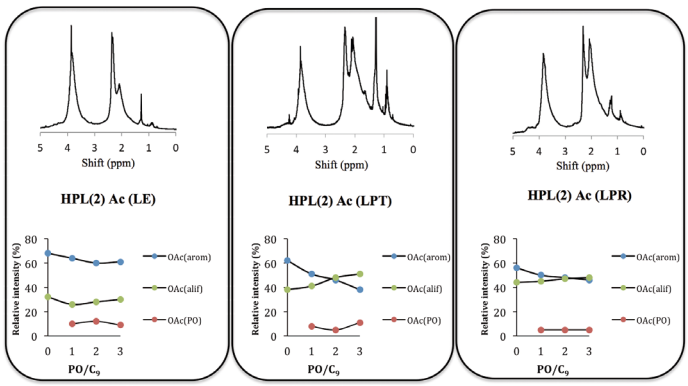

Fig. 3. (A) 1H-NMR spectra of hydroxypropyl Acetosolv lignin acetates illustrating aromatic and aliphatic signal features. (B) evolution of acetoxy groups of acetylated Organosolv hydroxypropyl lignin in function of molar equivalent. Note: Relative intensity is based on acetate signals.

Solubility test

Solubility of HPL samples shows dramatical changes in selected solvents (Fig. 4). In general, lignin modification decrease solubility in MeOH and acetic acid, while solubility increase slight in water/NaOH. HPL solubility virtually is less affected in protic than aprotic solvents. Surprising LE- exhibited the highest solubility changes.

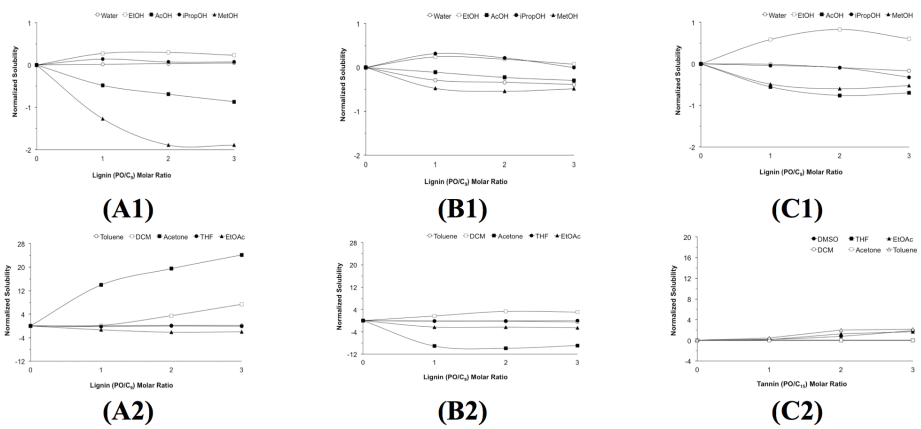

Fig. 4. Normalized solubility of HPL in different solvents at 21 ° C using the native lignin solubility as reference. (1) Protic solvents and (2) aprotic solvents. (A) LE-. (B) LPR-. (C) LPT-.

General trends show that hydroxypropylation decreases the solubility in polar solvents to increase the solubility in acetone and DMSO. Considering the solubility behavior, and the composition of the tested lignin, specific site of derivatization in term of aliphatic or aromatic –OH seems to affect the interaction of lignin derivatives with organic solvents.

Thermogravimetric analysis

Changes in thermal resistance of lignin in function to PO-charge was noticed (Fig. 5).

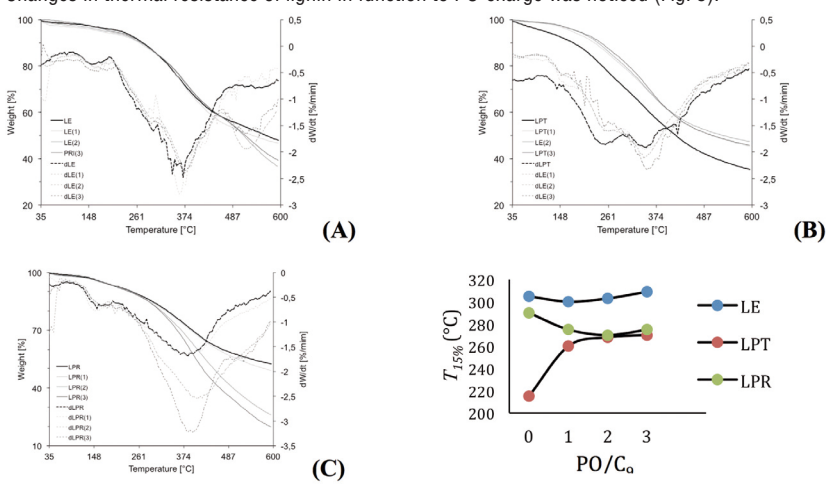

Fig. 5. Thermal decomposition of native lignins and HPL at 5 °Cmin^{-1} in N_2 atmosphere.

Thermal resistance of LPT-based derivatives was improved in the entire range of temperature. However, negligible differentes between LE-based derivatives estability were observed. In contrast, derivatization decreased the thermal estability of LPR-based HPL at high temperature (300 and 600 °C). Considering the results the highest PO-uptake the highest thermal resistance regardless the lignin-type.

Conclusions

Oxypropylation at room temperature of several Acetosolv lignin from *Eucaliptus sp., P. radiata*, and wheat straw obtained under pilot-plant conditions were performed for a first time. Spectroscopic and functional-group analysis provides valuable insight regarding reaction efficiency ratio and structural-properties relationship. A varied range of derivatization efficiency in function of the chemical structure were established. Lignin from wheat straw showed the best performance in term of enhanced properties. The highest S/G ratio favors the maximum hydroxypropylation efficiency at room temperature.

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Use of organosolv lignin modified by alkaline catalyst in adhesive resins: Evaluation of the behavior

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Abstract
The organosolv lignin from Eucalyptus globulus has been modified under alkaline conditions (NaOH, 173 °C, 33 min and 8,6 %) defined as optimal conditions using chemometric analysis. The modified lignin showed an increase in hydroxyl phenolic group determined by ^{31}P-NMR from 2830 to 5270 (µmol per gram of lignin) and decrease in the molecular weight according to SEC, from 1855 to 1581 (g/mol). The modified lignin was used to substitute 50, 60 and 70 % of phenol in lignin-phenol-formaldehyde resol (LPF) resins for plywood manufacture. The adhesion behavior of the resins obtained was evaluated using Automated Bonding Evaluation System (ABES) compared with a pure phenol-formaldehyde resin (PF) as control. The shear strength was tested a different temperature (100-160 °C) and pressing time (5-300 s). The new resins (LPF) showed a similar behavior with conventional pure phenolic resin; increase in the shear strength with pressing time (5 to 300 s) and temperature (100 to 160°C). Also, the maximum bond strengths reached after different pressing time and temperature, showed different bonding ability for LPF compare with PF, confirming that lignin could be possible to replace untill 60 % of phenol without loss of bond ability according to ABES, while, a decreasing of shear strength was observed when the replacement of phenol by modified lignin higher than 70 %.

Introduction
Actually, the lignin have been considered to be a raw material from lignocellulosic biomass with high potential of valorization that could play a key role in the further development of cost effective biorefinery process for biofuels, chemical and production of biobased material. The structural characteristics offers a wide range of alternatives for chemical and enzymatic modifications, however, in the lignin could be carried out depolymerization process to generate low molecular fractions remaining the oxy aromatic nature. The lignin could play a central role as a new chemical feedstock in a biorefinery concept [1]. Lignin can be used as a green alternative to many petroleum-derived products, such as oil-based resins, rubber additives, thermoplastic blends [2] carbon fibers [4,5] and activated carbon [3], as well as for generation of vanillin, vanillic acid, dispersing agents, polymer filters, DMSO, phenol, and ethylene, etc. [4]. The use of lignin as a wood adhesive has also been evaluated in several studies [5,6]. It has been suggested that the high content of phenolic hydroxyl groups in organosolv lignin from softwoods could be a potential source for the production of phenolic, epoxy, and isocyanate resins [7]. As is known, lignin macromolecules have multiple functional groups influencing the reactivity of lignin, such as methoxyl, phenolic and aliphatic hydroxyl, benzyl alcohol, noncyclic benzyl ether, and carbonyl groups [8].
For this purpose, the aim was to study the effect of an alkaline catalyst on lignin depolymerization and on the structure of newly generated compounds, with the goal of optimizing conditions to increase hydroxyl phenolic groups in organosolv lignin and use the organosolv lignin modified to replace phenol in lignin-phenol-formaldehyde (LPF) resol resins and evaluate their bonding capacity.

Experimental

The organosolv lignin was recovered by acidic precipitation from ethanolic black liquor derived from organosov pulping of E. globulus, which was carried out in a batch reactor (4550 Parr Instrument Company, Illinois, USA). The study of influence of different variables was realized using a factorial design. The variables and ranges studied in the process were temperature (T: 142–181 °C), time (t: 33,3–66.8 min), and concentration of NaOH used as alkaline catalyst (C: 3.3–11.7% w/w). The model was subscribed to a factorial design central composite with star points and the experimental design was optimized to a response variable, defined as the free hydroxyl phenolic groups determined by phosphorous nuclear magnetic resonance (^{31}P-NMR). The influence of each variable was determined using response surface methodology (RSM) and the Modde software (Umetrics, Sweden) was used for data processing. Free hydroxyl phenolic groups were determined by ^{31}P-NMR [9] and the molecular weight and molecular weight distribution of acetylated lignin was carried out using SEC (size exclusion chromatography) [10]. The resin synthesis was developed using directly the modified lignin solution as replacement of phenol in different substitution level of 50, 60 and 70 % w/w in lignin-phenol-formaldehyde (LPF) resins. Finally the adhesion performance of the new resins obtained were tested using Automated Bonding Evaluation System (ABES). The performance of LPF was compared with a pure phenol-formaldehyde resin (PF), using ABES at different temperatures (100-160 °C) and pressing times (5-300 s). The test condition were, wood veneers; (*fagus sylvatica*), moisture content between 6-8%. Specimens were prepared with a thickness of 5 mm (±0.04) and 20 mm (±0.05) by 117 mm (±0.05). The spread amount of adhesive was 180 g/m^2.

Results and discussion
Optimal factorial design
The factorial design applied to optimize the response corresponding free hydroxyl phenolic groups in modified lignin determined by ^{31}P-NMR was obtained, and the response polynomial for NaOH, was defined using multiple linear regressions, where Y_{pOH} represent the independent variable (response), defined as hydroxyl phenolic (OH-Phe) in the model, and the linear coefficient, quadratic coefficient, and interaction coefficient for dependent variables X_1 (temperature), X_2 (time), and X_3 (concentration of alkaline catalyst) are described in the following equations:

$$Y_{pOH(1)} = 5194.1(\pm115.9) + 159.5[X_1](\pm47.1) + 67.6[X_2](\pm61.5) + 377.6[X_3](\pm58.0) - 572.1[X_1]^2(\pm61.5) - 340.0[X_3]^2(\pm76.4) + 346.6[X_1X_3](\pm61.5) - 90[X_2X_3](\pm61.5)$$

Different coefficients indicated in the equation, were evaluated and showed that all variables influenced the model, and interactions between them were also observed. The influence of temperature, time, and alkali concentration studied by analysis of variance (ANOVA) for a confidence level of 95% showed that second-order polynomial models presented a high significance. The optimization of free hydroxyl phenolic group generation by alkaline catalysis was obtained and the contour diagram describing the estimated response surface for free OH-Phe shown in the Figure 1, with the reaction time fixed at 50 min. The results indicated that the maximal region of OH-Phe, found close to 5100 (µmol per gram of lignin), could be obtained when sodium hydroxide is used as alkaline catalyst under specific conditions.

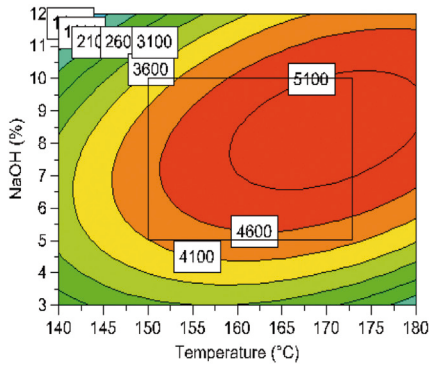

Figure. 1. Estimated response surface for free hydroxyl phenolic groups (µmol/g of lignin) obtained as a result of the optimization of the experimental design using NaOH as catalyst at a fixed reaction time of 50 min.

The optimal reaction conditions determined by the model, is showed in the Table 1. The experimental free OH-Phe in modified lignin determined by ^{31}P-NMR reached 5270 (µmol per gram of lignin) while, the maximal free OH-Phe predicted by the model was 5383 (µmol per gram of lignin) and the lignin-control was 2830 (µmol per gram of lignin).

Table 1. Free OH-Phe groups estimated and obtained under optimized reaction conditions.

Variables/sample	Lignin-NaOH
Alkali catalysts (%)	8.6
Temperature (°C)	173
Time (min)	33
Free phenolic groups predicted (µmol per g of lignin)	5383
Free phenolic groups obtained (µmol per g of lignin)	5270

Characterization of lignin and modified lignin

The hydroxyl groups content (aliphatic, carboxyl, and phenol) in lignin, determined by ^{31}P-NMR (Table 2) showed that an increase in the OH-Phe groups is mainly associated with an increase in OH-syringyl, condensed phenolic OH, and OH-guaiacyl, when compared to the amount of hydroxyl groups obtained under alkaline treatment of organosolv lignin compared with untreated lignin (lignin control). Also, variations of aliphatic and carboxyl hydroxyl groups were also observed, indicating that different changes in the structure of lignin occur as a result of alkaline treatment.

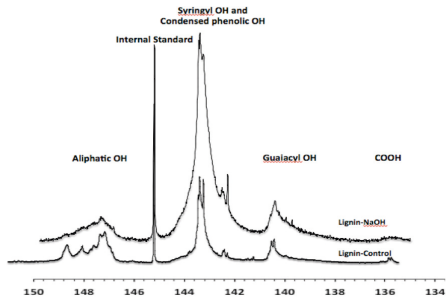

Figure 2. ^{31}P-NMR spectra of control and alkaline lignin

	OH (μmol/g)					
	Aliphatic	Condensed and syringyl OH	Guaiacyl-OH	Carboxyl-OH	Total phenolic	Total OH
Integrated shift range (ppm)	150–146.5	144.4–141.0	140.4–139	135.6–134.2		
Lignin-control	1820	2130	700	90	2830	4740
Lignin-NaOH	540	3960	1310	120	5270	5930

Table 2. Hydroxyl groups in control and alkaline lignin determined by ^{31}P-NMR.

Effect of alkaline treatment on the molecular weight of organosolv lignin

An important feature for the development of lignin-based products is the molecular weight and polydispersity of lignin derivatives; thus, the determination of these characteristics in lignin is a useful tool to estimate chain lengths for future applications. The results of molecular weight distribution obtained by GPC of acetylated lignin (lignin control) and after alkaline treatment are shown in Table 3. These results indicate that the M_w and M_n for lignin modified in optimal conditions is lower compared to lignin-control without important changes in in the polydispersity.

Table 3. Molecular weight effect of alkaline treatment of LOS. [a)]

Sample	M_w (g/mol)	M_n (g/mol)	M_w/M_n
Lignin-control	1855	815	2.3
Lignin-NaOH	1581	639	2.5

a)Polidispersity (Mw/Mn), Weight average (Mw) and number average Molecular Weight (Mn).

Adhesives performance evaluation.

The resins were prepared with different substitution level, 50 %, 60% and 70 % w/w of phenol by lignin, using the lignin-NaOH liquor fraction. The adhesive performance was evaluated by Automated Bonding Evaluation System (ABES) at different temperature and pressing time maintaining the viscosity between 300-450 cps. The measure of shear strength for LPF resins was compared with a pure phenol-formaldehyde resin (PF). The shear strength curves for resins with 50, 60 and 70 % of lignin-NaOH instead of phenol (LPF 50%, LPF 60% and LPF 70% respectively) are shown in Figure 3. In general, all resins showed a similar behavior in the initial state, with a rapid nearly linear increase of shear strength versus pressing time at different temperature and on the other hand, a decrease of bonding rate at higher pressing time. Moreover, LPF 50 % and LPF 60 % showed a better capacity of bonding ability than LPF-70 %, with maximum values close to 6.0 MPa.

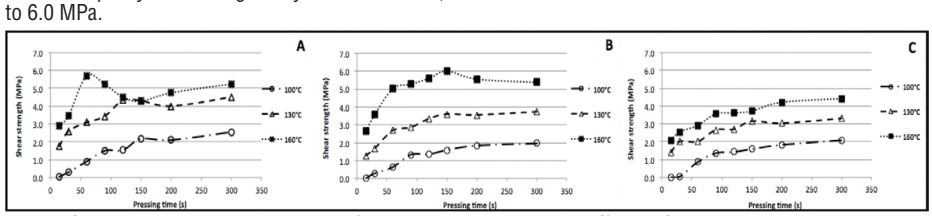

Figure 3. Shear strength development of LPF resins (LPF 50% A, LPF 60 % B, LPF 70% C) by ABES at different temperature and pressing time.

The adhesion performance of all LPF resins compared with PF control at different temperature, (Figure 4). The resins with lignin shown a similar and very low shear strength development in all resin at cure temperature of 100 ºC. However, with higher temperature of 130 and 160 ºC, the shear strenght of the new resins LPF, increased reaching maximum bonding capacity similar with the resin PF control. Thus, among all resins LPF formulated with by lignin-NaOH instead of phenol, LPF 50 % and LPF 60% showed a high potential as wood adhesive.

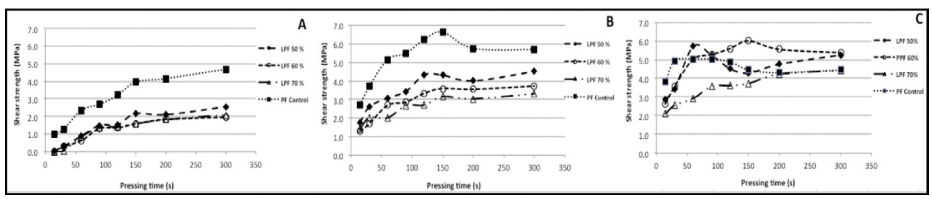

Figure 4. Shear strength comparison of LPF and PF resins at different press temperature (100 ºC A, 130 ºC B, 160 ºC C) using ABES.

Conclusions

The results show that under defined conditions of alkali catalyst concentration, temperature, and reaction time, optimized using chemometric tools, it was possible to obtain chemical changes in lignin organosolv from E. globulus for be used instead of phenol in conventional phenol formaldehyde resins. The modification of lignin by alkali catalyst produce a variation in the content of OH-functional groups, such as an increase in free OH-Phe, and carboxyl-OH groups, and a decrease in aliphatic-OH groups and a decrease in the molecular weight compared with unmodified lignin. The use of lignin modified for the synthesis of resins, showed that could be possible to replace untill 60 % of phenol by alkaline treated organosolv lignin. Under specifics temperature and pressing time, the LPF reached a maximum bonding capacity, close to shear strength performance of pure PF resin. Finally, the LPF resins formulated would be used to wood board manufacture in order to evaluate the real potential of LPF resins as replace of conventional resins used nowaday and generate an alternative of valorizing lignin to produce new chemical compounds and materials.

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Novel synthetic and natural adhesives: performance evaluation using ABES

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Introduction

Adhesives are involved in several industrial processes and comprise a wide range of applications (e.g., construction, aerospace, automotive, marine and bio-medical). Despite significant scientific progress, the design and modeling of joint adhesives remains under constant research. The mechanical behavior of composite materials is very complex due to inter- relationships among different factors, such as adhesive-substrate interaction, substrate nature, surfacing characteristics and operational conditions in board manufacturing. To address some of these issues, the Automated Bonding Evaluation System (ABES) was developed; it optimizes resources and improves the accuracy of board laboratory manufacture. ABES shows how strength is developed and affected by temperature, adhesive type, as well as substrate nature and process conditions. The present study illustrates the potential of ABES for several adhesives and test configurations. By varying the adhesives and substrate conditions it is possible to obtain information about adhesion performance and adhesive bond development under a wide range of conditions. Moreover, this contribution intends to explore how adhesion affects the design of board composite materials.

Experimental

An ABES instrument was used in order to evaluate several adhesives and wood veneers as substrate. The wood veneer samples were stored in a conditioned chamber at 20 °C and relative humidity of 53% prior to testing; two dimensions (0.7 and 2.6 mm) were tested. The probes were cut into 117 mm × 20 m strips using a pneumatically driven sample cutting device for ABES sample preparation (supplied by Adhesive Evaluation Systems, Corvallis, Oregon). In this study, a new overlapped area of 4 mm^2 was proposed, in comparison with the standard of 1 mm^2 that is most often used. The change in overlap area was introduced to reduce the effect of the small area and possible surface defects which affect adhesive performance and induce drawbacks. Figure 1 illustrates the general scheme of ABES for bonding evaluation.

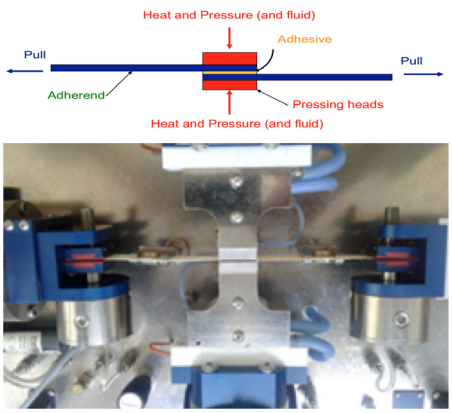

Figure 1. General scheme of equipment and traction of wood veneer using ABES.

For the bonding test, the adhesive was applied manually and the spread rate was controlled in an analytic balance. After the desired temperature was reached, adherent pairs of strips were mounted in the system with an overlapping area of 1 or 4 mm^2 (depending on the test), then pressed together at 1.2 N/mm^2. After the pressing time was elapsed, the probe was pulled with a standard loading rate of 1 kNs^{-1}. The bond strength was tested in shear mode, the system being digitally controlled and pneumatically driven, as illustrated in Figure 2.

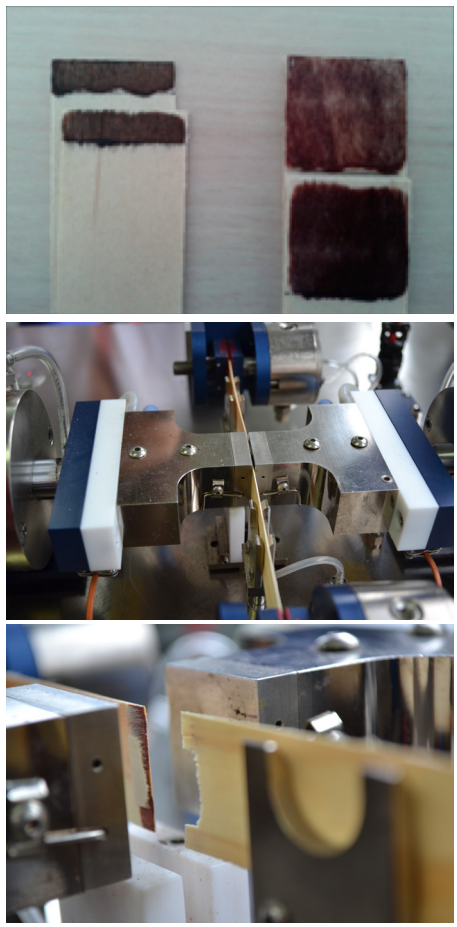

Figure 2. ABES Testing using standard and new area for bonding evaluation.

The first step of this study was to validate the change of the test area (1 to 4 mm^2) and the thickness of the veneer (0.7 to 2.6 mm), the last one corresponding to *radiata* pine veneer, widely utilized in industrial processes. Table 1 summarizes the test conditions.

Table 1. General ABES testing ccharacteristics.

Parameter	Value
Adhesive, type	Commercial PF and tannin*
Thickness, mm	0.7 and 2.6
Press temperature, °C	135
Press time, s/mm	12 - 150
Spread rate, g/m^2	180
Substrate	Strips and veneer

*Tannin adhesive formulation developed in UDT used for plywood manufacture.

ABES bond strength performance test.

Increasing test area.

The new area configuration (4 mm^2) was evaluated using standard thickness for new tannin-based and commercial adhesive (Figure 3a). In both cases the shear strength response increased for the higher area. At the same time, better consistency was observed for three repeated tests, as shown in Figure 3b.

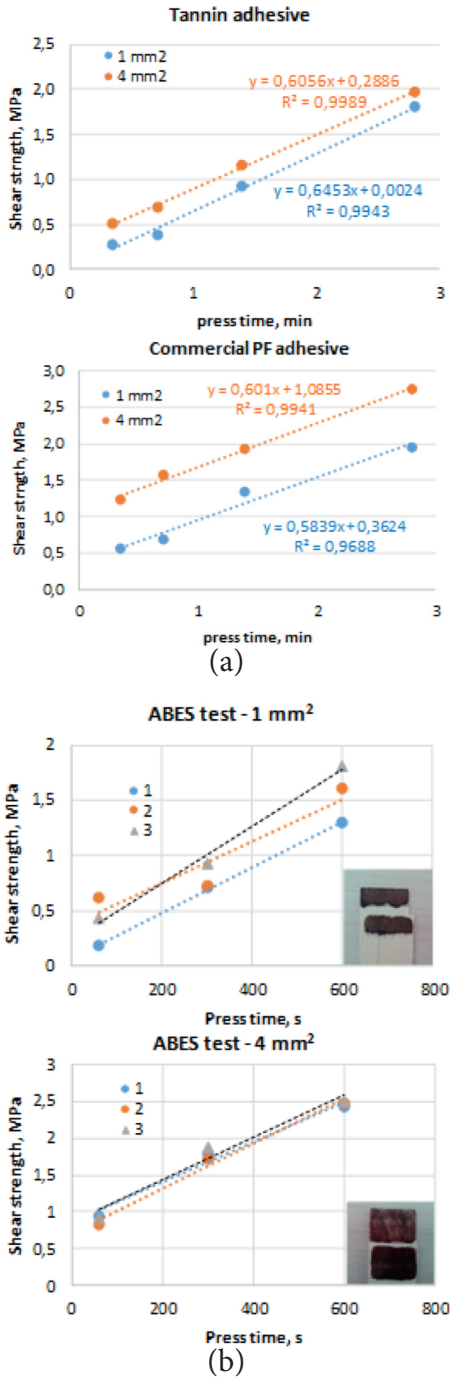

Figure 3. ABES comparison
a)Different areas for two adhesive systems; b)Response with three repeated tests.

Effect of crosslinking agent.

Two different probe thicknesses were evaluated, using industrial veneer samples of radiata pine (2.6 and 0.7 mm). The results show that the ABES methodology proposed here is a good alternative for the study of adhesives with industrial veneer for plywood manufacture. The development of bond strength, including features of the veneer (roughness, moisture content and nature), was close to real conditions for adhesive application (spread rate: 180 g/m^2; press temperature: 135 °C and veneer thickness: 2.6 mm). The evaluation test for veneer of 2.6 mm is therefore adequate for adhesive performance evaluation (Figure 4).

Based on previous results, probe configuration for ABES was established in order to obtain consistent response for industrial radiata pine veneer (2.6 mm and overlap area of 4 mm^2).
The next step was to study the ABES performance of tannin adhesives, with a crosslinking catalyst, to study the press temperature and tannin type effects.

Results and Discussion

Effect of catalyst on the performance of radiata pine tannin-based adhesive.

The effect of crosslinkers (hexamine) and/or "catalysts" was evaluated over tannin-based adhesive performance, considering radiata pine veneer thickness of 2.6 mm (Figure 4). At the same time, the effect of different crosslink percentages was evaluated, as shown in Figure 5.

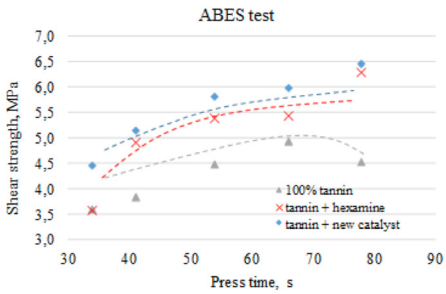

Figure 4. ABES evaluation for different adhesive systems based on tannin with and without hexamine and catalyst.

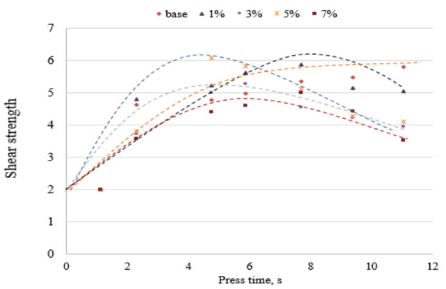

Figure 5. ABES evaluation for different catalyst contents in the new tannin adhesive.

The addition of hexamine, the traditional crosslinking agent used in tannin-based adhesives, was as expected. A higher shear strength with respect to tannin-based adhesives without a crosslinking agent was observed. Catalyst addition increased the curing rate as well, but beyond 5% a negative effect on adhesive performance was observed; it is attributed to dry-out potentiated by high catalyst content. In this sense too, ABES is a good tool to detect changes in adhesive formulation.

Effect of press temperature on the performance of radiata pine tannin-based adhesive.

The effect of press temperature was analyzed for the new tannin adhesive, as summarized in Table 2.

Table 2. ABES test conditions for new tannin adhesive.

Parameter	Value
Adhesive, type	Tannin*
Thickness, mm	2.6
Press temperature, °C	100, 135 and 150 °C
Press time, s	12 to 72
Spread rate, g/m^2	180
Substrate	*radiata* pine veneer

*Tannin adhesive formulation developed in UDT used for plywood manufacture.

As expected and shown in Figure 6, the shear strength development is faster at higher temperatures. The adhesives tested have very different curing behaviours, depending on the press temperature; this is observed clearly using ABES.

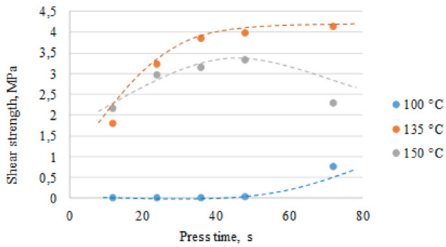

Figure 6. ABES for tannin-based adhesives at different press temperatures.

The corresponding pressing conditions, at the industrial level, should be adapted according to these results. In each case, for tannin-based adhesives, high temperature was needed for good performance. Therefore, for the new tannin systems, 135 °C is the recommended press temperature for higher bond strength results. At 100 °C it is not possible to produce the curing reaction, and a higher temperature, such as 150 °C, pre-curing phenomena likely compete with adhesive diffusion which can impede veneer penetration prior to curing.

Effect of tannin type on adhesive performance.

Comparative analyses for commercial PF resin with adhesives based on different tannin sources (radiata pine, mimosa and quebracho) are shown in Figure 7. Radiata pine tannin exhibited similar behavior to the commercial PF adhesive.

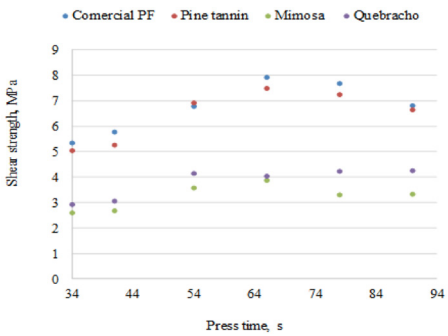

Figure 7. Comparative results from ABES of adhesives used in plywood manufacture.

Conclusion

The ABES instrument is a powerful predictive tools for adhesive performance evaluation, including conditions that approach industrial requirements. Its application could obviate the need to use more expensive resources for research and development in the adhesives field. It is possible to cover a wide range of formulations and substrates in less time and this allows one to identify, in a first stage, the most promising formulations. Although ABES cannot replace board manufacturing and characterization according to standard tests, it can minimize the number of runs to be performed in the process and product design stages. The ABES testing equipment using 2.6 mm veneer for plywood manufacture is thus a good tool for industrial-level prediction of adhesion performance.

Acknowledgement.

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Biochar-based materials for the sustainable catalysis and photocatalysis

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Abstract

Applications of biochar-based materials in catalytic and photocatalytic processes are presented in this work. Sawdust from a soft wood was used to prepared biochars by carbonization, thermochemical and physical activation and these biochars were used as a co-support for the H_2 photoproduction on Au-TiO_2/biochars under visible irradiation. A remarkable increase in the photocatalytic activity of the composite up to a factor about 3 times higher than the commercial catalyst free of biochars was found and ascribed to the surface pH of biochars. Biomass-derived molecules such as furfural, chitosane, and saccharose were used as carbon source to prepare hybrid C-TiO_2 materials by solvothermal synthesis. It was found that carbonized hybrid TiO_2-C supports led to an important enhancement in the catalytic activity of Pd-based catalysts in the electrooxidation of formic acid with a maxima density power up to 3.3 times higher than the same catalyst supported on a commercial carbon. In addition, Pd-based catalysts showed that hybrid Biochar-TiO_2 supports can be designed to control the selectivity of phenol hydrogenation (up to 100% yield) to cyclohexanone or cyclohexanol by controlling the chemical nature of the biochar-based supports. Enhancements up to a factor about 2 and 5 times higher photocatalytic activity that the commercial semiconductor were found in the photodegradation of methylene blue under visible irradiation by using S-doped and N-doped Biochar-based/TiO_2 materials [1].

1. Introduction

Biochar-based materials have gained an important role in the sustainable society of XXI century [1]. This is mainly due to the effective application in different catalytic [2,3] and photocatalytic [4] processes. For example, the use of biochar-TiO_2 hybrid materials showed an additional approach because these materials are in agreement with the green chemistry principles [5]. For example, these materials have showed important and potential application in hydrogen photoproduction by using solar irradiation [6], polluted water and air remediation [7-12], and in selective photooxidation [10]. C-TiO_2 is a non-toxic and biocompatible material and it is relatively cheap and easy to prepare in one-step procedure [7,13]. The objective of this work is to show that heteroatoms-doped biochar-based hybrid materials prepared under eco-friendly conditions can be used in different photocatalytic processes related to polluted water remediation and clean energy production.

2. Experimental

Synthesis of non-doped and S- or N-doped biochars have been described elsewhere [1,6,8,12]. Au–TiO_2/AC photocatalysts preparation was performed as follows [6]. Au was firstly deposited at basic pH on a commercial TiO_2 (Avonik, ex-Degussa) and then Au-TiO_2/C hybrid materials were prepared by a slurry method [9,10]. C-doped TiO2 hybrid materials were prepared by solvothermal synthesis from mixtures of furfural, chitosan and saccharose with titanium isopropoxide [2,3,7]. Characterization was performed by N_2 adsorption–desorption isotherms, X-ray photoelectronic spectroscopy (XPS), surface pH (pHPZC), infrared spectroscopy (FTIR), X-ray diffraction (XRD), UV-vis/diffuse reflectance spectra (UV-Vis/DR), and electron microscopy (TEM/STEM). Experimental conditions for the H_2 photoproduction, and methylene blue (MB) photodegradation tests have been carefully described elsewhere [6-8,11-12].

3. Results and Discussion

A summary of the kinetic results of MB degradation are given in Table 1 and 2. In presence of S- and N-doped biochars, the photoactivity increased up to about 2 and 5 times higher than on TiO_2 alone. The photoactivity of the biochar depends both on the texture and on the S and N content [11,12]. The photoactivity was confirmed by PS and CV analysis [11] suggesting that S incorporations decrease the energy band gap in carbon-based materials. S- [11] and N-doped [12] carbons are not only photoactive buth also they photoassist to TiO_2 by electron transference of electrons from π* orbital in S- or N-doped biochars to the conduction band of the TiO_2.

Table 1. Summary of kinetic results for the MB photodegradation on S-doped carbons

Sample	v_o [a] (μmol.L-1. min-1)	v_{rel} [b]	k_{app}x10^{-3} [c] (min-1)	R [d]	φ_{photo} [e]
C (6. 3 mg)	0.291	1.04	6.00	0.9740	1.3
C-S (6.3 mg)	0.579	2.1	10.28	0.9693	2.2
B (6.3 mg)	0.262	0.93	4.00	0.9870	0.9
B-S (6.3 mg)	0.536	1.9	8.56	0.9917	1.9
TiO_2 (62.5mg)	0.281	1.0	4.61	0.9849	1.0

[a]Initial rate: $C_{eq}.k_{app}$. [b] Initial activity relative to TiO_2: $v_{o\text{-}AC}/v_{o\text{-}TiO2}$. [c]Apparent rate constant (k_{app}). [d]Square regression factor. [e]φ_{photo} defined as $k_{app\text{-}AC}/k_{app\text{-}TiO2}$

Table 2. Adsorption in the dark (Ads_{dark}) of MB, apparent first-order rate constants (k_{app}), square regression factor (R^2), photocatalytic activity relative to TiO_2 (φ_{photo})

Photocatalyst	Ads [a] (%)	k_{app} x 10^{-3}(min^{-1})	R^2	φphoto[b]
PK	4	1.7	0.9078	0.38
PN	2	2.0	0.9376	0.44
PNA	5	2.3	0.9840	0.51
PNO	2	1.3	0.9637	0.29
TiO_2	22	4.5	0.9858	1
TiO_2-PK	28	22.5	0.9871	5.0
TiO_2-PN	23	17.1	0.9931	3.8
TiO_2-PNA	15	23.9	0.9887	5.3
TiO_2-PNO	16	16.2	0.9914	3.6

[a]After 60min adsorption . [b]φ_{photo}= ($k_{app\text{-}f}/k_{app\text{-}TiO2}$).

H_2 photoproduction results are given in Table 3. It can be seen an increase up to 3 times higher photoactivity on Au-TiO_2/C than that on Au-TiO_2 and clearly much higher than that on neat TiO_2 which is non-photoactive under visible irradiation. This increase was attributed to an increase in the plasmon resonance of gold [8] due to the transference of electron density from the conduction band in TiO_2 modified by the interaction with oxygen functional groups on AC [8,11,12]. The stability of photocatalytic activity of these materials was studied following the kinetics of H_2 production during consecutive photocatalytic runs and No gold lixiaviation during reaction was detected and photoactivity remains constant at least for three consecutive runs.

Table 3. First-order rate-constants (k_{reac}) for the hydrogen photoproduction

Sample	k_{reac} [a] (mM.min^{-1})	R^2
TiO_2-P25	b	b
Au-TiO_2-P25	0.044	0.9790
Au-TiO_2/AC_{CO2}	0.115	0.9840
Au-TiO_2/AC_{N2}	0.094	0.9965
Au-TiO_2/AC_{ZnCl2}	0.062	0.9730
Au-TiO_2/AC_{H3PO4}	0.052	0.9890

[a]k_{reac} obtained from the kinetics of hydrogen production. [b]No photoactivity was detected.

Table 4. Kinetic results of phenol hydrogenation on Pd-based catalysts. Initial activity, maxima phenol conversion (Ph_{conv}), selectivity of cyclohexanone ($S_{C=O}$) and cyclohexanol (S_{OH}).

Catalyst	Pd[a] (wt %)	Activity [b] (μmol.gPd^{-1}.s^{-1})	Ph_{conv} (%)	$S_{C=O}$ (%)	S_{OH} (%)
Pd/TiO_2-P25	1.1	45.5	99	98	2
Pd/Fu-TiO_2-C	1.0	71.0	99	97	3
Pd/Sac-TiO_2-C	0.9	40.3	99	93	7
Pd/Fu-TiO_2-C-C	0.9	156.2	99	9	91
Pd/Sac-TiO_2-C-C	1.0	60.8	99	8	92
Pd/TiO_2-AC_{CO2}	0.9	62.1	99	24	76
Pd/TiO_2-AC_{N2}	1.0	48.3	99	19	81
Pd/TiO_2-AC_{ZnCl2}	0.9	91.1	99	96	4
Pd/TiO2-AC_{H3PO4}	0.9	62.2	99	89	11

[a]wt % of palladium measured by ICP chemical analysis. [b]Phenol converted (μmol)/Pd_{weight}.sec.

The results of selective phenol hydrogenation [3] on Pd-based catalysts supported on TiO_2-C are given in Table 4. The nature of the support can be modified to direct the reaction either to cyclohexanol (~100% yield) or to cyclohexanone (~96% yield). High selectivity to cyclohexanone is obtained with Pd on more polar TiO_2-C supports (4), while when these are transformed into hydrophobic TiO_2-C the catalyst becomes selective to cyclohexanol. This remarkable change in selectivity was attributed to the stabilization of charges in the semiconductor by the electron transference from carbon materials, which is clearly enriched when it is thermal treated at high temperatures, thus becoming in a basic and hydrophobic support. In short, selective phenol hydrogenation is easily controlled by controlling the functionalization of the hybrid support. Figure 1, shows the mechanism proposed for the selective hydrogenation of phenol to cyclohexanone [3].

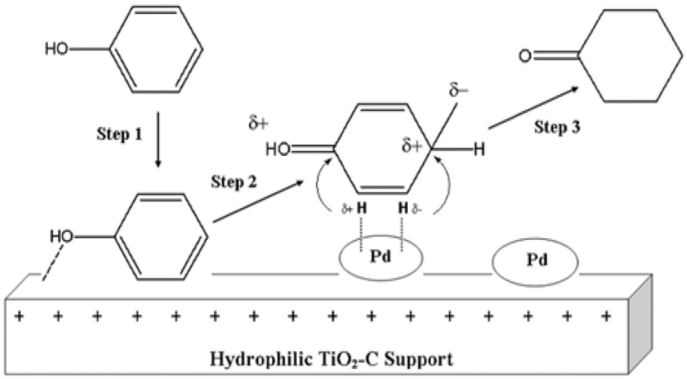

Figure 1. General pathway for selective hydrogenation of phenol to cyclohexanone [3].

Finally, we have found in a recent work [2], that carbonized hybrid TiO_2-C supports prepared by solvothermal synthesis from mixture of titanium alcoxides and biomass-derivative molecules such as furfural, chitosan, and saccharose, led to an important enhancement in the catalytic activity of Pd-based catalysts in the electrooxidation of formic acid with a maxima density power up to 3.3 times higher [2] than the same catalyst prepared on a commercial carbon. This results can be seen from figure 2.

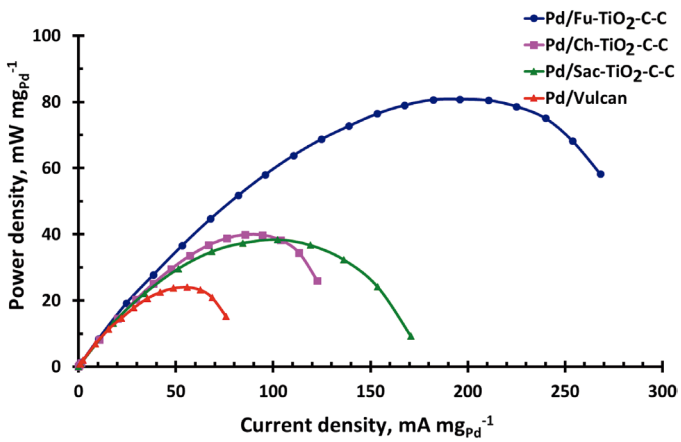

Figure 2. Power density developed on Pd-based catalysts as a function of current for the different Biomass-derived-TiO_2 supports and comparison against a commercial carbon.

Conclusions

Different biochars and hybrid biochar-TiO_2 materials were prepared by different methods and studied in several catalytic and photocatalytic processes. An important increase in the photocatalytic activity of Au-based catalysts supported on TiO_2-C materials was found in the H_2 photoproduction under visible irradiation. The composite showed up to a factor about 3 times higher than the commercial catalyst free of biochars was found and ascribed to the surface pH of biochars. It can be concluded that biomass-derived molecules such as furfural, chitosane, and saccharose are usefull to prepare hybrid C-TiO_2 materials by solvothermal synthesis and these hybrid TiO_2-C supports led to an important enhancement in the catalytic activity of Pd-based catalysts in the electrooxidation of formic acid and at the same time, hybrid Biochar-TiO_2 supports can be designed to control the selectivity of phenol hydrogenation (up to 100% yield) to cyclohexanone or cyclohexanol. Enhancements up to a factor about 2 and 5 times higher photocatalytic activity that the commercial semiconductor were found in the photodegradation of methylene blue under visible irradiation by using Biochar-based/TiO_2 materials. In summary, it can be concluded that biochars-based materials show new perspectives for the sustainable catalysis and photocatalysis related with clean energy production, green and selective catalytic processes, and for the environmental remediation of polluted water by solar technology. This approach is showed in a schematic way in the Figure 3.

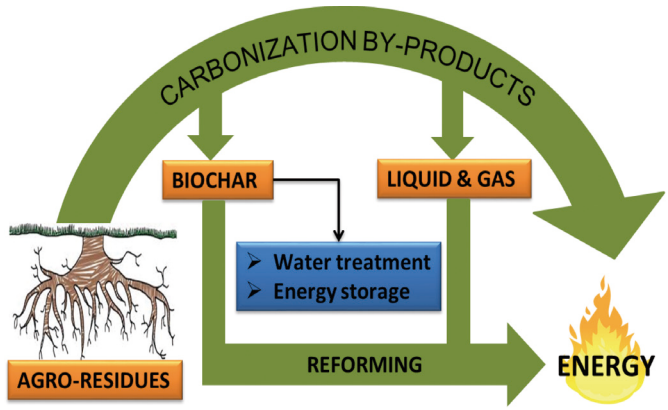

Figure 3. Production of biochar-based materials and applications for the clean energy production, green and selective catalysis, and for the environmental remediation of polluted water by solar technology.

Acknowledgment

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Carbohydrates and cellulosic fibers applications

Between two stools: the paper industry in a change
Frank Miletzky, Papiertechnische Stiftung, Germany

Frank Miletzky

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Abstract
The paper industry is in a state of dramatic structural change. Millions of tons of production capacity for graphic paper have been shut down in the previous decade, in particular in North America and Europe. Reason was the relocation of regional production capacity in response to changes in global newsprint and magazine paper demand caused by the omnipresent digital revolution.
The industry is still looking for answers and solutions to meet these challenges. There are two basic approaches to overcome the current situation: up-stream and down-stream scenarios. Forest owners are following the up-stream path, i.e. enhancing the value of forests, wood and wood components to create multiple profits via platform chemicals, bio-energy, cellulose, hemicellulose and other engineered products. Paper producers owning no extensive woodlands have to look for other solutions: in particular in developed countries and mainly in Europe, so called "urban bio-refineries" seem to be viable alternatives. The predominant fibre source used in these countries is recycled fibres. Within EU countries, close to 70 per cent of the fibrous raw materials used for papermaking are based on paper for recycling. The real value of recycled fibres is greatly underestimated today. Improved separation and refining processes would make it possible to use recycled fibres also in high-tech applications. Additionally, fibre engineering techniques based on completely new approaches could enable the use of recycled instead of virgin fibres in full-scale applications.
Paper itself (as defined by us today) or fibre based materials like nonwovens offer great potential to generate value in sectors like building, automotive and aircraft or for lightweight construction in general, thanks to attractive features like the global availability of bio-based raw materials, recyclability, high strength-to-mass-ratio, high productivity levels and easy processing. Furthermore, paper technology offers opportunities to treat manifold other types of fibrous materials besides of cellulose, e.g. basalt, glass, plastic or carbon fibres.

The paper industry has been in a state of fundamental change for more than a decade. In the graphic paper segment, profitability is low despite extremely productive paper machines. Many paper companies are struggling to survive, and their situation is really dramatic. Financial strength has decreased continuously since approx. 2000, as illustrated by the development of global share indices in forest and paper sectors compared to basic industries. Forest & paper shares have not profited from the commodity boom (Fig. 1).
The question today is whether or not the paper industry is able to implement an innovation and adaption process to sustainably renew itself and regain profitability. The next question is which unique assets of paper can provide the basis and are used as fundamentals for this (Tab.1).

CEPI, the European association of paper producing industries, has issued a 2050 Roadmap towards a "low-carbon bio-economy" in 2011. This roadmap addresses today's greatest challenges in the field of production: -80 % CO_2 emissions, +50 % value added by current and new products based on so-called "break-through technologies". The document manifests a visionary strategy. It reflects also present constraints and drawbacks that make it difficult to turn the ideas into sustainable pillars of the sector's renewal as a sustainable, innovative industry and bio-economy in the face of climate change. In order to realize the vision, a so called "Two Team Project" was launched, resulting in eight innovative concepts that

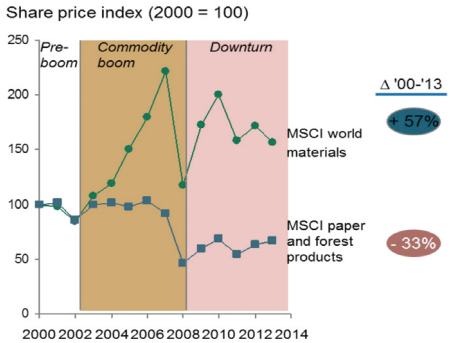

Fig.1. Global downturn of the P&P share values (courtesy of R. Haslehner, BCG: PTS Coating Sym-posium 2013)

will show – after finishing the R&D work - how the necessary breakthroughs could be made. Some of the concepts are still under investigation, some have provided already interesting results, but still on the level of basic research.

Strengths	Weaknesses
Maximum productivity	Inflexible processes (scale!)
Bio-based raw materials	High-tonnage production lots
Specialized technical products	Raw material & energy intensive
Globally present & available	Low level of integration
Cost efficient	One-dimensional value creation (basic industry)
	Capital intensive

Tab. 1: Characteristics of the paper industry today

As the CEPI activity focusing on technology and processes, a next project with the programmatic title "Paper & Fibre World 2030" has been launched by the German associations of paper producing, paper converting and supplying industries.
First step of the project was the evaluation of nearly 100 international studies on global trends carried out by scientific institutions, think tanks, NGO's and political bodies. Using a clearly defined procedure, topics were identified that hold potential for fibre based solutions in application areas like building, energy supply, mobility, health care, food, logistics and other. Links to paper or paper-based materials could be identified in many cases, but lightweight construction, functionalisation and compound formation with other systems were areas providing concrete starting points.

It helps to think outside of the box here: In the textile industry, visionary action has led to a significant renaissance of textiles for manifold applications, e.g. for hygiene or technical products like aircraft components. It was not conceivable 20 years ago that airplane components would be made from technical textiles one day!
Applications like this became a main driver leading to two digit growth rates and profitability in recent decades. Based on fibre consumption technical textiles achieved more than 25 % of the global textile production representing a 250 bn $ market in 2012 (Commerzbank. Corporate Sector Report "Technical texiles", 2014). These concepts can also point the way to the future of wood and paper companies, as long as they understand themselves and act as parts of a bio-based value chain and real bio-economy.

Summing up, answers and solutions must be found to meet the following challenges:

1) Developing more flexible machines and processes for more valuable products

2) Integration in the overall value chain by multiple utilization and higher participation

3) Gaining access to completely new application areas

1) Flexibility
Many enterprises of the paper sector pursue a strategy of adaption (Fig. 2), i.e. they respond adaptively to market developments and try to access segments with higher profit margins like packaging or specialty papers. This can be a successful strategy if they adjust not only their product portfolios, but all business activities to the new market situation and „rules", including the development of sustainable, innovative products. Packaging continues to be a commodity market where overcapacity can soon or later be expected to result in similar changes and developments like in the graphic paper segment today. By contrast, the specialty paper segment still offers some unique selling propositions – to companies capable of reading and understanding the market and adjusting their products to the specific requirements of customers and end users. Good examples can be found in areas like filter materials, nonwoven wallpapers and technical products like electrical papers or coated abrasives.

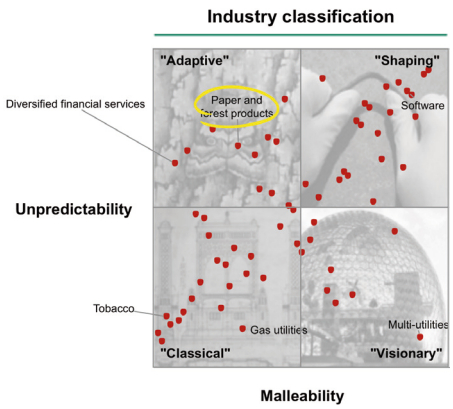

Fig.2. Position of the P&P sector R.Haslehner BCG. PTS Coating Conf. 2013

2) Integration
Paper companies basically have two options:
Up-stream integration based on wood. This option is of interest especially to integrated mills owning woodlands. Forests are seen as the origin of added value, i.e. wood is the first stage of the value chain and starting point for multiple value creation. Sulphite pulp mills were almost bio-refineries 100 years ago, manufacturing marketable products like bioethanol, high-protein feed yeast, lignin products, furfural or acidic acid parallel or complementary to cellulose pulps. Their value creation was sufficient, but dropped in the second half of the previous century when petroleum and coal and the products derived from them became cheaper and more easily available. Companies lacked the know-how and technology to separate valuable materials like vanillin or mono phenolic substances from material mixtures.

Current concepts like the wood-based „Nordic Biorefinery" rely on kraft pulping; organosolv processes have not yet become commercially viable for full-scale applications (Acetocell, Milox, ASAM, Organocell). All have been unable to solve the basic problem of a technology that is specially designed for the main process of cellulose production. More recent developments (LignoBOOST etc.) indicate, however, that they can serve as basis for value creation as well.

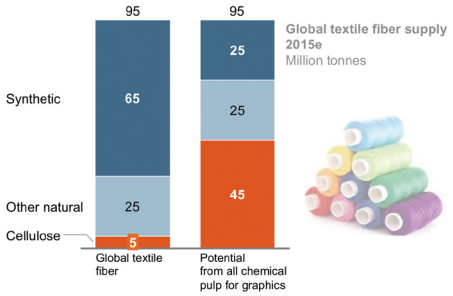

Fig.3. Hypothetical example: current cellulose prod. for graphic paper could significantly sub-stitute synthetic fibre (courtesy of Peter Berg, McKinsey, 2015

The Two-Team Project of CEPI has led to an outstanding breakthrough concept as well: DES stands for Deep Eutectic Solvents, a method that is expected to enable the dissolution of wood into its main components cellulose, hemicelluloses and lignin at room temperature and atmospheric pressure. The technology is still in the stage of basic research, but the sector is pinning great hopes on it.

Up-stream integration based on recycled fibres. This option focuses on integration into urbane areas, especially conurbations that produce large amounts of paper for recycling and for which it makes sense to develop recycled fibre based bio refineries. Paper for recycling is collected municipally or by private firms, pre-sorted into standard grades and supplied to paper mills today.

The composition of individual grades varies greatly, especially in terms of fibre and ash conetents. Clearly defined formulations are therefore almost impossible to achieve, and the quality can only be optimised by monitoring the process situationally. Moreover, the recycling process leads to high amounts of residues that are usually incinerated or otherwise disposed of, for example minerals, fibres and fibre fragments, additives, admixtures, contaminants and impurities, plastics etc. A specifically designed, efficient treatment process that is capable of simultaneously producing high-quality fibres and ethanol or bio-energy whilst separating most of the synthetic materials, additives, minerals and perhaps even ink particles would make these bio-refineries economically viable. Chemical or industrial parks would be suitable locations offering the necessary infrastructure. Several bio-refinery concepts have been planned, engineered and calculated, and suitable locations have been found as well, but at least in Germany they are far from being implemented.

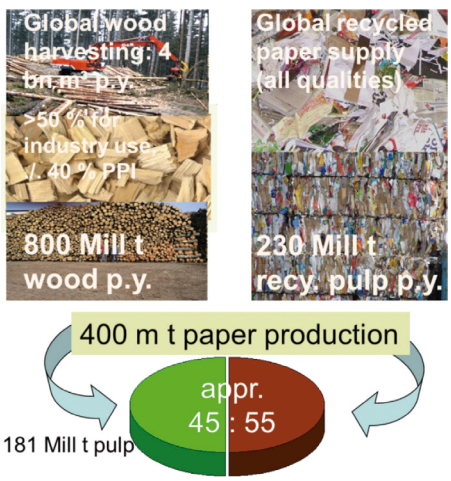

Fig.4. Global cellulosic fibre potential. VDP Report 2014

Down-stream integration. Extending the value chain towards customers means integrating production and converting, as demonstrated by the example of leading corrugated board companies. This can result in more added value provided that conditions and performance are adequate. The application of CPS (Industry 4.0) is expected to lead to significantly higher productivity and flexibility in this context.

3) New application areas

Adapting existing systems and solutions to changing requirements cannot be the only strategy for the future.

To identify applications and market opportunities that will really be sustainable and profitable in future, we must look further ahead and into other areas. We need to investigate and develop paper materials to make them suitable for high-quality applications and design solutions.

The market is broad and ready for this – successful examples can already be found in the specialty paper segment.

Lightweight construction has been one of the main growth drivers recently. The subject has certainly been surrounded by some hype, but there are also a number of profound economic and technical arguments in favour of it. Main drivers are the growing mobility in all areas, i.e. logistics, transport and all other applications where weight reduction can lead to significant energy savings.

Lightweight construction combines lightweight materials with specially adapted design solutions and manufacturing technologies. Development was mainly driven by the changing demands of mobility sectors (automotive, aerospace) here, focusing first on the use of lighter materials (aluminium; SpaceFrame ® technology) and at present mainly on CFC, FRP and similar fibre composites based on high-strength filaments. Because of the close links with textile technology and high innovativeness of textile producers, applications were mainly seen in the textile industry first. Moreover, methods of textile manufacturing turned out to be excellently suitable for filament treatment to achieve the well-defined properties and high quality needed for precursors offering the desired strength.

Fibre composites evidently hold the key to more lightweight but equally functionable designs – which raises the question why the paper industry's powerful technological platforms should not enable it to take a more prominent role in the area of fibre composites, and what it takes to create successful solutions for this area.

When looking for new solutions in the field of fibre composites, we tend to focus too much on filaments, forgetting that all three components – material, design and manufacturing technology – must be optimally designed and combined. We need to check, for example, if and where it is possible to establish solutions based on „short cut fibres". The trend towards lighter structures remains unbroken – we need to keep on developing new solutions to meet market demands here. Users in the mobility sector, for example, accept considerable extra costs in order to save weight. What is true for the vehicle itself cannot be wrong for the transported good. This brings us back to packaging. What we have in mind here is often just the end use packages on supermarket shelves. Industrial packaging, however, involves high-tech design solutions with great development potential. The same applies to architecture and interior design. What we are talking about here, though, is premium high-volume rather than niche products, which require first of all innovation and new network structures.

The paper industry should see lightweight construction as one of its future markets, and we are well advised to intensify our research and development activities in this area. We need new networks to transfer our bio-based solutions into innovative systems, i.e. a creative mixture of various disciplines to meet the present challenges. This must also be reflected by our training and recruitment practices.

Conclusions

Bio-refineries offer a huge field of innovation. But they have to be discussed in the context of an overall bio-economy. It is necessary to consider multiple approaches to use wood and other bio-based materials originating from wood or annual plants either for platform chemicals or as materials or integrated utilization concepts. We should keep in mind that some hundred million tons of bio-based materials like paper, wood or agricultural products are currently underused and not recycled on the same level of value added. This potential has to be utilized in future concepts. Recycling processes must not only be improved quantitatively, but also qualitatively. In particular by-products and functional additives have to be reused on the same level of value creation. The paper industry is changing dynamically and develops towards a part of bio-based economy. New application areas require greater knowledge and an increased awareness of the scientific profiles and networks needed to really make progress on industrial scale.

Ethanol-water fractionation of wheat straw and saccharification of the cellulosic residue

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Lignocellulose biorefineries have emerged as a sustainable alternative in comparison with the production of first generation ethanol, because they do not compete with the foodstuffs for the arable land and valorize low value agro-forestry residues [1]. In this scenario, lignocellulose fractionation into: cellulose, lignin and hemicelluloses has become a bottleneck due to the costs of the fractionation itself and the generation of substances which could inhibit the further enzymatic stages [2]. Fractionation with solvent-water mixtures appears as an alternative to the acid hydrolysis. In this work two fractionation procedures: ethanol-water and diluted sulfuric acid were applied to wheat straw and compared in terms of cellulose yield and energy consumption.

Wheat straw (100g) were fractionated with an ethanol-water mixture (2/1 v/v) or alternatively, with a diluted sulphuric acid solution. The experiments were carried out in a 6 L reactor provided with liquor recirculation, temperature control and sampling. For both set of experiments, the variables were liquor/solid ratio, temperature and time at constant temperature. For ethanol-water fractionation, the effect of adding a 1% of sulphuric acid (on dry straw) was also tested. Liquid samples were taken at different time and sugars analysed by HPLC, using a Hi-Plex-H column at the conditions recommended by Agilent. Solid fraction were removed at the end of the experiments, washed and weighed. Enzymatic saccharification tests were made with a mixture of cellulases (Celluclast 1.5 L plus Novozym 188 1:1.1 v/v; 200 FPU/g dry solid).

Table 1: Pretreatment conditions

	Experiment	Glucose	Xylose	Arabinose	A.A	HMF	Furfural	Lignin
	Raw material	50,07	29,32	2,69	2,51	-	-	12,06
EW (2/1 V/V)	EW1	67,93	16,08	0,63	1,69	0,62	1,84	15,81
	EW2	76,70	9,25	0,43	1,13	0,77	0,85	12,00
	EW3	72,25	11,30	0,32	1,42	0,68	0,94	10,46
	EW4	69,61	11,19	0,64	1,77	0,85	1,50	10,70
	EW5	62,87	18,33	0,72	2,48	0,72	2,09	13,07
	EW6	38,24	23,42	3,19	2,24	0,43	1,88	13,95
DSA	DSA1	69,64	6,69	0,00	0,91	0,73	0,65	20,58
	DSA2	65,77	6,79	0,22	1,02	0,63	0,70	25,28
	DSA3	54,93	13,41	0,57	1,88	0,62	1,64	22,32
	DSA4	51,19	20,46	1,06	2,98	0,55	2,56	17,31

Results have shown that, the yield of the solid fraction is mainly dependent on the fractionation temperature and ranges from 46% (170ºC) to 75% (130 ºC). In comparison with the diluted sulphuric acid pre-treatment, and for a similar energy input, the ethanol-water liquor is more selective to remove lignin and produce a solid residue of higher carbohydrate content. This method maintains the cellulose percentage a 3-5% higher and reduces the lignin in the same amount. Lignin has been reported as a possible inhibitor in the further enzymatic saccharification of sugars [2] and so, must be maintained as low as possible. Another difference is related with the hemicelluloses distribution in the residual solid. While the acid hydrolysis dissolves most of the xylans, ethanol-water delignification (at about 150ºC) preserves most of the xylans in the solid phase, what avoids the generation of furfural. Both solid fractions showed similar ability to be enzymatically hydrolysed.

Table 2: Composition (% dry matter) of raw material and pretreated substrate.

	Experiment	Catalyst	Tempera-ture (ºC)	Time (min)	Factor H	% Yield (Pretreat-ment)
Ethanol-Water (EW) (2/1 v/v)	EW1	-	170	90	1473	63,19
	EW2	H_2SO_4 (96%) 1%	170	90	1435	46,23
	EW3		170	60	956	47,93
	EW4		160	45	344	48,46
	EW5		150	45	140	58,49
	EW6		130	45	23	74,63
Diluted Sulfuric Acid (DSA)	DSA1	H_2SO_4 (96%) 1%	170	90	1475	51,06
	DSA2		170	60	1092	50,97
	DSA3		150	60	185	54,49
	DSA4		130	60	28	72,59

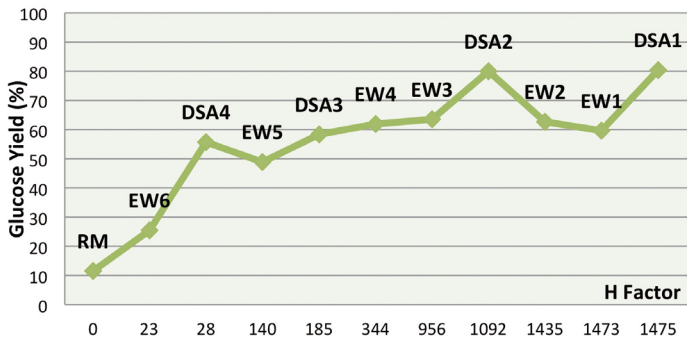

Figure 1: Glucose Yield vs. H Factor for the fractionated solids.

For a similar energy input, the diluted acid hydrolysis has shown better glucose yield than Ethanol-Water (Fig. 1), what, apparently, contrasts with the better cellulose selectivity which exhibit the last one. Figure 2 shows that the solid residue produced by acid hydrolysis has better quality to be enzymatically hydrolyzed, what would explain the better glucose yield.

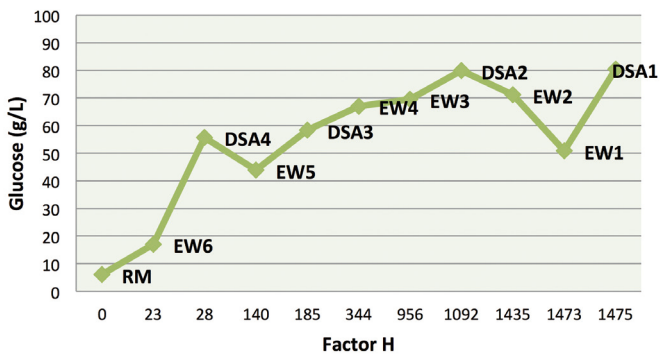

Figure 2: Glucose production at the enzymatic saccharification stage.

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Determination of hemicellulose extraction conditions from alkaline-sulfite pretreated sugar cane bagasse with a crude enzymatic extract from *Bacillus pumilus*

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Abstract
Sugarcane bagasse was pretreated by a chemithermomechanical process (CTM), using alkaline/sulfite solutions and the enzymatic extraction of hemicellulose between washed and unwashed pretreated bagasse was compared. The maximum hemicellulose extraction was obtained for the washed (25.7%) and unwashed (18.5%) bagasse pretreated at 10% w/w Na_2SO_3 and 5% w/w NaOH, the highest chemical loading tested. Increasing the enzyme load from 5 to 20 U/g did not increase the extraction of hemicellulose. Washing the material after the pretreatment enhanced the enzymatic extraction, suggesting that some inhibiting compounds were present.

Introduction
The production of ethanol from sugarcane is already consolidated in the Brazilian market. However, possibilities to better use the biomass have been studied over the years, converting the ethanol mills in biorefineries. Currently, after grinding sugarcane to produce sugar and ethanol, 125 kg of bagasse are generated per ton of processed sugarcane (1) which is currently burnt to produce steam and heat. This residue consists of 40-45% glucose, 25-27% hemicellulose and 19-24% lignin (2). To add value to this biomass, development of the technology, especially regarding to hemicellulose extraction and processing, is required.

Hemicellulose is a complex carbohydrate, mainly 4-O-methyl-glucuronoarabinoxylan in sugarcane bagasse, and its use is important in the biorefinery concept. The extraction of this xylan can be performed by various procedures, and the chosen method depends on the targeted product (3,4). Enzymatic extraction of xylan has gained attention, mainly based on the use of endoxylanases (5-7). These enzymes act on the main chain of xylan, releasing xylo-oligosaccharides (XOS), which have a great potential for use in pharmaceuticals, agriculture and food (8). Bacteria of the species *Bacillus pumilus* are promising for this application, since they produce low or insignificant levels of cellulases and β-xylosidases whereas the endoxylanases produced are active at high temperatures and neutral to alkaline pH (9,10)

As sugarcane bagasse has a compact structure and is recalcitrant to enzymatic hydrolysis; a pretreatment to facilitate access of the enzymes to the polysaccharides is required to increase the efficiency of hydrolysis (11). Thermomechanical process using alkaline sulfite facilitates the separation of the macromolecules by weakening the bonds that keep the structure of the plant cell wall. In this pretreatment, the cellulose is swollen, the fibers are mechanically separated, and the lignin is sulfonated and partially removed (12).
This study aimed to characterize the hemicellulolytic system of Bacillus pumilus in order to isolate hemicellulose from pretreated sugarcane bagasse as xylo-oligomers, resulting in a residual substrate less recalcitrant to the hydrolysis by commercial cellulases.

Experimental
Bacterial strain, inoculum preparation and xylanase production
The strain was maintained in culture medium described by Mandels and Stenberg (13) and stored in refrigerator at 4 °C. The inoculum was prepared by transferring cells from the subculture to 250 ml Erlenmeyer flasks containing 25 ml of maintenance medium (13). The inoculated flasks were incubated at 45 °C, 200 rpm for 20 hours. The enzyme production was carried out under the same conditions of the inoculum preparation, replacing the glucose carbon source by wheat bran, oat spelt xylan, sugar cane bagasse or chlorite-delignified bagasse. Culture pHs were adjusted to 8.5 or 9.5 with NaOH. The inoculum load was 8% v/v, corresponding to 108 cells/ml and the flasks were incubated at 45 oC for 24 h at 200 rpm. The supernatant was analyzed for xylanase, endoglucanase, β-xylosidase, β-glucosidase and arabinofuranosidase activities.

Enzymatic assays
The xylanase activity was determined according to Bailey et al. (14), and endoglucanase was performed according to Ghose (15) using carboxymethylcellulose as substrate (CMC - Sigma). Reducing sugars were measured using 3,5 dinitrosalicylic acid (16). The enzymatic activities of β-glucosidase, β-xylosidase and arabinofuranosidase were performed according to Tan et al. (17). All activities were determined in 50 mM sodium phosphate buffer, pH 8.0 and 50 ° C.

Chemithermomechanical pretreatment of sugarcane bagasse
A series of pretreated bagasses were prepared by controlled cooking with the alkaline sulfite liquor at a bagasse/liquor ratio of 1:10 (w/v). The alkaline sulfite liquor corresponded to loads of 1.25, 2.5 and 5 g of NaOH (per 100 g of dry bagasse) combined with 2.5, 5, and 10 g of Na_2SO_3 (per 100 g of dry bagasse), respectively. Impregnated biomass was cooked at 120 oC for 120 min followed by refining on a disk refiner (Regmed MD-300 with 0.1 mm disk clearance and 250 Wh of energy consumption) (18). Part of the pretreated bagasse at the harshest condition was washed with distilled water up to reach pH 7. The characteristics of the washed and unwashed pretreated materials are given in Table 1.

Chemical composition
Raw and pretreated sugarcane bagasses were ground to 20 mesh and extracted with 95% ethanol for 6 hours. Approximately 300 mg of the extracted materials (dry mass) were hydrolyzed with 3 ml of 72% (w/w) sulfuric acid at 30 °C, for 1 h. The acid was diluted to 4%, by adding 79 mL of water and the mixture was heated to 121 °C at 1 atm, for 1 h, in autoclave. The resulting material was cooled and filtered through a porous glass filter number 3. The solids were dried at 105 °C until constant weight, to determine the mass of insoluble lignin. The filtrate was analyzed by UV spectroscopy, using wavelength of 205 nm and molar extinction coefficient of 105 L/g/cm, to determine the concentration of soluble lignin. At this wavelength, there is almost no absorbance of furans. The monomeric sugars in the filtrate were quantified by HPLC, using a BIO-RAD HPX-87H column at 45 °C eluted at 0.6 mL/min with 5 mM sulfuric acid. Sugars were detected using a temperature controlled refractive index detector (19).

Extraction of hemicelulose by enzymatic treatment
The pretreated bagasse samples were suspended in phosphate buffer pH 8 (50 mM) (5% dry matter) and submitted to hemicellulose extraction based on the enzymatic treatment at 50 °C during 24 h under stirring. The enzymes corresponded to the culture broth from *Bacillus pumillus* grown in wheat straw medium. The enzymes loading tested were 5, 10 and 20 U of xylanase per gram of bagasse. The treatment temperature was evaluated in the range of 30 °C to 70 °C using a fixed enzyme loading of 20 U/g. Aliquots were withdrawn at regular intervals, boiled for 5 min at 100 °C and centrifuged at 4750 g for 25 minutes.

Pre-extracted xylans were treated with sulfuric acid for chemical characterization (20). The released monomers (xylose, arabinose and glucose) were analyzed on HPLC (Waters) using sulfuric acid 0.005 mol/L as eluent at a flow rate of 0.6 ml/min with a refractive index detector at 35 °C (Waters 2414) and a BioRad HPX-87H column at 45 °C (19).

Extraction of hemicellulose with sodium hydroxide
Some samples of the residual solids recovered after the enzymatic treatments were further extracted with NaOH. Alkali loads were varied from 10 to 70 % (w/w). The extraction was carried out for 30 min at 30 oC (21).

Results e Discussion
Xylanase production of Bacillus pumilus
Carbon source is one of the essential constituents of the microbial fermentation medium which affects the growth and metabolism. The commercial oat spelts xylan, wheat bran, sugarcane bagasse and chlorite-delignified bagasse supported high xylanase production by *B. pumilus*. The highest xylanase activity (38.4 U/ml) was obtained in 1% of wheat bran at pH 8.5. Other studies report wheat bran as a very suitable substrate for the production of xylanase on a commercial scale because it contains nutrients required for microbial growth and arabinoxylans for the induction of enzymes, and it has a lower cost than commercial xylan (22-24). The crude enzymatic extract was also characterized for presenting low β-xylosidase and β-glucosidase activities and no endoglucanase or arabinofuranosidase were detected. These data corroborate previous studies reporting the production of cellulase free xylanases in cultures of *B. pumilus* (9, 10).

Effects of the pretreatment process parameters on xylan extraction
Table 1 shows the solids yield and the chemical composition of pretreated materials. The solids yields of unwashed pretreated materials were always higher than 84%. Pretreated materials were enriched in cellulose and hemicellulose due to the partial solubilization of lignin. Values in parentheses show the percentage of removed components in the pretreatment liquor. The solubilization of the components was higher after washing. However, the washing procedure released mainly carbohydrates from the pretreated material.

Pretreated materials were subjected to enzymatic xylan extraction using the crude cultured broth from *B. pumilus* as enzyme source. Unwashed materials from all pretreatments were subjected to enzymatic hydrolysis for 24 h at 5% (w/w) solids content, using 5 U/g bagasse, 10 U/g bagasse and 20 U/g bagasse. Table 2 shows the total xylan and arabinosyl recovery after enzymatic treatment. The highest total xylan extraction (18.5%) was obtained for the bagasse pretreated with 10%/5% (sulfite/alkali) and the maximal xylanase load tested. However, the use of enzymes on bagasse pretreated with lower chemical loadings was less efficient and no differences in the extraction of xylan and arabinosyl groups were observed when enzyme loads were increased. In fact, the ratio of arabinose to xylose in the original materials was very similar, but it differed in the hydrolysates of the xylanase treated bagasses. The xylan in bagasse pretreated with the highest chemical load presented the highest proportion of arabinose substitution.

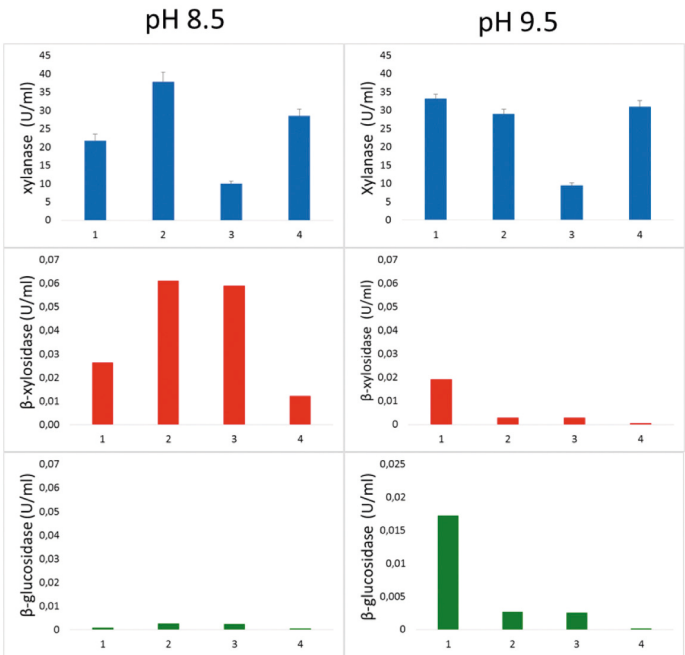

Figure 1: Effect of various carbon sources (1- xylan oat spelts; 2- wheat bran; 3- bagasse; 4- chlorite delignified bagasse) and pH on enzyme production in submerged culturing of *Bacillus pumilus* after 24 h of incubation at 50 ºC.

Table 1: Process yield and chemical composition of sugarcane bagasse submitted to alkaline-sulfite pretreatments performed at increasingly chemical loads

Chemical load[1]		Bagasse Components (%)			
Na_2SO_3	NaOH	Yield (%)	Lignin	Hemicellulose	Cellulose
0	0	100	22.0±0.1	23.8±0.4	42.5±0.2
2.5	1.25	86.7	20.8±0.4 (17.9)	22.4±0.2 (18.4)	39. 4±0.1 (19.8)
5	2,5	84.1	18.1±0.6 (31.3)	23.9±1.0(15.5)	43.8±1.8 (13.4)
10	5	86.2	13.8±0.4 (46.1)	25.3±0.4 (8.7)	45.5±0.6 (7.9)
10	5	73.92	12.6±0.2 (57.6)	25.8±0.2 (20)	47.3±0.5 (17.8)

[1] g/100g of bagasse; [2]washed bagasse. Values in parentheses represent the percentage of each component dissolved from original sugarcane bagasse.

Table 2: Effect of enzyme dose on xylan extraction of sugarcane bagasse submitted to alkaline-sulfite pretreatments performed at increasingly chemical loads by the crude enzymatic extract of *Bacillus pumilus*

SCB pretreated with Na_2SO_3/NaOH and refined	Enzymatic loading (U/g bagasse)	Extraction (% w/w)		
		Xylan	Arabinosyl residues	Xyl/Ara (w/w)
2.5% /1.25%	5	3.2±0.2	7.9±0.2	6.4
	10	3.2±0.3	7.9±0.6	6.5
	20	3.6±0.3	8.5±0.3	6.2
5% /2.5%	5	4.6±0.7	8.0 ±0.7	6.5
	10	5.2±0. 5	8.0±0.6	7.4
	20	6.3±0.2	8.3±0.2	7.4
10% /5%	5	13.6±0.6	32.9±3.0	4.4
	10	15.8±0.6	34.3±2.1	4.9
	20	18.5±0.3	38.9±2.4	5.1

SCB: sugarcane bagasse.

Even with similar hemicellulose contents, the enzymatic xylan extraction varied among the pretreated materials (Table2). This difference was attributed to the presence of lignin, which probably reduced the access of the enzymes to the substrate in the materials pretreated at lower chemical loadings.

There was no evidence for the presence of monomeric sugars in the hydrolysates of enzyme treated materials. This observation was in agreement with the low levels of β-glucosidase, β-xylosidase and arabinofuranosidase detected in the crude enzymatic extract. These results suggest that *B. pumilus* enzymatic extract has desirable characteristics for xylo-oligomers extraction.

The xylan extraction yield increased with the treatment temperature up to 50 oC. At higher temperatures, thermal deactivation of the enzymes seemed to occur since extraction yields decreased again (data not shown).

Effect of a second-stage alkaline extraction

After hydrolysis of the pretreated bagasse with xylanase for 24 h, the remaining solids were subjected to a second stage of alkaline extraction, at 30 °C, for 30 minutes. This procedure aimed to assess if partially xylanase-degraded xylan, still remained in the material, could be extracted in the second stage. The results showed that significant improvements in xylan extraction or savings in chemicals can be obtained when the alkaline extraction is preceded by enzymatic treatment with xylanases (data not shown).

Effect of washing the pretreated bagasse

One of the pretreated materials (10% Na_2SO_3/5% NaOH) was selected to evaluate the effect of a water washing after the pretreatment step. For this assay, 20 U of xylanase/g of bagasse was used. Xylan extraction yield was, in general, higher for the washed material than for the unwashed material, but the products identified in TLC were the same. On a percentage basis, the xylan extraction yields from unwashed and washed pretreated materials were 18.5% and 25.7%, respectively. This was also observed for the extraction of arabinosyl groups (40.4% and 50.45%, respectively). It is probable that byproducts remained in the unwashed pretreated material caused the inhibition of enzymes (25, 26). However, this increase in extraction should be studied for its viability, due to the larger volume of water in the washing step. This is not only expensive but also a major source of the water borne pollutants.

Conclusions

Although some alkali/sulfite loadings tested resulted in pretreated bagasses with similar hemicellulose content, the extraction efficiency varied, depending more on the lignin content than on the enzyme loading, suggesting that the presence of lignin limited the extraction of hemicellulose. The highest hemicellulose extraction yields were obtained at hydrolysis carried out between 40 ºC and 50 °C. Xylo-oligossacharides, with different degrees of polymerization were present in all hydrolysates, even in the conditions that resulted in low extraction yields. Monomeric sugars were not observed. The data suggest that the xylanase from *B. pumilus* might be suitable for industrial applications, considering the use of the hemicellulose fraction in the biorefinery concept.

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Addition of poly-electrolytes on recycled fiber for paper sheet formation

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Abstract
Colloidal phenomena have been under study for several decades, especially the interactions in suspensions of virgin fibers and cationic polyelectrolyte. However, research involving the adsorption of polyelectrolytes on secondary fibers and their impact on the distribution of fibers is limited. For this reason, the need to increase the knowledge the behavior of secondary fiber in fiber suspension. The interpretation of the results of this phenomenon will reveal the impact on the uniformity of fiber distribution. This investigation studied the adsorption mechanism of four polyelectrolytes polydimethyldiallylammonium chloride (poly-DADMAC) from high and low molecular weight, and its effect on the distribution of secondary fiber in paper sheet. The results achieved and its analysis is useful in design and structuring of paper sheet, to know the contact time and polyelectrolytes concentrations suitable to improve the consolidation of the fibrous network in paper. The study builds on the modified model of Langmuir with collision theory. Measurements of the adsorption kinetics were performed by polyelectrolyte titration using a particle charge detector Mütek PCD 03. The distribution was evaluated using an image analyzer using a Leica software suite LAS V4 and stereomicroscopy MZ7,5 working in an area of 78.72 mm². The results demonstrated that the required concentration of low molecular weight polyelectrolyte was 14,6 mg g^{-1}, to have a full coverage of the fiber surface in a contact time of 10 min. The pictures showed that in absence of polyelectrolyte there is a 65.4% area covered by fibers uniformly distributed. The analysis of fibers treatments with polyelectrolytes using the theoretical model reported that higher molecular weight polyelectrolyte require more time to achieve coverage of fiberss

Introduction

This paper studied the adsorption mechanism of four polyelectrolytes: polydimethyldiallylammonium chloride (poly-DADMAC) of high and low molecular weight and its effect on secondary fibers distribution during paper sheet formation. The information is useful in the design and structure of the paper when recycled fiber is involved, to find the contact time and polyelectrolyte concentrations to improve the consolidation of fibrous network. The study builds up on the implementation of the modified Langmuir model with the collision theory.

The formation of paper sheet is a continuous process, in which the cellulosic fibers, organic and inorganic fines and chemical additives consolidate a great three-dimensional network; on the basis of a mechanical intertwining of fibers and the chemical interactions between the various elements of the fibrous suspension. Paper sheet can be observed with a greater perspective on a smaller scale, based on: individual chemical flocs of fibers present in the consolidated structure, mechanical links and structures of the inner walls of fiber, microfibrils orientation on every wall, until to reach the α-cellulose molecule. Each of these levels directly influences on the paper structure and properties. For example: Uniform distribution of fibers into the structure, physical properties.

The increasing incorporation of secondary fiber as raw material is mainly due to environmental and economic concerns. The use of secondary fiber faces challenges as the natural aging of fibers and the fibers induced characteristics during the previous process, as well as adhesives contaminants, etc.

Understanding these challenges is a necessity in the production systems in order to produce papers with acceptable use properties. An alternative to match the physical properties between a paper made with high virgin fiber and a high recycled fiber content is the application of mechanical means of shear forces, flow velocity, turbulence, etc., [1]. However, these are limited by the presence of mineral fillers and fine particles of fibers (fines), which affect drainability during the consolidation of the network, [2]. Therefore it is necessary to add additives in the fibrous slurry which allow to increase the strength of paper in both dry and wet. These additives are mainly cationic polyelectrolyte because the cellulosic fibers have negative charges.

The principal function of the additives in fibrous suspension is retaining dissolved colloidal particles such as fine and inorganic fillers, furthermore, additives neutralize the dissolved ions in the aqueous system called anionic trash [3]. The retention and neutralization is originated because of electrostatic charges of all components in fibrous suspension. Through these actions, the additives to be added to the formulation of fibrous suspension, significantly improve the uniformity of consolidation the fibrous [4]. PolyDADMAC has been used mainly as a fixative cationic fine particle deposit process derived from recycled fibers [5].

Migration or diffusion of a polymer in a porous surface occurs as a function of a concentration gradient of electrostatic charges on the particles involved (cationic or anionic). The anionic charges, which are found in the pores of fibers act as driving forces to adsorb the cationic polyelectrolyte [6]. This diffusion can be hindered by steric and electrostatic hindrance. In this sense Zezin [7] suggests two mechanisms to explain the process of diffusion of a polyelectrolyte in the pores of fiber wall. The first mechanism assumes that the electrostatic attraction between the oppositely charged polyelectrolyte with the pores of the fiber wall is strong enough to achieve diffusion. The polyelectrolyte therefore could diffuse through a layer previously adsorbed and then set in the first charge available in the pores, this theory was supported by Horvath [6]. They analyzed by fluorescence microscopy adsorption of cationic dextran and pDADMAC copolymers, and concluded that the diffusion of the polyelectrolytes is accomplished by electrostatic attraction mechanism. The second proposed mechanism is called "relay race" which involves adsorption of the polyelectrolyte on the fiber surface, once adsorbed is flat conformation covering the pores of the fibers [8].

Experimental

Raw material used

The fibrous material used in this research was paper with a first cycle of use and paper with "n" cycles of use. They were analyzed according to TAPPI [9] techniques in its ash content (TAPPI T-211), moisture content (TAPPI T-412) and degree of drainability "Freeness" (TAPPI T-227). Having determined these properties, a proportion of fibrous mixture was established for subsequent experiments involved in this research. The ratio of the mixture was 70 units fibers with n cycles of use and 30 units of first generation fibers.

Four cationic polyelectrolyte were used: polydimethyldiallylammonium chloride (polyDADMAC), classified according to their molecular weight: high molecular weight Mw = 400,000-500,000 (ApM), medium molecular weight Mw = 200,000-350,000 (MpM) and low molecular weight Mw = 150,000-200,000 (BpM); the fourth polyelectrolyte Mw = 350,000-400,000 (CATIOFAST®111). This last will generate a comparison perspective (ApM), (MpM) and (BpM) of the phenomena occurring in fibrous suspension in the process of design and structure of the paper. The turning radius of Catiofast, BpM, ApM and MpM are $1.93x10^{-6}$, $8.6x10^{-7}$, $1.66x10^{-6}$ and 7.22x10-6 [Å] respectively.

The adsorption kinetics was evaluated by polyelectrolyte titration using a particle load detector Mütek PCD 03. The fiber distribution was evaluated using an image analyzer composed of software Leica LAS suite V4 and stereomicroscopy MZ7,5 in a working area of 78.72 mm².

Britt Jar and a flat tube was designed through which fibrous suspension passed, Figure 1. The Britt jar was used to control the homogeneity of the fibrous suspension as a function of the stirring speed.

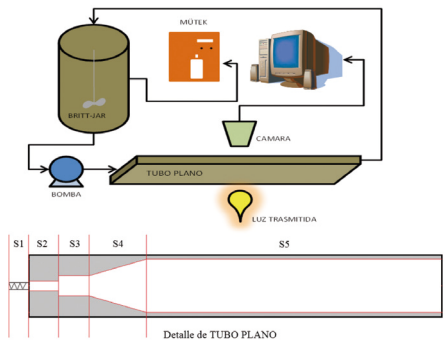

Figure 1, Experimental equipment

Results and discussion

The evaluation of the fibrous raw material outcomes are reported in Table 1, wherein difference shows between these different sources of fibers, ash content, refining degree, fiber length, fine, and Kink and content Curl Index.

Table 1, Fiber characterization

	Fiber characterization						
Raw Materials	Moisture, %	Ash, %	CSF	L, mm	IK, 1/ mm	IC,	Fines, %
First generation	4.8	0.83	650	1.8	0.74	0.06	15
"n" cycles	4.8	4.01	480	1	0.69	0.045	21

The analysis of water used in the study are in Table 2, it allows to evaluate the behavior of polyelectrolyte in the interaction with the ions from the process water.

Table 2, Initial evaluation of charge potential of the polyelectrolytes in presence of water

Ionic demand µeq L-1		
Polyelectrolyte	Tap water	Deionized water
CATIOFAST	2163,65	1693,82
Low molecular weight, BpM	1951,58	1647,85
Mean molecular weight, MpM	1586,24	1612,52
High molecular weight, ApM	1372,64	1584,89

With this information it is worked with a block design wherein the response variable was the ionic demand (μeq L-1) and the controlled factor was the type of water used for ion demand.

According to analysis of variance for ionic polyelectrolyte demand was obtained the information of Table 3:

Table 3. ANOVA Table for neutralizing the ionic demand of the fibrous suspension by the addition of polyelectrolyte

Source	Factors Sum of Squares	Gl	Mean Square	Reason-F	Value-P
MAIN EFFECTS					
A: Polyelectrolyte	242606,00	3	80868,5	1,69	0,3385
B: Kind of water	35806,21	1	35806,2	0,75	0,4507
RESIDUE	143555,00	3	47851,8		
TOTAL (CORRECTED)	421967,00	7			

The quantity of polyelectrolyte for saturating fibers depends on the ionic demand of the fibrous suspension, in this study a demand of 0.02507 μeq g^{-1} was obtained for involved fiber. When using 5 g o. d. of fibers, the ionic demand is 0.11235 μeq. This last data was the setpoint to determine concentration of polyelectrolyte expressed as mg g^{-1} (milligrams per gram of polyelectrolyte fiber) for saturating fiber and neutralize the system Figure 2.

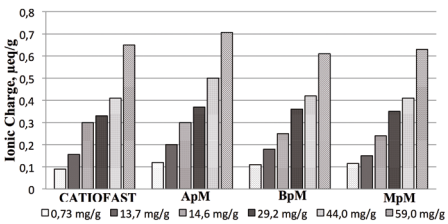

Figure 2, Ionic charge demand in suspension

Results of applying the linear model of Langmuir isotherms to the experimental data of the adsorption equilibrium of polyelectrolytes on fibers are presented in Figure 3. In this, it appears that the relationship between the inverse of the relative concentration of polyelectrolyte on fibers (1/Γ) and the inverse of the value of relative concentration of polyelectrolyte ($1/\gamma_{\infty}$) have a good arrangement because in all R^2 were 0.99.

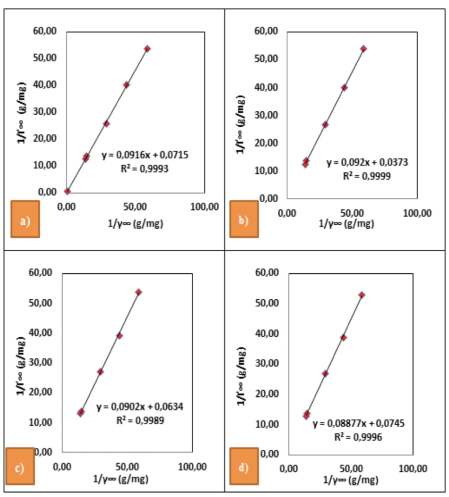

Figure 3, Linear form of the adsorption isitherm of the polyelectrolytes on recycled fibers, a) Catiofast, b) ApM, c)MpM and d) BpM

Also, the calculated value of maximum amount of polyelectrolyte which may be adsorbed on fibers are shown in Table 4.

Table 4. Determination of values of the parameters: R^2, Γmax and K.

Polyelectrolyte	R^2	K	Γ_{max}
CATIOFAST	0,9993	0,0916	13,98 mg/g
(ApM)	0,9999	0,0920	25,80 mg/g
(MpM)	0,9989	0,0902	15,72 mg/g
(BpM)	0,9996	0,0887	13,42 mg/g

The obtained results with the theoretical model using experimental values of the coverage factor, here is generally notice that there is no good compromise between them. However, polyelectrolyte of low molecular weight (BpM) is the one most to approaches the theoretical model (Figure 4) . In comparison to medium and high molecular weight.

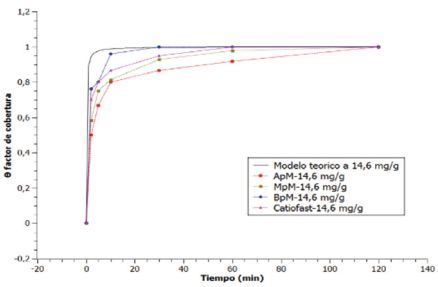

Figure 4, The Surface Factor of coverage of polyelectrolytes on fiber as function of time

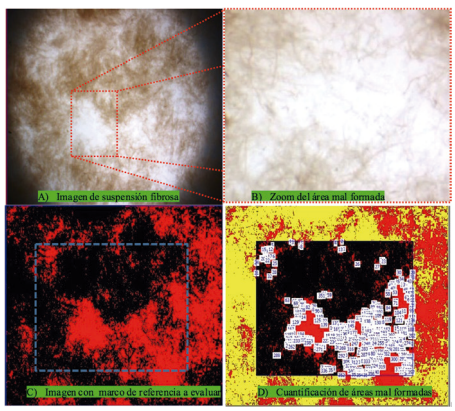

Figure 5, Outline the ajust process of parameters for image analysis

the image above composed of 4 images shows: A) fibrous slurry; B) extension of irregularly distributed area (25X); C) Selection of the framework) D: quantifying irregularly distributed area. Regions with a greater flocs presence, a framework situated in the middle of the image (C) was established, in order to prevent disturbance of focus lens, since in the corners of images obtained are shadow because of the camera lens. This framework had an area of 37.65 mm^2. Finally in the image (D) were quantified the fields unevenly distributed in function of color ranges with parameters (RGB), with this process were obtaining respectively values of (R = 247, G = 250 and B = 236).

Once determined the parameters of image analysis, it was proceeded to calculate the irregularly distributed are for each polyelectrolyte, and it was obtained that when a concentration of 14.6 mg g^{-1} (= 1) was used at different contact times in fibrous suspension irregularly distributed area decreases as seen in Figure 6.

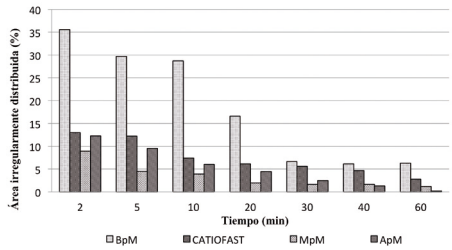

Figure 6, Area distribution

Conclusions

In this study in the ranges of variables studied, the modified Langmuir collision theory allowed todescribe the adsorption kinetics of polyelectrolytes on secondary fibers. However, modification of this model does not take into account the impact of harmful substances such as anionic trash of the suspension. The percentage of the distribution of suspended fibers showed a strong dependence on molecular weight of the polyelectrolyte. In general, the high percentages of irregularly distributed area were found when low molecular weight polyelectrolyte is used as to when polyelectrolytes of medium and high molecular weight were used. It was also noted that an increase in the contact time with fibrous suspension from 2 to 60 minutes will be significant, as it generate a decrease in the percentage of irregularly distributed area. The results show that the concentration of 14.6 mg g^{-1} of polyelectrolyte of low molecular weight (BpM) is sufficient for total coverage of the surface of fibers in 10 minutes of contact. The images showed that absence of polyelectrolyte there is 65.4% surface covered with uniform fiber distribution. Comparing treatments in the fibers with the theoretical model reported that polyelectrolytes with higher molecular weight require more time to achieve the coverage of fibers.

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Biodegradable films formed by polyelectrolyte complexes of xylan and chitosan

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Abstract
The objective of this work was to obtain potentially useful biodegradable films by using natural polyelectrolyte complexes (PECs) obtained by combining anionic xylan (Xyl) and cationic chitosan (Ch) solutions. The effects of different mass ratios of polyelectrolytes (30% Ch:70% Xyl; 25% Ch:75% Xyl; 20% Ch:80% Xyl and 15% Ch: 85%Xyl) with 10% glycerol as plasticizer were evaluated.
Polyelectrolytes were characterized by determining the charge density, molecular weight and degree of deacetylation of chitosan. Taking into account these results, PECs were prepared by adding the anionic Xyl on the cationic chitosan solutions, which is under continuous stirring, at pH 5.0 and 0.01N NaCl. It was found that the low charge densities of the different complexes obtained were always positive.
Films were produced by casting the suspensions of PECs. Solubility, mechanical properties, water vapor transmission rate (WVTR) and oxygen permeability were evaluated. The results show that the lowest value of solubility, WVTR and oxygen permeability was found in the case of the film with higher amount of xylan. The mechanical properties of the films depended on the proportion of Ch:Xyl used. When increasing the amount of chitosan in the films, the stress at break and the Young´s modulus were observed to decreased, whereas the opposite effect was observed in the case of strain at break.

Introduction

In order to assure a more sustainable use of our resources, it is essential to replace non-biodegradable plastic-based packaging materials by polymers from renewable and biodegradable materials. In this sense, biorefinery industry offers a viable alternative, using as a source, lignocellulosic biomass from trees, grasses, cereals, and other plants [1].

Xylan (Xyl) is an abundant component found in most lignocelluloses, with a great potential to be used for obtaining biomaterials such as films. This hemicellulose cannot be used alone for producing films due to its low molecular weight, high glass transition temperature, and low solubility [2]. A possible alternative for improving this situation is its combination with a cationic polyelectrolyte to form polyelectrolyte complexes (PECs). The combination of two polyelectrolytes yields in a complex with interesting and unique physicochemical properties. Chitosan is a renewable and natural cationic polyelectrolyte derived from chitin, with good film-forming properties under acid conditions [3]. Today, it is receiving a great deal of interest for medical and pharmaceutical applications [4].

In this work natural polyelectrolyte complexes of xylan and chitosan, at different mass ratios, were obtained and characterized. A high proportion of xylan was considered. Additionally, theses complexes were used to prepare films and their physical and barrier properties were evaluated.

Experimental

Characterization of chitosan and xylan

Chitosan was supplied by Sigma Aldrich (product number 448877). The viscosity average molar mass was determined using a viscometer cannon-feskes number 75 and the value obtained was M_v: 189.8 kDa. The degree of deacetylation of chitosan was determined using a potentiometric titration [5]. A solution of 2.5 g/L of chitosan in low concentration of acetic acid (0.25 %w/w) was prepared.

The anionic polyelectrolyte used was 4-O-metyl-glucuronoxylan isolated from beechwood by alkaline extraction and supplied by Sigma Aldrich (X-4252). The average molar mass was Mw: 18.7 kDa and polydispersity of 1.8 determined by size exclusion chromatography (SEC). Fresh xylan solutions were daily prepared at 4.0 g/L concentration by adding the commercial solid xylan to 10–4 N NaOH solution prepared in 0.01N NaCl and the solution was heated in a bath at 95ºC for 15 min, according to Linder et al. [6].

The charge densities of chitosan and xylan solutions were determined by polyelectrolyte titration method [7] and using streaming current measurements (Chemtrac ECA 2100) for determining the equivalence point. The titrations were performed at ionic strength of 0.01N NaCl and pH 5.0. The total content of uronic acid of xylan was determined using the Scott method [8].

Characterizations of the polyelectrolyte complexes

Construction of the streaming current curves. Selection of the different Ch:Xyl ratios

Complexes were formed by using a syringe pump for adding the anionic polyelectrolyte solution (2.5 g/L xylan) at a dosage rate of 40 ml/h on a solution of the polyelectrolyte of opposite charge (0.07 g/L chitosan), which is under continuous stirring (300 rpm). The pHs of the solutions were adjusted to 5.0 in 0.01N NaCl. Taking into account these curves, different cationic complexes were selected for preparing complexes with different charge densities to obtain the films.

Charge densities of the complexes

The charge densities of PECs were also determined by polyelectrolyte titration method [7]. The titrant used was a 200µN PVSK standard solution.

Preparation of the films

Formation of the complexes selected for film preparation

Complexes were formed as described above, except that a dosage rate of 90 ml/h was used. Table 1 shows the different cationic complexes prepared for the study. When glycerol (10 wt%, relative to the total mass) was used as plasticizer, it was added to the chitosan solution. The complexes suspensions were sonicated for 2 min using a Sonics & Materials ultrasonic homogeneizer (750 Watt, 50% amplitude) and then, they were heated for 10 min in a water bath at 75ºC to remove air bubbles [9].
The PEC suspensions were cast into polypropylene Petri dishes and placed in a fan-forced air circulation oven at 40ºC for approximately 48 h. Then, the films were allowed to dry in a conditioned room at 50% humidity and 23ºC. When necessary, they were stored in a desiccator containing a saturated solution of magnesium salt, $Mg(NO_3)_2$ in order to obtain a relative humidity of 50 %H.R.

Table 1. Mass utilized to form the different complexes for films preparation

Sample code	wt% Chitosan	wt% Xylan
100% Ch	100	---
30 % Ch: 70 % Xyl	30	70
25 % Ch: 75 % Xyl	25	75
20 % Ch: 80 % Xyl	20	80
15 % Ch: 85 % Xyl	15	85
100% Xyl	---	100

Evaluation of film properties

Solubility

The conditioned films were cut into samples of 1.2 cm^2. They were weighed and simultaneously their moisture content was determined by gravimetry in a vacuum oven at 60ºC for 24 h (Wdry). The film samples were placed in solutions at different pHs (buffers 3.0; 5.0 and 7.0) for 24 h. Then, they were removed, weighed and their moisture contents were again simultaneously determined (Wfinal). Solubility was calculated according to equation (1).

$$Solubility = \left(\frac{W_{dry} - W_{final}}{W_{dry}}\right) * 100 \qquad (1)$$

Physical properties

Film density (g/cm^3) was determined by dividing the grammage (g/cm^2) by the thickness (cm). Grammage was determined according to TAPPI T410 and the thickness was measured using a micrometer *Testing Machines Inc.* Model Nº 46-43 Series 400, according to TAPPI T411. The average thickness value from 10 measurements was used to calculate the density.
For evaluating the Young´s modulus, stress and strain at break of films, an Instron Universal Testing Machine model 3344 with a load cell of 1000 N was used according to the ASTM D882. For each treatment, 5 bone shape samples were cut according to the ASTM D1708-9.
All films were conditioned for 4 h at 50%H.R. and 23ºC prior to testing.

Barrier properties

The water vapor transmission rate (WVTR) was measured using a device designed as describe in ASTM E96-98. The device consisted in a cup of polypropylene with an area of 39.6 cm^2, which was filled with 10 mL of distilled water (water method) and the different films were mounted on the top. The cup was placed in controlled conditions (23ºC-50%H.R.) and its weight monitored every 30 min. An average of ten measurements is reported for each sample.
Oxygen permeability of the films was measured according to ASTM (D 3985-02) with an Oxygen Permeation Analyser MODEL 8001 (Systech Instrument) equipment. The analysis was made at 50 %H.R.

Results and discussion

Characterization of the polyelectrolytes

Table 2 shows the charge densities of chitosan and xylan at pH 5.0 and 0.01N NaCl. Charge density of chitosan resulted ten-fold higher than the charge of xylan. The table also shows a rather high degree of deacetylation for chitosan.

Table 2. Characterization of chitosan and xylan at pH 5.0 and 0.01N NaCl.

	Charge density [(a)] (meq/g)	Degree of [(a)] deacetylation (%)	Total content of [(a)] uronic acids (meq/g)
Chitosan	+4.03 ± 0.01	79.6 ± 0.7	---
Xylan	-0.39 ± 0.04	---	-0.71 ± 0.02

[(a)]The standard deviations of the means from three replicates of the trial are indicated.

Characterization of the polyelectrolyte complexes

Streaming current (SC) curves, charge densities and sizes of the complexes

Figure 1 shows the effects of the addition of xylan to chitosan solutions on the streaming current signal at 0.01N NaCl and pH 5.0. The figure shows that, the SC zero signal, related to the neutrality, is reached when the charge ratio Xyl:Ch is 1:0.58. The deviation from the stoichiometric expected ratio 1:1 indicates that free cationic charge of chitosan remains into the PECs. In the figure, the cationic complexes selected for the study are also shown.

The charge densities of the different cationic PECs are shown in Figure 2. All of them have low cationic charge densities. Particularly, the PECs prepared with a mass ratio of 15%Ch:85%Xyl have a charge density, close to neutralization.

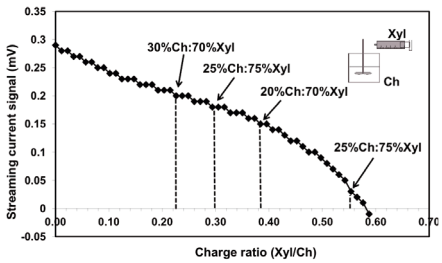

Figure 1. Effect of the addition of xylan solution on chitosan solution on the streaming current signal at 0.01N NaCl and pH 5.0. The amount of xylan and chitosan used for preparing the different complexes are indicated.

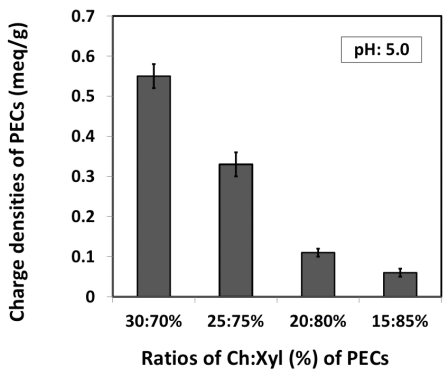

Figure 2. Charge densities of the complexes (PECs) formed with different amount of Chitosan:Xylan (Ch:Xyl) at pH 5.0 and 0.01N NaCl.

Evaluation of films

Film physical properties and solubility.

Table 3 shows the effect of the addition of 10 wt% of glycerol as plasticizer on the film properties prepared with the different PECs. The properties of the films prepared with 100% chitosan and 100% xylan, as well as a description of the appearance of the film are included. In general, the films with plasticizer were more flexible and easy to handle. When no glycerol was added, the higher the amount of xylan, the lower were the stress and strain at break values. Nevertheless, when glycerol was added to the film, the increase in the amount of xylan, increased the stress but the strain at break values was observed to decrease. In this case, the film was more rigid, as it is shown in the Young's modulus results.

Although the film prepared with 100% chitosan shows high stress and strain at break values, they were opaque and difficult to test because they wrinkled and moistened during the test in the conditioned room. On the other hand, the film prepared with 100% xylan was too brittle and fragile to evaluate, in agreement with observations reported by other groups [2].

Figure 3 shows the effect of the increasing amount of xylan in the stress-strain curves of the films prepared with different mass ratios of Ch:Xyl and 10% glycerol.

Figure 4 shows the solubility of films at different mass ratios of Ch:Xyl and pHs. At pH 7.0 the solubility slightly increased when compared to pH 5.0, for all ratios. At these pHs, the lowest value of solubility was found with a ratio 15%Ch:85%Xyl into the films, i.e., when the proportion of xylan in the film was the highest. At pH 3.0 the results were all similar.

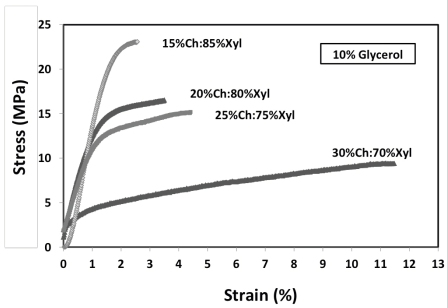

Figure 3. The stress – strain curves of the films formed at different mass ratios of chitosan/xylan (Ch:Xyl) (100%Ch:0%Xyl; 30%Ch:70%Xyl; 25%Ch:75%Xyl: 20%Ch:80%Xyl and 15%Ch:85%Xyl) and with 10% of glycerol.

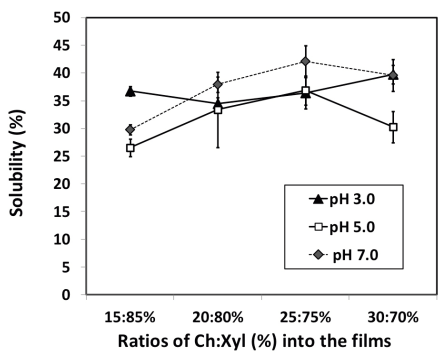

Figure 4. Solubility of the films formed at different mass ratios of chitosan:xylan (Ch:Xyl) (30%Ch:70%Xyl; 25%Ch:75%Xyl: 20%Ch:80%Xyl and 15%Ch:85%Xyl) and pHs (3.0; 5.0 and 7.0).

Table 3. Physical properties of the films with and without the addition of 10% of glycerol as plasticizer and different ratio of chitosan:xylan (Ch:Xyl).

	Sample code	Density (g/cm3)[a]	Stress at break (MPa)[b]	Strain at break (%)[b]	Young´s mod. (MPa)[b]	Appearance
Without glycerol	100% Ch	1.14 ± 0.16	17.0 ± 0.7	6.9 ± 2.1	2188 ± 67	opaque, wrinkled
	30%Ch: 70%Xyl	1.08 ± 0.15	13.3 ± 1.3	3.8 ± 0.6	1156 ± 186	brittle, more transparent
	25%Ch: 75%Xyl	0.56 ± 0.05	4.0 ± 2.4	2.0 ± 0.4	496 ± 88	brittle, transparent
	20%Ch: 80%Xyl	0.45 ± 0.05	3.6 ± 1.9	1.2 ± 0.2	365 ± 137	not homog., brittle
	15%Ch: 85%Xyl	0.59 ± 0.14	2.2 ± 0.4	1.1 ± 0.2	609 ± 71	not homog., brittle
	100% Xyl	--	--	--	--	too brittle, too fragile
With 10% glycerol	100% Ch	1.01 ± 0.08	16.6 ± 3.21	30.5 ± 6.9	363 ± 101	opaque, too wrinkled
	30%Ch: 70%Xyl	1.31 ± 0.15	12.2 ± 2.23	10.9 ± 3.2	347 ± 63	more transparent
	25%Ch: 75%Xyl	1.45 ± 0.07	12.0 ± 3.86	4.7 ± 1.4	844 ± 271	transparent
	20%Ch: 80%Xyl	1.12 ± 0.09	17.5 ± 3.34	3.1 ± 0.8	1478 ± 207	not homogeneous,
	15%Ch: 85%Xyl	1.04 ± 0.07	20.9 ± 5.38	2.2 ± 0.2	1539 ± 184	not homogeneous,
	100% Xyl	---	---	--	--	too brittle, too fragile

(a)The standard deviations of the means from ten replicates of the trial are indicated.
(b)The standard deviations of the means from five replicates of the trial are indicated.

Barrier properties

Table 4 shows the water vapor transmission rate (WVTR) and oxygen permeability (OP) of the films prepared with different mass ratios of chitosan and xylan and with 10% glycerol. Both, the WVTR and the oxygen permeability decreased when the amount of xylan in the film was increased, indicating the potential of xylan to be used in films preparation.

Table 4. Water vapor transmission rate (WVTR) and oxygen permeability (OP) of the films measured at 23ºC-50%H.R. and 23ºC-50%H.R., respectively.

Sample code	WVTR(a) (g/h m^2)	OP (mm cm^3/day m^2 atm) x10^{-3}
30%Ch: 70%Xyl	21.3 ± 0.8	26.8
25%Ch: 75%Xyl	21.1 ± 1.9	14.0
20%Ch: 80%Xyl	18.9 ± 1.3	17.2
15%Ch: 85%Xyl	19.6 ± 0.3	Below 1.1

(a)The standard deviations of the means from three replicates of the trial are indicated.

Conclusions

At pH 5.0, acceptable films from complexes of chitosan and xylan with high proportion of the last one can be obtained. The addition of glycerol as plasticizers allowed obtaining more easily to handle films.

When glycerol was added to the film, the increase in the amount of xylan, increased the stress at break, but the strain at break is decreased and the film were more rigid. Particularly, the film prepared with 30 wt% chitosan and 70 wt% xylan and 10 wt% glycerol (relative to the total mass) was the most transparent, uniform, deformable and flexible film evaluated.

Nevertheless, the presence of high amount of xylan into the films is beneficial for their low solubility, water vapor transmission rate and oxygen permeability.

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Foamed packaging made from cellulose acetate

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Abstract
The contribution shows results of extrusion foamed externally plasticized cellulose acetate (plCA) and its potential for producing thermoformed trays. Using a mixture of HFO-1234ze and ethanol as blowing agent, foam density and density reduction in the range of standard PS foam sheets were obtained. The foam constitutes a fine and homogeneous morphology with closed cells. The plCA foam sheets were successfully thermoformed to packaging trays. The results show that plCA is a promising biopolymer for extrusion foaming and can be a suitable alternative to PS.

Introduction
Today, polystyrene (PS) is the predominant polymer for producing extruded foams for various applications including insulation boards or thermoformed packaging. However, PS is derived from petrochemicals, it is non-renewable, non-biodegradable and its monomer styrene is considered to be toxic. The use of renewable resources, the reduction of packaging waste, and the minimization of emissions becomes more and more important with respect to a more sustainable economy. Consequently, considerable research has been conducted on foaming bio-based polymers, especially thermoplastic starch or poly(lactic acid) (PLA) in recent years (e. g.: Lee et al. 2007, Pilla et al. 2009, Richards et al. 2008, Zhang and Sun 2007). These biopolymers, however, exhibit several drawbacks with respect to certain foam applications such as trays filled with hot food contents. Poor melt processing and restricted melt properties, embrittlement over time, limited heat distortion resistance for PLA, and insufficient moisture resistance for starch are their main disadvantages.

Cellulose acetate (CA) on the other hand is a biodegradable and bio-based organic cellulose ester (Figure 1). CA is also odorless, non-toxic, and hypoallergenic. Thermoplastic CA, which generally has a degree of substitution (DS) of around 2.5, exhibits excellent optical, mechanical, and thermal properties comparable to those of neat PS. It also provides higher heat resistance and flexibility, as well as better melt properties than PLA or starch. Hence, CA may be a promising biopolymer to replace PS in certain foam applications, for example extrusion foamed trays for hot contents.

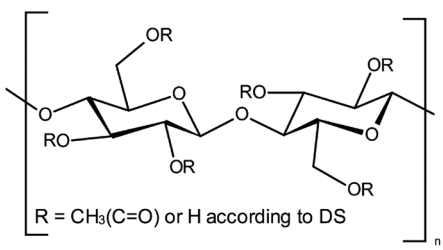

Figure 1: General chemical structure of cellulose acetate (DS: degree of substitution)

Due to the strong hydrogen bonds between the non-substituted free OH groups, unmodified CA shows a narrow processing window between melting and decomposition temperature. As a result, CA must be modified to achieve sufficient thermoplasticity. The most common way is external plasticization using low molecular weight plasticizers to yield plasticized CA (plCA).

Despite its long history, no systematic research has been conducted on foam extrusion of thermoplastic plCA and only few publications are available concerning plCA foams (Deanin and Berner, 1996, Willett and Shogren, 2002).

Previous research was focused on the investigation of the rheological properties and the general foamability of plCA using chemical blowing agents (Zepnik et. al, 2011, Zepnik et. al, 2011a, Zepnik et. al, 2012, Zepnik et. al, 2012a). However, physical foaming is state of the art for producing thermoformed trays. As a consequence this paper gives a summary of our research on foam extrusion of externally plasticized CA using physical blowing agents (PBA).

Materials and methods
Externally plasticized CA (plCA) was obtained from FKuR Kunststoff GmbH (Germany) under the trade name BIOGRADE® C 7500 CL. CO_2, N_2, and 1,3,3,3-Tetrafluoropropene (HFO- 1234ze, supplied by Honeywell International Inc., USA) were selected as PBA. Different amounts of a 5 % talc masterbatch made from the BIOGRADE® C 7500 CL were added as nucleating agent. The talc used was supplied by Mondo Minerals B.V., Netherlands (Finntalc M05SL-AW). It has platelet geometry with a specific surface area of 9.5 $m^2 g^{-1}$ and a median particle size d_{50} of 2 µm.

Foam extrusion tests were conducted at the Institute of Plastics Processing (IKV) at the RWTH Aachen University. A 60 mm single screw foam extruder of Barmag Oerlikon Textile GmbH & Co. KG, Remscheid, Germany, with a length to diameter ratio L/D of 40 was used. The extruder is equipped with a mixing screw optimized for the foam extrusion process. A rod die was used for first experimental series to study the expansion behaviour, foam density and foam morphology in dependence of PBA and nucleating agent content. In further trials to produce foamed films in an industrially relevant process the extruder was equipped with an annular die, diameter 50 mm.

Results
Extrusion foaming using CO_2 and N_2 as physical blowing agents
The expansion ratio (D/D_0) of extrusion foamed plCA rods with CO_2 and N_2 as a function of PBA content is shown in Figure 2.

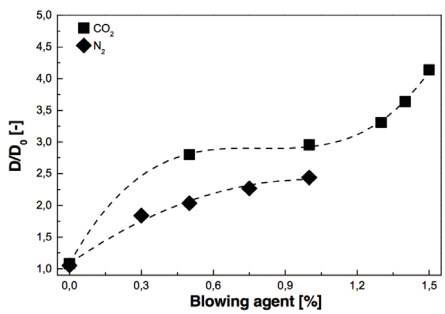

Figure 2: Expansion ratio (D/D_0) of extrusion foamed plCA rods as a function of PBA 3,5 type and content

CO_2 leads to higher D/D_0 in comparison to N_2. Due to the limited solubility of N_2, supersatura- tion in the extruder and an early loss of N_2 occur. Consequently, a smaller amount of N_2 is still dissolved in the plCA melt, which can contribute to foaming at the die.
Cell size was investigated in dependence of blowing agent and nucleating agent, as shown in Figure 3. As expected, average cell size decreases continuously with increasing talc content independent of the inert gas type used. Talc when used as a nucleating agent leads to heterogeneous cell nucleation. As a result, cell growth is predominated by cell nucleation at this low content of PBA. An increase in talc content causes an increase in cell nucleation rate. However, leveling off can be seen at 0.8 wt.-% indicating that further increase in talc content may not lead automatically to further decrease in foam density.

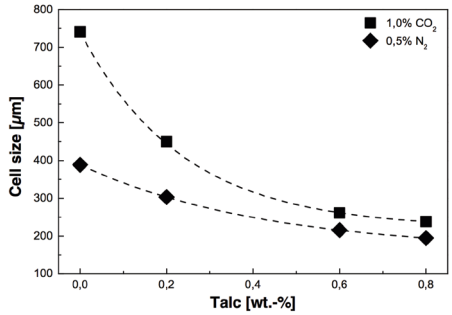

Figure 3: Influence of 700 nucleating agent content (talc) on cell 600 size of extruded plCA rods foamed with CO_2 and N_2

The optical microscopy images in Figure 4 confirm the quantitative analysis of the cell size. When externally plasticized CA is foamed with PBA (CO_2) only, closed but relatively large cells can be found. However, when talc is added, fine and homogeneous foam morphologies can be achieved. This agrees well with literature data for PS foaming (Han et. al, 2003). Cell nucleation due to talc is predominant at low CO_2 content. At higher CO_2 content, cell nucleation is more influenced by CO_2.

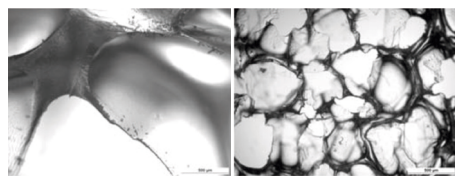

Figure 4: Optical microscopy images of extrusion foamed plCA using 1 % CO_2 (left) and 1 % CO_2 + 0.8 wt.-% talc (right)

Figure 5 compares SEM images of the foam morphology as a function of the inert gas type. N2 used as a PBA produces a broad inhomogeneous cell size distribution with high amount of small cells but also extremely large cells to some extent. This may be an explanation for the lower average cell size of N2 when compared to CO2, as it was shown in Figure 3.

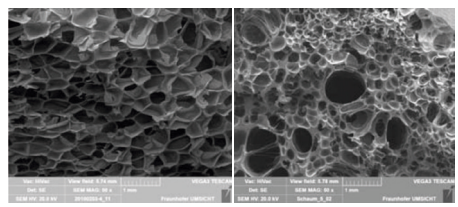

Figure 5: SEM images of ex- trusion foamed plCA, 1 % CO_2 + 0.6 wt.-% talc (left) and 0.5 % N_2 + 0.6 wt.-% talc (right)

CO_2 use yields a more homogeneous morphology with a narrow cell size distribution when compared to N_2. The average cell size is higher for CO_2 at low talc contents (Figure 3). However, at talc contents higher than 0.6 wt.-% no significant differences in the average cell size can be found for CO_2 and N_2.

Extrusion foaming using HFO-1234ze as physical blowing agents
Despite CO_2 being an effective blowing agent for plCA, its high volatility and fast diffusion are disadvantageous for producing thermoformed trays from extrusion foamed sheets. After foam sheet extrusion, CO_2 evades rapidly and no rest gas can give stability in the following thermoforming process. Therefore, higher molecular weight blowing agents having lower diffusion coefficients are more promising for producing extrusion foamed sheets for thermoformed packaging. If additionally claims

for zero ozone depletion potential and low global warming potential are made, the choice of a suitable hydrofluoroolefin is reasonable. With regard to a good solubility in plasticized CA, 1,3,3,3-Tetrafluoropropene (HFO-1234ze, supplied by Honeywell International Inc., USA) was selected as PBA for further trials.

Table 1 shows the physical foam properties, strand diameter *D*, expansion ratio D/D_0, foam density ρ_f and foaming ratio ρ_p/ρ_f in dependence of HFO-1234ze concentration and talc content. As seen from Fig. 1 and Table 1, strand diameter D and expansion ratio D/D_0 increase considerably with increasing content of HFO-1234ze. Higher amount of dissolved blowing agent results in stronger expansion at the die. By comparison, an increase in talc content does not have similar strong influence on the diameter D and expansion ratio D/D_0 of the strands. When 2 % HFO-1234ze is added as blowing agent, the density of pICA decreased about three-times. Talc when used as nucleating agent improves cell nucleation rate and leads to higher cell density and higher porosity P. As a result, foam density decreases continuously with increasing talc content within the range studied in this paper.

TABLE 1: Strand diameter *D*, expansion ratio D/D_0, foam density ρ_f and foaming ratio ρ_p/ρ_f of extrusion foamed plasticized CA in dependence of HFO 1234ze concentration and talc content.

	Composition (%)	D (mm)	D/D0 (-)	ρ_f (kgm^{-3})	ρ_p/ρ_f (-)
HFO-1234ze / talc	0/0	6.5	1.08	1310	1.0
	2.0 / 0.0	10.9	1.82	427.9	3.1
	2.0 / 0.2	17.3	2.88	220.8	5.9
	2.0 / 0.6	17.9	2.98	182.8	7.2
	2.0 / 0.8	18.1	3.02	160.2	8.2
	2.5 / 0.8	20.6	3.43	120.5	10.9
	3.0 / 0.8	23.9	3.98	108.6	12.1

An additional increase in HFO 1234ze concentration at constant talc content results in further density reduction. The more HFO 1234ze is injected and dissolved in the pICA melt, the more blowing agent is available for expansion at the die and density reduction. However, premature supersaturation must be avoided which sets an upper limit for the PBA content. Maximum porosity P of 92 % was achieved for extrusion foamed plasticized CA using 3 %HFO 1234ze and 0.8 wt.-% talc. The foam density of a commercial XPS film from Inde Plastik (Germany) produced with 4 %butane as PBA is 88 kg m^{-3} which corresponds to a foaming ratio ρ_p/ρ_f of 11.9. Thus, the density reduction obtained for extrusion foamed pICA is as high as for the standard XPS film. However, due to the lower density of neat PS of 1050 kg m^{-3} the XPS foam offers a lower foam density.

Further improvements of pICA foam structure and density were achieved using ethanol as a co-blowing agent. The following table 2 shows the results reached after transferring the extrusion foaming to a semi-technical foamed sheet production line using an annular die, followed by a cooling mandrel, slicing-up the tubular foam sheet and pulling the resulting flat foam sheet using a calander roll (figure 6).

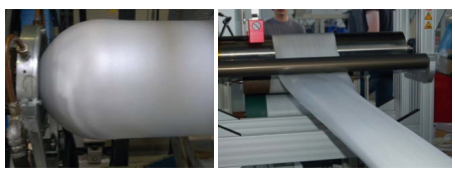

Figure 6: Semi-technical pICA foam sheet production

Table 2 compares typical characteristics of the extruded plasticized cellulose acetate (XCA) sheet with standard extruded polystyrene sheet (XPS). As can be seen, the density is close to an industrially produced XPS sheet, whereas the blow up ratio is lower and thus the sheet thickness is higher.

TABLE 2: Physical properties of XCA sheet in comparison to an extruded XPS sheet (Density neat pICA: 1310 kg m^{-3}, neat PS: 1050 kg m^{-3})

Property		XPS sheet	XCA sheet
PBA	[-]	butane	HFO 1234ze + ethanol
Nucleating agent	[-]	talc	talc
Density	[kg cm^{-3}]	88.0	115.5
Porosity	[%]	91.6	91.2
Film thickness	[mm]	1.5	2.4
Blow-up ratio	[-]	5:1	3:1

The XCA sheet shows fine and homogeneous foam morphology with polyhedral closed cells, as shown in figure 7 (a). The morphology is similar to that of the extruded XPS sheet (figure 7 (b)). The average cell size of the XCA sheet is 286 µm and for the conventional XPS sheet it is 193 µm. The average cell wall thickness is 1.6 µm for both materials. However, the lower blow up ratio achieved for the XCA results in less homogeneous stretching of the sheet. Thus, slightly higher cell anisotropy (aspect ratio) is observed for the XCA sheet than for the standard XPS sheet, namely 1.44 for the XCA and 1.36 for the XPS.

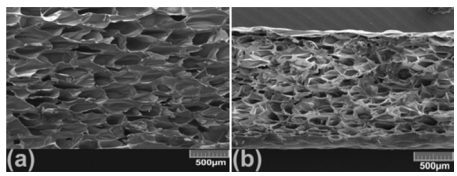

Figure 7: (a) Foam morphology of extruded XCA sheet, (b) of extruded XPS sheet.

Tensile properties of the XCA sheet were measured in machine direction (MD) and in transverse direction (TD). Table 3 compares the specific stiffness and specific strength of the extruded XCA sheet with the standard XPS sheet. The stiffness of the XCA sheet is similar to the XPS sheet, while the specific strength of the conventional XPS sheet is noticeably higher in both directions, MD and TD. Additionally, the ratio between MD and TD is lower for the standard XPS sheet. The higher blow up ratio of the standard XPS sheet leads to stronger and simultaneously more homogenous stretching, consequently resulting in lower anisotropy of the foam structure (see figure 7).

TABLE 3: Tensile properties of extruded XCA sheet in comparison to extruded XPS sheet

Property			XCA sheet	XPS sheet
Specific tensile modulus	MD	[(MPa)/(kg m^{-3})]	0.89	0.89
	TD	[(MPa)/(kg m^{-3})]	0.61	0.76
	MD/TD	[-]	1.46	1.17
Specific tensile strength	MD	[(kPa)/(kg m^{-3})]	28.7	36.4
	TD	[(kPa)/(kg m^{-3})]	21.6	34.1
	MD/TD	[-]	1.33	1.07

Acknowledgments

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Biorefineries

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Poster presentations

BIOREFINERIES BASED ON LIGNOCELLULOSICS. HOW TO AVOID THE ERRORS FROM 20th CENTURY OIL REFINERIES DESIGNS.

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Until the invention of gasoline engines in the late 19th and beginning the 20th centuries the oil as raw material was just a source of what we know today as low value products (for lighting, heating, lubricants, etc). The gasoline at that time was a waste, usually evaporated to the environment. The design of the refineries in the beginning was not an integral strategy and just a step by step addition of products depending on the knowledge of the oil chemistry and market. It is interesting to note that the real forces driving the evolution of refining technologies today is the environment concerns. Goals as better catalysts, reduction of byproducts and integral use of all the fractions are just examples of the criteria for refiners today. However, it sounds like new products based on oil fractions are not the priority today. In the case of biorefineries, and especially when lignocellulosics are used as raw materials, there is totally another context. The evolution of oil refineries processing, organic chemistry, biotechnology, materials understanding and other fields are applied today to biorefineries design. But the most important difference is how the sustainability is changing from being just a desired concept during design stages to be more and more a restriction for future designs. Moreover the wide products profile of modern biorefineries responds to the increasing demand for biobased products replacing those coming from oil, but demonstrating always the process sustainability.
In this work, the most important aspects of biorefineries design based on lignocellulosics are discussed making emphasis in what was learnt from oil refineries and what kind of errors should not be repeated. For this purpose, the essence of the biorefinery was studied and the influence of the 6 more important strategies of analysis was evaluated: hierarchy, sequence, integration, scale scenario and sustainability. The results can be considered as guidelines for designing modern biorefineries based on lignocellulosics.

SUSTAINABILITY ASSESSMENT OF BIOREFINERY SCENARIOS ANNEXED TO SOUTH AFRICAN SUGAR MILLS

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The sugar industry has to “re-invent” itself to ensure long-term economic survival and opportunities for job creation and enhanced community-level impacts, given increasing pressure from fluctuating and low global sugar prices, increasing energy prices and sustainability demands. We propose biorefineries for re-vitalisation of the sugar industry using low value lignocellulosic biomass (sugarcane bagasse, leaves and tops) annexed to existing sugar mills, producing a spectrum of high value platform chemicals along with biofuel, bioenergy and electricity. Opportunity is presented for greener products, to mitigate climate change and overcome economic challenges. Xylose from labile hemicellulose remains largely underutilised and conversion to value-add products a major challenge. Insight is required on pretreatment and/or extraction to optimise production of cellulosic ethanol together with lactic acid, furfural or biopolymers from sugarcane bagasse, leaves and tops. Experimental conditions for alkaline and pressurised hot water extraction, dilute acid and steam explosion pretreatment of sugarcane bagasse and harvest residues were investigated to serve as basis for developing various process scenarios under a sugarcane biorefinery scheme. Dilute acid and steam explosion pretreatment was optimised for maximum hemicellulose recovery, combined sugar yield and solids digestibility. An optimal range of conditions for alkaline and liquid hot water extraction of hemicellulosic biopolymers, as well as conditions for acceptable enzymatic digestibility of the solid residue after such extraction was established. Using data from the above, a series of energy efficient biorefinery scenarios are under development and modelled using Aspen Plus® software, to simulate potential factories to better understand the biorefinery processes and estimate the CAPEX and OPEX, environmental impacts, and overall viability. Rigorous and detailed sustainability assessment methodology was formulated to address all pillars of sustainability. This work is ongoing and to date, models have been developed for some of the processes which can ultimately be combined into biorefinery scenarios. This will allow systematic comparison of a series of biorefinery scenarios to assess the potential to reduce negative impacts on and maximise the benefits of social, economic, and environmental factors on a lifecycle basis.
Keywords— biorefineries, lignocellulose, xylose, lactic acid, furfural

APLICACIÓN DE SIMBIOSIS INDUSTRIAL PARA LA GENERACIÓN DE BIOENERGÍA EN UN CLUSTER AGROINDUTRIAL BAJO EL ENFOQUE DE BIOREFINERÍAS

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La implementación de simbiosis industrial, como modelo de gestión para el aprovechamiento de los materiales residuales, tiene como objeto utilizar los residuos de una industria como materia prima de otras, lo cual no es una práctica muy común en Latinoamérica. Sin embargo, hoy en día, los empresarios se están dando cuenta que esta materia prima que ellos antes enviaban a los sitios de disposición de residuos, puede ser utilizadas o transformadas para la obtención de subproductos que pueden introducir nuevamente a sus procesos o venderlos a otras industrias como materias primas, lo que les generaría disminución de los costos de producción e incremento de las ganancias económicas. Esta nueva forma de visualizar los residuos, desde el punto de vista ambiental, tiene muchas ventajas ya que permite hacer uso eficiente de los recursos y cerrar los ciclos de materia. Algo similar se busca en una biorefinería, la cual tiene como objeto integrar procesos para aprovechar los residuos orgánicos como materias primas para producir productos con mayor valor agregado (simbiosis industrial). Lo cual indica que si las empresas hacen la planeación de su producción bajo este concepto, estas serán más competitivas. En el Estado de Guanajuato, la actividad agroindustrial juega un papel muy importante en la economía regional, pero su crecimiento no se ha planificado bajo los conceptos de la sustentabilidad, lo cual hace que grandes cantidades residuos orgánicos no sean aprovechados y terminen dispuestos en los vertederos. Actualmente existe en el Municipio de Pénjamo Guanajuato un Clúster Agroindustrial propiedad del Grupo BEGULA el cual posee alto potencial para implementar un modelo de aprovechamiento de sus residuos. Por esta razón el objetivo de esta investigación fue identificar a través de la metodología que propone la simbiosis industrial, cuales son las sinergias existentes y cuales podrían establecerse en el futuro dentro de clúster con un enfoque de biorefinería. La investigación se llevó a cabo en cuatro etapas: primera, revisión de los procesos de producción de las empresas; segunda, caracterización cualitativa y cuantitativa de los residuos; tercera, identificación de las sinergias existentes y cuarta, identificación de sinergias que pudieran establecerse en un futuro. Una de las principales sinergias identificadas fue la producción de biogás a partir de las aguas residuales provenientes de la matanza, lavado de jaulas (CICABA, S.A. de C.V) y acondicionamiento de vísceras (VSB, S. de R.L. de C.V.). En esta operación se genera 1,300 m3 de biogás/día el cual se utiliza como combustible en sus calderas, que a su vez suministran vapor al túnel y tinas de escaldado de cerdos, lo cual ha permitido bajar el consumo de combustible alterno en aproximadamente 70% diariamente (10-12 horas de producción), generándoles un ahorro económico de 1,176,410.00 MXN/año (81,131.75 USD/año). Otra de las sinergias establecidas dentro del clúster es la producción de biogás a partir de residuos orgánicos en la planta de producción de harina de carne (PESABA, S.A. de C.V). En esta sinergia los residuos

orgánicos provenientes de la matanza de cerdos (líquido ruminal, restos cárnicos y agua residual) son fermentados en un biodigestor anaerobio. El volumen de biogás generado diariamente es más baja que la anterior (800 m^3 de biogás /día), ya que el proceso se encuentra en la etapa de estabilización. Actualmente, el biogás generado solamente satisface entre 4 y 5 horas de la demanda diaria, pero en un futuro, se espera que el biogás producido pueda sustituir, entre el 70-80%, del combustible que se utiliza en el proceso. Otra sinergia que ya se tiene visualizada es la generación de biodiesel a partir de grasa residual recuperada en las trampas de grasa en sala de sacrifico y planta de tratamiento de aguas residuales. Como se puede observar, la metodología que propone la simbiosis industrial permite detectar sinergias para el aprovechamiento de residuos orgánicos para la generación de bioenergía como está sucediendo en este clúster, el cual ya está haciendo su planeación para la generación de biocombustibles y subproductos con una visión de biorefinería y ecología industrial.

SOCIO-TECHNICAL PERSPECTIVES ON BIOREFINERIES: NICHE MARKETS AND POTENTIAL PATHWAYS FOR BIO-BASED DEVELOPMENT AGENDAS

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Biorefineries have emerged as a key component in the transition from the oil-based economy to the bio-based economy, or bioeconomy (EuropeanCommission, 2013; Holladay, Bozell, White, & Johnson, 2007; Werpy, Petersen, Aden, & Bozell, 2004). In fact, several advanced economies as well as a few developing nations have supported their development in economic and political agendas. In this context, we posit that biorefineries shall not be conceived as mere "artefacts", but rather, as a societal function associated with production and consumption that is fulfilled by socio technical systems consisting of "knowledge, technologies, user needs and markets that interact dynamically within networks" (Geels, 2004, 2005). Building on the concept of socio-technical systems, we analyze the transition from oil to biomass economy by using the case of biorefineries, in order to identify niche markets that may play a significant role in this challenge and contribute to potential industry and policy agendas.

"The Stone Age did not end for lack of stone, and the Oil Age will end long before the world runs out of oil"

Sheikh Zaki Yamani

This quote by a former Saudi Arabian Oil Minister, depicts the immense belief in technology and its ability to shape and transform markets. In the case of alternative energy sources, countries and private actors made significant investments. Between 2009 and 2011 approximately 44,5 billion dollars were invested in "Clean Tech" in the United States (Eilperin, 2012). In other countries such as Chile, investment by public agencies and companies attained 40 million dollars, which was significant for national R&D expenses (CORFO, 2010). Despite the fact that technology had advanced to levels in which building an industry was possible, a confluence of several factors, among which were the financial crisis, low cost shale gas production and fluctuating prices in commodities, but also the interaction between policy, investors, entrepreneurs and companies, made the sector colapse worldwide.

Similarly, biorrefineries are expected to play a key role in the production of carbohydrate-based chemicals, materials and energy products from lignocellosic biomass. As sources of biomass are diverse and may originate in agriculture, forestry and algal crops main streams, but principally waste streams, biorrefineries require significant interaction with other agents for transforming and producing bio-based products. Furthermore, due to technical and logistic matters, these products are seldom able to compete on price, thus requiring support from regulations and consumer groups in order to achieve faster diffusion rates (Rogers, 1983).

All these actors have their own vested interests, resources, strategies and values. With the recent experience of Clean Tech we will analyze the networks in which biorrefineries are positioned in order to identify the key actors and markets in which bio-based products may succeed.

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REUSE OF RESIDUES FROM PULP AND BIOMASS ENERGY INDUSTRIES: ALTERNATIVE FOR MINIMIZE THE USE OF MINERAL FERTILIZERS

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The pulp and biomass energy industries produce as a main residue the fly ashes in their combustion boilers, those equipments generate between 2 to 6% of ash by ton of burned bark. A pulp mill that produces 115,000 tons of cellulose per month, generates almost 3,800 tons of fly ashes in that period, that is to say, it generates 33 kg of fly ashes per ton of pulp produced. The latter is equivalent to disposal costs from 20 to 40 USD/ton of fly ash, which represents a cost between 76,000 to 152,000 USD per month only in concepts of disposal [1].
The aim of this work was to elaborate a soil and crop amendment using the residues from pulp and biomass energy industries. These residues were selected because of their capacity to improve the physical, chemical and biological properties of soils, besides of being a source of micro and macro nutrients that are required by plants [2]. Moreover, in Chile, 49% of soils present erosion [3], and the costs of fertilization to improve soils represents between 40 and 60% of the production costs in agriculture. Thus, the use of this technology will have a high impact in this sector.
Two different soil amendments were elaborated (A & B), and each one was evaluated in wheat crops by adding different doses: 1, 2 and 4 ton/Ha. All treatments had an additional 50% of the traditional amount of mineral fertilizer used in wheat. The soil used was an Alfisol located in Chillán, in the VIII region of the country. The principal parameters studied were the grain yield obtained in the harvest and the quality of the grain. Both parameters were also evaluated in a control (C) with no fertilizer or amendment, a treatment that used 100% of the mineral fertilizer dose (F-100), and another that used half of fertilizer dose (F-50).
Regarding the production of grain, the highest value was observed in the F-100 treatment with approximately 6,900 Kg/Ha. However, the grain yield of treatments A-4 and B-2 showed no significant differences (P>0.05) compared to the F-100 yields. Additionally, the grain quality (Hectoliter weight) for F-100 was of 73.9 Kg/100 L, which showed no significant difference (P>0.05) with any other treatment, indicating that the wheat quality was similar. These results suggest that A-4 and B-2 could be used as soil amendments, replacing in a 50% the use of minerals fertilizers in Alfisol.

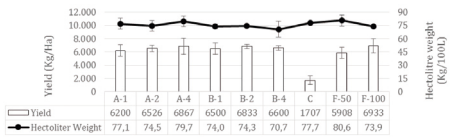

Fig 1. – Yield and seed quality for different soil amendment doses (A and B), compared to the common use of fertilizers in wheat (F). Amendment doses were 1, 2 and 4 tons per hectare; C: control treatment; F-100: common fertilization; F-50: half of common fertilization.

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ENERGY BALANCE AND EQUIVALENT CARBON EMISSIONS OF TWO LOW-INVESTMENT BIOMASS HARVESTING SYSTEMS APPLIED IN A SITUATION OF MIXED FOREST OF SEMI-NATURAL ORIGIN

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Abstract
This study compares the cost, energy consumption and equivalent carbon emissions of two low-investment biomass harvesting systems, likely to be used by owners of small and medium-sized forests in central-southern Chile. It also focused on harvesting the natural regeneration of Acacia melanoxylon, characterized by a high density, around 6,900 trees ha^{-1}, with average diameters at breast height (DBH) of 5.3 to 5.6 centimeters, under the cover of a plantation of Eucalyptus globulus established in 2004 in the Los Ríos region, Chile. Whole-tree harvesting method was used, which included felling and skidding activities. Time-motion studies were performed, productivity models were developed and costs were calculated. Energy consumption was estimated utilizing the Gross Energy Requirement (GER); evaluation including human labor, oxen food, mechanical supplies and fossil fuels. The equivalent carbon emissions were estimated by using the factors of fuel and lubricant emission, mechanical supplies and the enteric fermentation of the oxen. The best performance was achieved by the manual harvesting system, with a cost, energy consumption and equivalent carbon emissions per dry tons of biomass (Mgd) of 36.1 USD Mgd^{-1}, 201.8 MJ Mgd^{-1} (energy balance of 91) and 8.0 kg CO_2eq Mgd^{-1}, respectively. In the semi-mechanized harvesting system, they were 42.0 USD Mgd^{-1}, 358.8 MJ Mgd^{-1} (energy balance of 51) and 48.9 kg CO_2eq Mgd^{-1}, respectively. The results indicate that the sustainability of mechanized equipment will depend on if they can reach the necessary productivity level to compensate their greater consumption of fossil fuels with respect to the alternatives with lower mechanization levels, which is related to its adaptation to the characteristics of the biomass and operating conditions.

Keywords: forest biomass, harvesting systems, energy consumption, greenhouse gas emission (GGE), natural regeneration of A. melanoxylon.

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IMPROVEMENT OF SUGARCANE STRAW: A REVIEW OF AVAILABLE TECHNOLOGIES FOR ENERGY AND NON-ENERGY PURPOSES

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In Brazil, sugarcane always figured as one of the most expressive cultures of production of sugar, ethanol, etc. Currently, the country stands out on the world stage as both producer and exporter of products from sugarcane [1]. In the future, it is expect that the greater demand for food and fuel due to population growth will push Brazilian production to keep up with this evolution [2].
One of the big questions of sustainability nowadays is the use of by-products from production processes. For every ton of processed sugarcane is generated, with 50% humidity, 280 kg of bagasse and 280 kg of straw [3]. While the bagasse is used to feed boilers or in the production of second generation ethanol, the straw is often "dismissed" by burning carried out before harvest, being very harmful to the environment and causing serious health problems to the population [4].
With the prohibition of practice of the straw burning in the state of São Paulo (Brazil) for mechanized areas already in 2015, as stipulated by the Environmental Protocol, it is necessary to give a destiny to this abundant residue in São Paulo sugarcane fields [5]. Thus, the objective of this study was to survey available technologies for the processing and transformation of sugarcane straw in different forms of energy and bioproducts in order to add value to this waste.
Regarding changes to energy generation, there are several ways: gasification, pyrolysis, liquefaction, briquetting (solid fuel), biodigestion, combustion (burn in boilers) and 2G ethanol. While the first three are in pilot scale development, the others are already commercial realities. As for byproducts, it can be mentioned uses like adsorbent for pigments and heavy metals, medicinal textile fiber, additive for cement, hydroponic green forage (type of feed), paper, obtainment of silicon carbide, animal feed and additive for latex paint .
Thus, there is a range of applications for the sugarcane straw, many of which present environmental benefits by replacing petroleum as well as economic. Finally, it should be noted that if there is interest in using any of these processes, it is necessary a thorough technical-economic study to ensure that the cost of the collection and storage of this material are offset, that is, it is necessary an evaluation of the sustainability of the project, considering social, environmental, economic and technical aspects.

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COLLABORATIVE INTERNATIONAL ENGINEERING EDUCATION, ENVIRONMENTAL TECHNOLOGY AND SUSTAINABILITY – DEVELOPING A MASTER'S OF SCIENCE PROGRAM IN PULP AND PAPER TECHNOLOGY IN URUGUAY

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Uruguay noticed increased international interest of their forestry sector in early 2000s. This implicated direct need to develop higher education in the country for which there was limited previous experience to cover the entire scope of the industry and related fields. Following international investments of a Finnish forestry company the partner for the educational development was also selected from Finland. Aalto University, former Helsinki University of Technology (TKK), has long tradition and experience in forest products technology and engineering education. Educational collaboration between the two countries started in 2004 with a goal to establish a Master's degree program in pulp and paper technology at the Universidad de la República in Montevideo, Uruguay. The project introduces a process for developing a tailor-made program for master's degree education in engineering. Most of the set objectives were reached in terms of increasing and sustaining knowledge in Uruguay. A productive research group has been established at the local university and several research projects have been launched since the initiation of the program.

Nowadays, engineers are being asked to do a lot of consideration of natural and environmental systems, and of social systems and institutions [1]. Design for sustainability involves the application of performance metrics using environmental, economic and social indicators and sustainable engineering includes life cycle thinking [2]. Numerous students have also been proceeding with their careers by improved skills and knowledge gained with the program. Career development has been strongest among those who have already been employed by the sector when entering the program and there are also cases of excellent work placements as follow-up and after graduation from the program. Flexibility towards students in personal curriculum planning was well appreciated however individual study projects were seen as some of the main practical challenges relative to personal scheduling and other commitments. The program succeeded to provide a holistic perspective into the field of forest industry however selection for elective courses and projects to increase specialism will be needed in the future. Moreover, steady and strong economic growth in Uruguay and current employment situation encourages work over graduate education which also has an impact on number of graduated students. Further development to make the program stronger with critical mass of students could include possibilities of combined and partially shared courses for an interdisciplinary approach. Personal and practical guidance service with field specific projects and internships were seen as additional steps to encourage students to participate in practical training and gaining suitable competences for work life.

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BUILDING CURRICULA FOR AN INTEGRATED APPROACH TO LIGNOCELLULOSIC BIOREFINERY PRACTICE AND RESEARCH

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Lignocellulosic raw materials conversion to biochemicals, bioenergy and biomaterials has the paradox of the very old and cumulative experience with very new and emerging technology and knowledge. The biorefinery is a very broad word but the concept is converging fast to third generation (multi-feedstock. multi-product, multi-process, multi-scale) and highly integrated technology and engineering. And its fundamental knowledge and research will grow steady and deeply by own way as the bioinspired, renewable and green sciences, often without the word "biorefinery" as the master keyword. So the first challenge is how to build a scope of knowledge with a strong root in the industrial practice as our proved sectoral strength, and another root in the rigorous science as proved as our academic strength, to improve research as highly quality and innovative knowledge generation. The lignocellulosic biorefinery research and education is a recent and rapidly growing activity and it is an usual matter of studies of forest engineering, chemical sciences, material sciences, physical and nanobio sciences, chemical engineering, mechanical and thermal engineering, and so on, with inherent difficulties to cover all the knowledge in only one program or to find only one person with all the expertise. The second challenge is how to elaborate a minimal program to give the knowledge catch up to the potential science and technology researchers. As the consequence, there are no centers where all the expertise is concentrated there. The fourth challenge is how to link the student simultaneously to several education centers. Finally, to build the best technological and scientific area with the best program and the best education program, we need the best candidates. The important and least formally considered question is how to make this education very attractive to young and clever researchers. The fifth challenge is how to turn this biorefinery area a long term attractive matter of research and innovative development, beyond fashion, grants, and job positions. The long history of experiences of almost 25 researchers from several Institutions, in network, collaborative projects, and graduate research works, is applied here to discuss in details all the aspects of curricula and to describe a proposal of the lignocellulosic biorefinery education program.

OPTIMIZATION OF THE SODA-ETHANOL DELIGNIFICATION IN THE BIOREFINERY OF RICE HUSK

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Lignocellulosic materials are an interesting alternative to obtain products with high added value, such as bioethanol, food and cosmetics additives, and adhesives, among many others. Rice hulls (RHs) is an abundant residue in the Argentine Northeast region (provinces of Corrientes, Entre Rios, Santa Fe, Formosa and Chaco). Rice production in Argentina in 2012/13 reached 1,397,242 tons, of which about 20% was RHs [1]. RHs is composed of 48.7% carbohydrates (34.1% cellulose and 14.6% hemicelluloses), 17.2% and 1.8% of insoluble and soluble lignin acid respectively, 15% of inorganic components, and others [2], so the application of the biorefinery concept, i.e. the separation and valorization of its individual components, is of high interest. Delignification of RHs pretreated with acid (previously optimized process) [2] was carried out using an Organosolv soda-ethanol process. This treatment is advantageous since the reaction is faster, it uses smaller amount of soda than the conventional soda process, and the ethanol can be recovered and reused. A Central Composite Experiment Design of two variables with three replicas of the center point was used. Soda concentrations between 9 and 17g per liter, and ethanol concentrations between 46 and 60% were evaluated. The liquor was placed in a reactor of AISI 316 stainless steel of 180ml, with a closing screw gauge. It was heated in a heat resistant silicone bath to 160°C. The reaction time was 60min, and it was necessary around additional 25min to reach 160°C. The mixture was then cooled in a cold water bath and filtered through a 100 mesh sieve. The solid was washed repeatedly to remove the remaining solution. The concentration of residual lignin (NREL process/TP-510-42618) in the pretreated solid was measured as response variable and delignification relative to initial lignin was calculated on this basis. The results were analyzed using multivariate analysis of variance (ANOVA). Statgraphics statistical software was used, with a level of significance of 95%. The adjusted model explained the 92% of the variability. Both variables showed significant effect ($p \leq 0.05$) on the residual lignin in the treated solid. The percentage of delignification ranged from 73% in the treatment with 13g/l NaOH and 46% EtOH; to 90% in the treatment with 13g/l NaOH and 53% EtOH. The optimal response corresponded to 13 g/l NaOH (central point) and 50% EtOH (-0.8 as coded variable), with a theoretical value of residual lignin in the solid of 1.61% (equivalent to 92% of delignification). In conclusion, the sequence acid / soda-ethanol allowed hemicelluloses extraction in the first stage and lignin extraction in the second stage. The residual solid was composed almost entirely of cellulose with approximately 25% of inorganic components, which will be separated in future studies, to fully implement the biorefinery concept.

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EUCALYPTUS SIDEROXYLON BARK AS A SOURCE OF HYDROPHILIC EXTRACTS

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Tree barks are complex biomass components with a large structural and chemical diversity among species that are today viewed as a potential resource for biorefineries. *Eucalyptus sideroxylon* A. Cunn. ex Woolls is a species native to Australia, that belongs to the ironbark group of eucalypts. The wood is very hard and used in construction. *E. sideroxylon* can be easily recognised by its hard, deeply furrowed, rough bark ranging from dark grey to black,.
The whole bark of *Eucalyptus sideroxylon* trees with 6 years of age was collected at a height of 1.30 m from an eucalypt arboretum located in the fields of the School of Agriculture, University of Lisbon.
The summative chemical composition of *E. sideroxylon* bark was analyzed by sequential determination of extractives (as the sum of dichloromethane, ethanol and water extractives), suberin (as the mass loss upon base-catalyzed methanolysis), lignin (as insoluble and soluble lignin following acid hydrolysis) and polysaccharide content (as the sum of individual sugars determined in the acid hydrolysate). The inorganic content was quantified as ashes.
Ethanol/water (50/50, v/v) extracts were prepared at 50 OC using an ultrasonic bath. Total phenolics content was determined by the Folin–Ciocalteu method, total flavonoids content by an aluminium chloride colorimetric assay and tannins content by the vanillin-sulphuric acid assay. Two methods were applied to determine antioxidant activity: the DPPH assay to determine a free radical scavenging activity and the FRAP assay to determine a ferric reducing power of bark extract.
The E. sideroxylon bark showed a remarkable high content of extractives, amounting to 55.7% of the initial dry bark, of which the polar compounds extracted by ethanol and water represented about 97% of the total extractives.
E. sideroxylon bark contained 1.9% suberin (4.6 % reported to extractive-free weight of bark) and total lignin represented 13.1% of the bark (29.6% of the extractive-free material). The sugar composition of the *E. sideroxylon* bark polysaccharides showed a comparatively high proportion of cellulose: the glucose content, corresponded to 80.1% of total monosaccharides and xylose represented 11.1% Ash content was 1.26 %,
The yield of ethanol-water extraction was 50.0%. The extract contained high levels of total phenolics (440.7 mg GAE/g extract or 219.9 mg GAE/g of bark), tannins (204.3 mg CE/g extract) and flavonoids (395.0 mg CE/g extract).
Regarding the antioxidant activity the extract of *E. sideroxylon* bark showed an exceptional DPPH scavenging activity (0.320 mg Trolox /mg extract, or 156.662 mg Trolox /g of bark) and lower IC_{50} value (IC_{50} of extract 0.120 mg /mL vs IC_{50} of Trolox 0.120 mg /mL). The ferric reducing ability of the extract revealed high FRAP activity (5247.0 mM Fe^{2+}/g extract).
These results allow to consider *E. sideroxylon* bark as an interesting source of phenolics and of antioxidant extracts.

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$AlCl_3$ CATALYZED ORGANOSOLV PULPING OF BEECH WOOD

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In view of the development of platform chemicals from lignocellulosic biomass, the pretreatment step is of special importance. Therefore several chemical pretreatment techniques have been developed (steam explosion, hydrothermal, acid pretreatment, lime pretreatment,…). In this work the so called Organosolv pulping is of special interest. In this pulping method lignocellulosic biomass is treated with a water/ organic solvent mixture. This treatment leads to a separation of the three main components in lignocellulosic biomass cellulose, hemicellulose and lignin.
In this work the usually taken Brønsted acid catalysts, like hydrochlorid acid or sulfuric acid, are replaced by the Lewis acid aluminium chloride. A comparison of the Brønsted and the Lewis acid catalyzed Organosolv pulping of beech wood leads to impressive results. Using the same molar amounts of catalysts the xylan hydrolysis is much faster if using the Lewis acid. The main products of the Brønsted acid catalyzed xylan hydrolysis are oligomeric and monomeric xylose. If using a Lewis acid the xylose reacts further to furfural and some other byproducts. The furfural yields are shown in Fig.1. The reason of the different furfural yields is a difference in the mechanism of the condensation of xylose to furfural. If using a Brønsted acid the condensation mainly takes place via cyclic intermediates of the xylose. If using a Lewis acid the ketose xylulose is formed as an intermediate.
Also in view of the other components cellulose and lignin the AlCl3 catalyzed Organosolv pulping leads to good results. The recovery rate of the cellulose is in the range of
90 % till 95 %. The Organosolv lignin is soluble in most of the organic solvents and has a low average molecular weight.
Comparing biorefinery concepts there is a huge advantage of using a Lewis acid compared to a Brønsted acid as a catalyst. For the conversion of C5-carbohydrates to high value added products there are no additional capital as well as energy costs required. If a Lewis acid is used the conversion to furfural takes already place in the pretreatment stage.

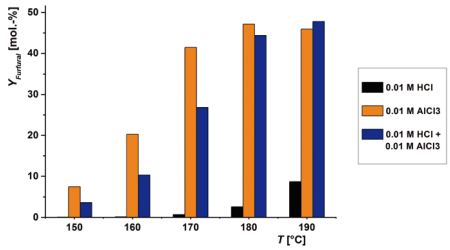

Fig.1 – Yield of furfural after the organosolv pulping of beech wood in dependence of the temperature. Reaction conditions: t = 60 min; Input: 1g beech wood and 5.6 ml solvent.

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DEVELOPMENT OF A HETEROGENEOUS ACID SOLID CATALYST BASED ON BIOCHAR WITH MAGNETIC PROPERTIES.

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Biochar a product derived from thermochemical conversion of lignocellulosic residues has been widely used as adsorbent material or remedial/ amendment for soil based in its physics and chemical properties [1]. Moreover, in the last years this material has been found useful as base for the preparation of acid heterogeneous catalyst to be applied for the synthesis of biodiesel. To obtain this catalyst, the biochar must be activated by introduction of SO_3H groups in its structure, which is involved in the esterification/transesterification reaction [2]. In despite of it good performance, this type of catalyst need to spend energy in terms of filtration or centrifugation for its separation from the products. Therefore, an interesting alternative is to provide magnetic properties to the catalysts [3]. The purpose of this research was to develop a heterogeneous acid solid catalyst based on biochar with magnetic properties. In this work, oat hull was used as raw material. The operation conditions of pyrolyzer were: 600°C, heating increase rate of 3.5 °C/min and residence time of biomass of 1.5 h. The biochar obtained was activated consecutively with HNO_3 8 M and concentrated H_2SO_4 at 140 °C for 4 h. Finally, the magnetization was carried out by co-precipitation method. Aqueous suspension containing biochar, $FeCl_3$ and $FeSO_4$ was prepared under vigorous magnetic stirring and precipitated by adding NaOH solution. Potentiometric titration method was used to measure total acidity and FTIR for identification of functional groups presents in the prepared catalysts. Preliminary results showed the formation of reactive groups in each activation phase, mainly nitrogen (such as NH_2, CN and NO), oxygen (COOH, C=O, CHO) and sulfate compounds (SO_2 and SO_3H), increasing the total acidity.

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PINE NUT SHELL AS A FEEDSTOCK FOR OLIGOSACCHARIDES PRODUCTION

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Pinus pinea (Italian stone pine) is widespread throughout the Mediterranean region and particularly in Portugal it occupies an area of about 78.000 hectares, of which 68% are located in the district of Setúbal. Annually in Portugal are produced from 600,000 to 700,000 tons of pine nuts, that also yields large amounts of residues, the pine nut shell. This represents about 80% of the fruit mass, corresponding to an annual production of about 480,000 to 560,000 tons.

Due to these large amounts of wastes and their geographical concentration, the use of pine nut shells as feedstock within the biorefinery framework can be a potentially advantageous alternative. In order to achieve it, fractionation processes must be applied. Autohydrolysis process was carried out to the selective removal of hemicelluloses, maximizing the yield of oligosaccharides, compounds with potential applications in food, pharmaceutical and cosmetic industries.

Autohydrolysis experiments were performed using a liquid-solid ratio of 3 (g/g), under non-isothermal conditions in the range of 150 to 230ºC. After treatment completion, liquid and solid phases were separated by filtration and both their compositions were characterized according to standard NREL protocols and HPLC using an Aminex HPX-87H column. Oligosaccharides are the major soluble products found and were categorized as acetylated xylo-, galacto- and mannan-oligosaccharides, and at the optimal conditions (210ºC) their maximum concentration is 15.4 g/L.

The enzymatic digestibility of autohydrolysis treated materials (sstandard NREL protocols using Celluclast 1,5L and Novozyme 188) was found to increase as a function of increased treatment severity, but reaching only 14 % for the severest condition.

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BIODIESEL FROM AGRO-INDUSTRIAL WASTES: AVOCADO OIL, A STUDY CASE

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Despite the great interest aroused by the use of biodiesel as a renewable energy source, the use of raw material directly linked to the food chain, makes biodiesel an expensive product. The use of cheap raw materials is an alternative to improve the economy of the production process and provide more affordable fuel. Apart from edible vegetable oils, other alternative sources of triglycerides with low cost, are the agro-industrial wastes. In this context, this paper refers to the utilization of wastes from the production of avocado as a potential source of raw material for the production of biodiesel. The avocado is a nutrient-rich food with a high concentration of oil. Avocado oil contains a high proportion of monounsaturated fatty acids (MFA) and polyunsaturated fatty acids (PFA) (60% and 20% respectively) and a low amount of saturated fatty acids (SFA) (30%). The fatty acid ratio is modified with fruit maturity, being that the proportion of MFA increases and the SFA decreases. In general, its SFA content is similar to oils of either sunflower, corn, olive, soybean and peanut, its MFA content is similar to that of olive and canola oil, and its PFA content is greater to that of olive and palm oil, but lower than that oils of either corn, cotton, soybean and sunflower [1-3]. Agro-industrial wastes of avocado (Persea americana Mill, var. Hass) were used. For the oil extraction, the whole avocado (peel, pulp and seed) was triturated, and the product was dehydrated. After that, the oil was extracted by the Soxlhet method using heptane as solvent. The content of avocado crude oil extracted was 32-38%. The fatty acid profile of the avocado crude oil was determined by gas chromatography. Transesterification of avocado crude oil (50 g) was carried out carried out under standard conditions using a KOH methanolic solution at 70 °C for 1 h with constant stirring. The reaction course was followed by thin layer chromatography. After cooling, the glycerol phase was separated. Methanol of the methyl ester phase was evaporated, and 50 ml of heptane was added. The mixture was twice washed with NaCl-saturated solution until pH = 7. As final step, the organic phase was dried with anhydrous sodium sulfate, and the solvent was evaporated. The yield of biodiesel was 90%.

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EVALUATION OF ORGANOSOLV PROCESS ON WHEAT STRAW FROM VALLE DEL YAQUI, SONORA

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With the actual demographic growth the use of paper and carton has increased in Mexico to the point of needing to import cellulose pulp from a different country. At Valle del Yaqui, localized in Mexican state Sonora, wheat straw residue are considered a pollution problem as they are burned indiscriminately, nevertheless they could be an option to cellulose production used in carton industry as well as national paper industry, this is capable thanks to the straw lignocellulosic nature. Lignocellulose is composed of a three polymer matrix (cellulose, hemicelluloses and lignin). To be able to divide wheat straw in their component they can be treated with chemical, physical and/or biological processes. Among these, the organosolv process consists of separating wheat straw into its components by high temperature and organic solvents [1], using acetic acid as solvent. The objective of this work was to optimize time variables and temperature for the pulp organosolv process using an experimental design of response surface, statistical software Design Expert 7 was used, creating a complex central design to evaluate the temperature effect and reaction time of wheat straw separation having a response Kappa number (residual lignin quantity in cellulose pulp) and pulp viscosity. For the separation reaction wheat straw samples, variety CIRNO, were taken from Valle del Yaqui. Experimental units were 30 g of wheat straw each, they were processed in 1 lt. steel reactors, mixed with aquose solution of acetic acid, delignification samples according to experimental design. Resultant phases were separated by filtration to retain the pulp. Liquor obtained in pulping was evaporated at 40 C using a rotavapor until achieving a 30-40% solid concentration, which distilled water was added after, to separate both fractions, hydrophobic (composed by lignin) and hydrophilic (composed by hemicelluloses). Data analysis was done with Design Expert 7 software. After treatments, two optimized responses were found to a less number Kappa with mayor viscosity possible: Proposal A with temperature parameter of 175.46 C during 60 minutes obtaining a Kappa of 10.08 and viscosity of 459.98 ml/g and Proposal B with temperature parameter of 161.38 C during 180 minutes obtaining a Kappa of 9.23 and viscosity of 452.79 ml/g. These proposal need to be evaluated [2] to be able to choose the most convenient process to obtain pulp from wheat straw destined to paper industry/carton in Mexico.

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5-HYDROXYMETHYLFURFURAL OXIDATION USING HYDROTALCITE SUPPORTED COPPER CATALYST

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Biorefineries involve multiple catalytic processes in order to convert biomass feedstock into valuable chemical products, such as alcohol oxidation [1,2]. A potential chemical platform alcohol derived is 5-hydroxymethylfurfural (HMF), which is a versatile key intermediate in new generation renewable based chemicals [3-5].

HMF transformation is an aerobic oxidation route that leads to 2,5-diformylfuran (DFF), 5-hydroxymethyl-furan-2-carboxylic acid (HMFCA), 5-formyl-2-furancarboxylic acid (FFCA), and 2,5-furandicarboxylic acid (FDCA) formation. These compounds are important intermediates as monomers for polymers production, being able to replace petroleum derived aromatic [3, 5-7].

Researchers are exploring a variety of catalysts with alternative metal types in HMF oxidation over a broad range of conditions. Some catalysts have been investigated, including V, Cu and Mn, and catalysts containing noble metals [1-11]. Various supports have been used in this reaction, including supports with basic functionality, like hydrotalcite (HT) due to a bifunctional catalyst nature [4,10].

The aim of this work was to investigate HMF oxidation over Cu/HT. The effects of O2 pressure, temperature and nature of base on HMF conversion and product selectivity were evaluated.

Cu-supported on HT (15 wt% Cu) was prepared by incipient wetness impregnation method described elsewhere [12]. Catalyst pretreatment was carried out ex-situ in a fixed bed quartz reactor under atmospheric pressure. The impregnated solid was dried under He flow (30 ml/min) at 150 °C for 1h and reduced under H2 flow (100 ml/min) at 500 °C for 1h. This system was cooled and catalyst was passivated with 5%O2/He (100 ml/min) at 0 °C. HMF oxidation reactions were carried out in a batch reactor (Parr 4842- 100 ml) with vigorous stirring (700 rpm). 4 mmol of HMF was dissolved in 42 ml of water and NaOH (2M) amount was added. Then, an amount of catalyst was added to the above aqueous solution. The reactor was closed and pressurized with nitrogen while heating. After that the reactor was purged with oxygen once and then pressurized to the required pressure. Reactants and products were analyzed by HPLC (Agilent 1260 Infinity) with Zorbax Eclipse Plus C18 column and DAD detector.

Catalyst was characterized by XRF, XRD, and CO_2-TPD. The wetness impregnation method was efficient and characterization results were in agreement with literature [12,13].

Table 1 lists HMF conversion and yield after 5 h of reaction. HMFCA was the major product and its highest yield was about 30%. Higher O_2 pressure favored FDCA yield, in the range investigated, as observed in previous studies [1,4]. Nevertheless, HMF conversion was independent of O_2 pressure (entry 1-3). Higher temperature favored HMF conversion (96%), but decreased oxidation products yield (entry 3-5). The significant influence on conversion and product distribution indicates an important role of OH- in the activation, oxidation and degradation of compounds at high base concentration [2,8,11], as was observed (entry 5-6). The formation of HMFCA was major to lower amounts of OH-. And also Na_2CO_3 favors HMF conversion and formation of oxygenates products (entry 6-7).

Table 1 HMF oxidation in water over 15 wt% Cu/HT after 5h reaction time

Entry	Temperature (°C)	Pressure (bar)	Conversion (%)	Yield (%)			Unknowns (%)
				HMFCA	FFCA	FDCA	
1	60	2.5	82.7	26.1	2.9	1.0	52.6
2	60	5	84.2	29.5	2.0	0.8	51.9
3	60	10	82.6	26.7	7.3	5.1	43.4
4	80	10	84.5	8.1	3.1	0.5	72.8
5	100	10	96.0	6.7	1.6	0.6	87.1
6[a]	100	10	30.5	10.5	0.0	0.0	20.1
7[b]	100	10	61.5	5.7	4.6	0.6	50.6

Reactions Conditions: HMF/Metal = 10 (mol/mol), pH=13.

[a] Reaction with NaOH (pH=11)

[b] Reaction with 0.83 wt% Na_2CO_3 (pH=11)

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BIORREFINERIA BASADA EN LA SEMILLA DE MANGO (*Mangifera indica L.*). VALORIZACIÓN DE ALGUNOS DE SUS COMPONENTES COMO BASE PARA UN ANÁLISIS DE CICLO DE VIDA

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Introducción

Al conocer la composición química de la semilla de mango se pueden obtener diferentes productos de interés, como biodiesel, productos con actividad coagulante-floculante e incluso productos con aplicación a la industria de los alimentos, como espesante o emulsificante (Torres y Col., 2012), cambiando así el enfoque de producción lineal por uno cíclico (Cervantes, 2007) y proporcionando un valor agregado a la materia prima y evitando el uso excesivo del suelo, agua, energía y otros recursos. En este trabajo se presenta el análisis proximal de la semilla de mango, así como los resultados de la prueba de uso de la harina y goma de semilla de mango, como coagulante-floculante de aguas residuales municipales. También se presentan algunos resultados sobre la capacidad emulsificante de la goma empleando aceite comestible o diesel. Se presentan las bases del análisis de ciclo de vida del biodiesel producido a partir de la semilla de mango. Esta será el primer paso para un análisis comparativo de los impactos ambientales al utilizar la semilla de mango como origen de una biorefineria.

Metodología

Se eliminó la drupa de forma manual para obtener la semilla, la cual fue cortada en láminas y se deshidrato en un secador de charolas a una temperatura de 49 °C durante 24 horas, posteriormente la semilla se pasó por un molino de discos para obtener una harina y se procedió a realizar el análisis químico proximal, el cual fue realizado mediante los métodos recomendados por la AOAC. Los lípidos obtenidos se sometieron a una transesterificación para obtener Biodiesel (BD). Se determinó actividad coagulante-floculante mediante una prueba de Jarras, determinando parámetros como color, turbidez, pH, volumen de lodos y DQO en aguas residuales provenientes de la planta San Juan Ixhuatepéc (Edo de México), antes y después de la aplicación de diferentes dosis de la harina desgrasada de semilla de mango. La capacidad emulsificante de la goma se evaluó en una prueba estándar, empleando aceite de cocina o diesel como la fase oleosa. Se empleó la harina desengrasada de la semilla y se midió la estabilidad de las emulsiones generadas. El análisis de ciclo de vida se hizo en base a las norma UNE-EN ISO 14040 y a la normas mexicanas, utilizando SIMAPRO 7.2 y con el método EIDP2013.

Resultados

El análisis proximal de la semilla de mango se muestra en la Tabla 1. Como puede observarse, la cantidad de carbohidratos contenidos en la semilla de mango es muy considerable y puede dársele diferentes usos. Los valores obtenidos en este trabajo se comparan aproximadamente con los de Pascual-Bustamante y col, aunque en este trabajo se encontraron más carbohidratos, más grasa y menos proteína que en aquel trabajo. Estas diferencias pueden ser debidas a diferencias en el estado de maduración de la fruta.

Tabla 1. Análisis Químico Proximal de la harina de la semilla de mago (g/100g B.S.).

Componente	Valor experimental	Pascual-Bustamante y col. (2007)
Proteína	5.46	2.2
Grasa	27.8	29.5
Cenizas	2.48	2.21
Fibra	ND	0.14
Extracto libre de Nitrógeno	62.35	85.95

En la prueba de jarras con la harina desengrasada se observó una disminución en los parámetros de turbidez, color, pH y conductividad, un aumento en el de volumen de lodos mientras que en los valores de DQO no se observan cambios significativos, obteniéndose un máximo del 4% de remoción en este parámetro.
Estas mismas pruebas fueron llevadas a cabo con la goma recuperada al precipitar la harina, pero los resultados en la prueba de jarras fueron inferiores y no se muestran aquí. En cuanto a las pruebas de emulsificaciòn, se encontró que la harina de semilla de mango resulto capaz de emulsificar tanto al aceite comestible como al diesel en mayor proporción de lo que hizo un aislado proteico proveniente de *Phaseolus*. Para el ACV se tomó como unidad funcional 100 kW generados a partir del biodiesel obtenido de la semilla del mango Haden.

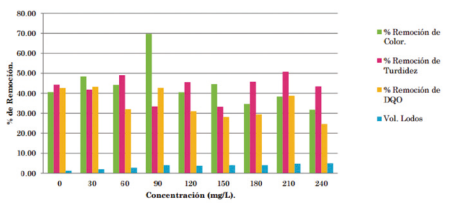

Figura 1. Resultados de la coagulación-floculación de aguas residuales empleando la harina desengrasada de semilla de mango.

Conclusiones

La semilla de mango es una fuente potencial de polisacáridos. Los lípidos de la semilla de mango representan una fuente alterna para la producción de BD. Los carbohidratos presentes en la harina de la harina de semilla de mango presentaron actividad coagulante-floculante la cual podría ser empleada para el tratamiento de aguas residuales. Por otro lado, la capacidad emulsificante de la harina la hace candidata para emplearse en alimentos, pero también como agente de lavado en suelos contaminados con hidrocarburos de petróleo o pesticidas, como se ha reportado para algunas gomas (Torres el al ,2012).

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EVALUATION OF CHEMICAL CHANGES IN LIGNOCELLULOSIC BIOMASS OF SACRED FIR WOOD ALONG AN ELEVATIONAL GRADIENT BY FTIR-ATR SPECTROSCOPY

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Lignocellulosic biomass in the form of wood has been used by mankind since the dawn of civilization, presenting a number of advantages such as being an energy source: its local availability, its accessibility and renewal [1]. However, lignocellulosic biomass is complex and heterogeneous [2], showing a compositional variation associated with various factors, for example: type of plant species, genotype, environment and its genotype-environment relationship [3]. Sacred fir (*Abies religiosa*) or Oyamel, is an endemic conifer of Mexico, its wood is considered to have high valued physical properties. This plant is developed under very specific geographical, climatic and ecological conditions, confined to high mountain areas in altitudinal gradients [4,5]. Therefore, the Oyamel grows in limited environmental conditions, and it is relevant for its use and conservation, to generate knowledge about the possible influence of environmental factors on their lignocellulosic composition.

Currently, various spectroscopic techniques are being used to determine the chemical composition of the wood. One such technique is infrared spectroscopy, also known as FTIR (Fourier Transform Infrared Spectroscopy), a very useful analytical method, widely used by scientists because it presents a series of advantages being a non-destructive technique and of high performance, which allows qualitative and quantitative analysis of wood samples, among other uses[6,7,8]. FTIR operates in the mid-infrared region, providing information about particular components of the cell wall through absorption bands, additionally, if the FTIR features the ATR device (Attenuated Total Reflection), IR spectra can be obtained by minimizing interference due to the presence of water [7].

Based on the above, the objective of this research was to evaluate, through FTIR-ATR spectroscopic analysis, if there is a difference in the lignocellulosic composition of Oyamel tree wood (36 trees), which was developed along an elevational gradient of 3,000 to 3,500 meters above sea level in the central area of Veracruz, Mexico.

FTIR spectra corresponding to the Oyamel wood were generated, detecting 16 peaks comprised amongst the region 3500-800 cm-1 (Figure 1). When statistically analyzing the intensity (absorbance) of 15 peaks, only statistically significant difference was found in the band of 2,891 cm^{-1} ($F = 4.833$, $P = 0.00233$), based on that reported in the literature, this peak corresponds to the C-H type bonds of methyl and methylene groups [7], which according to the results, increase in intensity in Oyamel tree wood developed in an altitudinal elevation of 3,200 meters. The results suggest that trees grown at this altitude, could present a different physical feature to the ones of other trees that grew in other dimensions

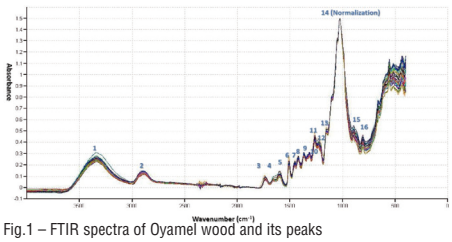

Fig.1 – FTIR spectra of Oyamel wood and its peaks

of the altitudinal gradient studied, probably due to the existence of different micro-climatic conditions and specific soil of this altitude, which could have an influence on the development of Oyamel wood. Additionally, this study confirms the usefulness of the analytical FTIR-ATR technique in the comparison and qualitative evaluation of the chemical composition of wood samples.

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EXTRACT FROM GRAPE CANE UNDER PILOT SCALE CONDITION: CHEMICAL CHARACTERIZATION AND ANTIOXIDANT CAPACITY

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The evidence of the health benefits of E-resveratrol (polyphenol) has increased over the last twenty years. This has caused the interest to study the levels of this and other stilbenoids residues in grapes, wine and winemaking. In Chile, more than 120.000 tons of waste each year winemaking occur, including pomace, seeds, stems and canes [1].
In this work an extract of *Pinot noir* grape canes was produced at industrial scale in a reactor of 750 L. Grape canes were chopped in a grinder Retsch brand model SM 300-2000 rpm, until 1 cm of length, and were stored in 1m^3 bags. The extraction solvent was a mixture of ethanol and water (80% v/v) [2]. Solvent mixture was prepared in an Intermediate Bulk Container (IBC) of 1 m3 by mixing 543.9 L of ethanol and 127.9 L of water. The extraction process was divided in different stages: a) maceration, b) heating, c) extraction, d) cooling, e) discharge and f) evaporation. Profiles and content of stilbenoids and other polyphenols (mainly procyanidins) were determined by HPLC-DAD-ESI-MS/MS. The recovery of total stilbenoids was studied by following the overall process (Fig. 1). Other chemical constituents as carbohydrates, organic acids, metals and lignin were also detected and quantified. Antioxidant capacity of the whole extract, using cells and cell-free assays was also evaluated.
The dry extract obtained under optimal conditions (T: 80 °C, t: 100 min, ratio S/L: 1:10) showed, a yield of 2.4 g stilbenoids by kg of dry grape cane and total stilbenoids concentration was 5.45% w/w. The antioxidant capacity was determined by ORAC was high (14760.66 µmol trolox equivalent/g of extract) However, additional antioxidant assays suggested synergistic or antagonistic effects between compounds.

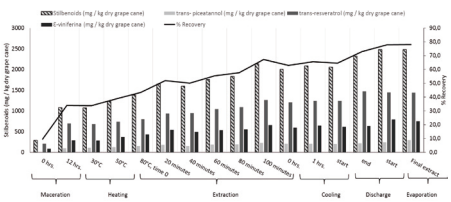

Fig.1 – Monitoring the concentration of each of the stilbene during the different stages of the extraction process under pilot conditions.

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FRACTIONATION OF LIGNOCELLULOSIC BIOMASS AND RECOVERY OF PHENOLIC COMPOUNDS ASSISTED BY IONIC LIQUID AND SUPERCRITICAL CARBON DIOXIDE TECHNOLOGIES

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The valorisation of renewable carbon source such as lignocellulosic biomass, is an opportunity to substitute the petroleum usage. However, technological limitations and environmental issues lead to unrelenting search and development of efficient, green and sustainable methodologies to apply in biomass processing and fractionation. This opens a window for the successful application of ionic liquids (ILs) as efficient tools for biomass fractionation and further valorisation [1,2].

Among the wide range of IL applications already existing for biomass processing [1,2], a differential and innovative process based on a three-step fractionation of lignocellulosic biomass into high purity samples of cellulose, hemicellulose and lignin is hereby presented [3]. Several ILs were examined and 1-ethyl-3-methylimidazolium acetate ([emim][OAc]) demonstrated the highest efficiency for entire biomass dissolution [4]. The maximal biomass dissolution with this IL allowed selective and efficient precipitation of each lignocellulosic fraction mediated by the addition of specific anti-solvents. Purity superior to 86% was obtained for each obtained fraction, which can be later directly used for other successive applications. The efficiency of the process is thereafter accomplished by recovering IL up to 96% of the initial content [3].

Another interesting finding was the change of [emim][OAc] colour from the original light yellow to a dark brown after the recovery from pre-treatment process. Solid phase extraction (SPE) was applied for the recovered IL using different type of polymeric resins and several phenolic compounds, such as vanillin, tricin, *p*-coumaric and vanillic acids, among others, were found to be present. The phenolic-rich extract still contained residual IL after SPE methodology. Therefore, subsequent extraction of phenolic compounds was successfully accomplished using supercritical CO_2, which produced a phenolic-rich sample free of IL.

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HEMICELLULOSES EXTRACTION FROM EUCALYPTUS WOOD UNDER ALKALI CONDITIONS

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Hemicelluloses are the second most abundant polysaccharide in the nature after cellulose. They can be used for in packaging- films, hydrogels or biomedical materials. In the case of eucalyptus wood, glucuronoxilanos are the main constituent. The effects of alkali concentration, time and temperature of the alkali treatment on the total dissolution of hemicellulose from eucalyptus wood are here analyzed. Samples of sliced wood are considered in order to minimize diffusion restriction for the hemicelluloses. Sugar concentration in the extraction liquor is determined by a rapid method and also by acid hydrolysis and HPLC-PAD method. A kinetic expression of xylan dissolution is found which could be useful for modeling the hemicellulose extraction process from wood chips.
Fresh *Eucalyptus grandis* of 6 years old were treated using a face-centered cube central composite design (Time: 10, 20 and 40 min; alkali concentration: 1, 0.5, 0.25 N NaOH and temperature: 110, 120 and 130ºC). The treated liquor was separated, filtered and quantitatively neutralized to pH 9, then centrifuged for 15 min to remove precipitate. The supernatant was filtered (fiber glass filter of 1.5 µm).
The carbohydrates concentration of the extract was determined as Sluiter et al. [1]. Samples were treated with sulphuric acid 0.03% for one hour at 121ºC. The sample was filtered and two aliquots of the supernatant were separated. One was neutralized with and diluted for HPLC carbohydrates determination. The other was used for soluble lignin determination.
Supernatant hemicellulose concentration was also determined by the phenol/sulphuric acid method proposed by Hodge and Hofreiter [2]. The xylose content was determinated by a calibration curve using xylose provide by Sigma Aldrich.
Soluble lignin was determined by spectrophotometric measurements at 240 nm using an absorptivity of 25 (L. g^{-1}.cm^{-1}) as was proposed by Sluiter et al. [1].
The alkali consumption was between 8 to 21 NaOH g/100 g of wood and strongly dependent on the nominal concentration of the solution.
Figure 1 shows the amount of xylose extracted by the alkaline treatment as a function of the H-factor.

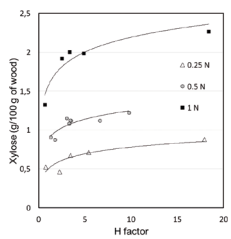

Fig.1: Xylose extracted (g/100g of wood) as a function of H-factor.

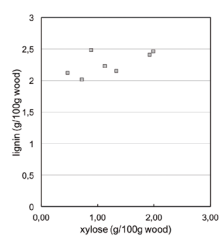

Fig. 2: lignin extracted during some treatment.

The amount of extracted xylose shows a strong dependence on the process variables, and is increased when treatment intensity (H factor and alkali concentration) is increased.
Figure 2 shows the amount of soluble lignin extracted for some of the treatments, as a function of the xylose extracted. The amount of lignin found in the extracted liquor resulted similar for the different treatments. Always more lignin than xylose is extracted.

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HYDROTHERMAL TREATMENTS OF CISTUS LADANIFER RESIDUES AFTER ESSENTIAL OIL DISTILLATION

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The use of forest understory biomass, including shrubs, is a particularly relevant issue, as they can become an environmental problem namely related to the risk of fire spread. An effective valorization of these biomass materials will therefore contribute to solve this problem. *Cistus ladanifer* (rock rose) is one example of a perennial shrub species that is mainly distributed in the Mediterranean countries. It is particularly interesting as a source of essential oils and bioactive compounds for cosmetics and perfumes. Most studies have been related to ecological and environmental issues or to composition and a bioactivity of its essential oils and exudates from the leaves. Few studies describe its lignocellulosic composition and no detailed chemical characterization of *Cistus ladanifer* (CL) is reported. A detailed study of the chemical composition of CL and the development of mild hydrothermal processes (autohydrolysis) for the selective hydrolysis of hemicelluloses, and production of xylo-oligosaccharides (XOS) and cellulose/lignin enriched solids can provide a new perspective for the use of residues from these species in a biorefinery framework.
The *C. ladanifer* residues (CLR) used in this work consist of 2 to 4-year-old plants previously extracted to obtain the commercial essential oils. The CLR showed the following average composition (dry mass basis, extractive-free): glucan 27.8%, xylan 13.8%, arabinan 4.3%, acetyl groups 4.6%, Klason lignin 29.7%, soluble lignin 3.0% and ash 4.3 %. CLR has also a significant amount of extractives (ca. 40%), suggesting an important additional potential for their valorisation.
In order to selectively hydrolyse the hemicelluloses, the CLR were subjected to autohydrolysis treatments. The raw material was mixed with water in a 6:1 liquid-to-solid ratio (w/w) and the effects of temperature (130-230ºC) on the composition of liquid and solid phases was evaluated and interpreted using the severity factor (log Ro).
The operational conditions leading to the highest recovery of oligosaccharides (20.5 g/L), corresponding to a yield of 23.8 g/100 g of CLR, were established at log R0 of 3.56 (205ºC). The highest glucan content of the solid residue was obtained for log R_0 of 4.0 (220ºC). There was no apparent lignin solubilisation in any of the conditions, which is an advantage of this hydrolytic pretreatment.
The results obtained showed that the hydrothermal processing of *C. ladanifer* residues is an interesting option for hemicellulose fractionation producing a XOS-rich liquid stream together with a lignin and cellulose rich solid stream that can be used for the production of biofuels and bioproducts. In addition, the chemical composition of CLR also suggests the potential of these residues for the production of a wide range of added-value compounds.

Acknowledgements
Junia Alves-Ferreira is grateful to CAPES Foundation, Ministry of Education of Brazil, Brasília – DF 700 40-020, Brazil (doctoral scholarship – Process 9109/13-7). This work was supported by QREN Project "Biomassa Endógena".
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OBTAINING SEED OIL OF *Pinus Radiata* BY SUPERCRITICAL FLUID ASSISTED WITH ULTRASOUND

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Traditionally obtaining lipid extracts performed by using hexane as a solvent due to its high yields, however the use of this solvent makes the resultant product has a restricted use[1]. Techniques such as extraction with supercritical fluids (Figure 1) can be a good alternative when humado obtain a product for consumption, which can increase their yields when combined with ultrasound[2]
In this paper the optimization is presented in the first part of pressure temperature conditions for obtaining oil from seeds of radiata pine, using supercritical CO_2, and in a second stage the working conditions ultrasound presented different potencies (Figure 2). The extract obtained characterized by GC-MS and compared with excito obtained by soxhlet.
The yields obtained by FSC were between 21-23% while by soxhlet was greater than 35%. Characterization by GC-MS present between the two different extraction techniques, however the main components of which were: oleic, linoleic and linolenic acid.

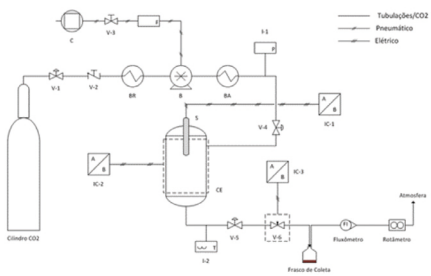

Figure 1: scheme supercritical fluid extractor.

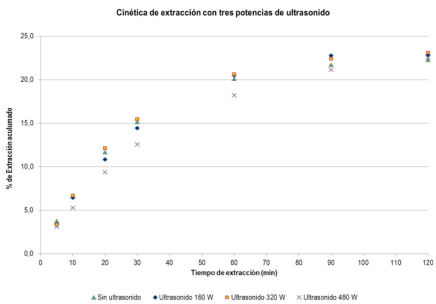

Figure 2: extraction kinetics with ultrasound.

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VALORIZATION OF HOLLOCELLULOSE FORM IVORY NUT LEFTOVERS UNDER THE BIOREFINERY CONCEPT

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Pontificia Universidad Católica del Ecuador

Abstract
Phytelephas aequatorialis is an endemic species from the tropical and subtropical regions of the occidental foothills of the Andes in Coastal Ecuador, which is widely distributed along habitats below 1,500 m.a.s.l. In Ecuador, this palm had been tightly related to the economy of the country until 1950s, when petro-based polymers replaced ivory nut manufactures, mainly that of buttons for fashion industry. The seeds' endosperm is whitish and hard, that is why it's commonly known as "vegetable ivory".
Currently in Ecuador, both the handicrafts industry and the buttons manufacturer workshops produce 400 t/month of residues are being used as firewood in bricks' ovens and industrial boilers. Occasionally the residues are also used as filler for animal feed. In the search for new chemical platforms for biorefineries, a research on valorisation of the ivory nut residues has been performed.
This biomass is especially rich in hollocellulose, which represents nearly 90%-93% of its weight, i.e. 82% mannan , 8% cellulose and 7-10% water. The mannan polysaccharides in the endosperm's cell walls are the reserve of energy for the embryo during germination and the structural scaffolds made of cellulose fibrils, provides support to the cell walls.
By using both chemical and biochemical approaches we were able to obtain several different products from the ivory nut leftovers. Herein we report three of them: a. fuel ethanol, b. mannan oligosaccharides, and, c. nanofibrillated cellulose. Ethanol is currently being processed in a pilot biorefinery located in Quito Ecuador, under the Netropical Center for the Biomass Research (CNIB) at the Pontificia Universidad Católica del Ecuador (PUCE). The potential uses and future applications of these products will be addressed in this presentation.

VALORIZATION PRIOR TO COMBUSTION: REMOVAL OF HEMICELLULOSES FROM EUCALYPTUS SAW DUST.

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The conversion of biomass into biofuels and chemicals products has gained interest due to the increasing energy demand, limited supply of fossil fuels and growing concern about the environmental impact of emissions of greenhouse gases.

This study is part of a project work that aims xylitol production from wood residues resulting from industrialization in sawmills at the north of Uruguay.

Previous studies have indicated that the production of liquid biofuels, biomaterials and bioenergy could profitably coexist in an integrated process. The goal is to make a preliminary extraction to the material to be burned in the boiler to produce energy. From the efficiency viewpoint , the hot water extraction removes the components of the raw material which haves lower heating value (hemicelluloses) and can become valuable if they transformed in other products such as ethanol or xylitol products. By extracting the hemicelluloses, the calorific heat of residual solids is higher per unit mass.

The aim of this work is to study the products formed in the water extraction of *Eucalyptus grandis* sawdust. We worked with the sawdust fraction less than 3 mm and with different conditions of temperature (80-170 ˚C) and time (30 - 480 minutes) for a L/W ratio of 7. Final pH, carbohydrates (mono and total), the carbohydrate molecular weight distribution and the presence inhibitors as furfural, HMF and lignin are analyzed. The production of other products such as acetic and formic acid is also analyzed in the extracted liquor,. Likewise, the calorific value of the original sawdust and the solid material obtained after extraction is also determined.

The best extraction conditions are between 165-170 ˚C and 30 minutes. In these conditions the content of xylose in the extracted liquor is 10% of the dry wood (67% of the xylose originally present in the wood) and the formation of furfural is less than 0.3% The compounds obtain with this conditions are in the form of monomers or oligomers with lower molecular weights 1000Da.
The high calorific value of the extracted sawdust is slightly higher (2%) than the starting sawdust

Additionally, with the experimental data obtained we modeled in Matlab the auto-hydrolysis kinetics of *Eucalyptus grandis* xylan.

AN ALTERNATIVE TO PRODUCE VALUE ADDED BIO-BASED PRODUCTS FROM SUGARCANE BAGASSE.

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Small-sized biorefineries are not capital intensive and have lower transportation cost, lesser movements of liquid and solid streams, and lower heat transfer problems than high-sized ones [1]. Xylitol production consists of the following stages: autohydrolysis of the hemicellulose of bagasse, concentration of spent liquor, acid post-hydrolysis, removal of inhibitors by adsorption, fermentation of xylose to xylitol, and xylitol recovery by crystallization. A simplified kinetic model was developed for the extraction of xylose in the autohydrolysis process. Kinetic constants *kh* and *k1* were determined, and activation energies Ea and *Ln(ko)* for the kinetic reactions were calculated from the Arrhenius equations. Experimental data obtained by Vallejos et al., [2] were used for the autohydrolysis pretreatment step. Temperature and time for the maximum extraction of xylans with minimal energy demand were determined with the model. The conditions and reactions for the other stages of the process were selected from updated bibliography. In addition, costs and benefits that could be obtained by exploiting the residual solid feedstock were estimated. Sugarcane is so far the most efficient raw material for bioethanol production, involving four major unit operations: enzymatic hydrolysis, fermentation of sugars into ethanol, and ethanol recovery [3]. Pellets production could be an interesting alternative for the hydrolysis residue [4]. In this work, the economics of xylitol production from hemicelluloses versus other alternatives was evaluated. Two alternatives for the use of the solid fraction were proposed: (1) ethanol production or (2) pellets production. Figure 1 shows a simplified block flow diagram of the process to convert lignocellulosic materials into xylitol. Autohydrolysis treatment and evaporations are energy intensive operations because of high temperatures and large amounts of water involved. The most expensive equipments for the process would be the fermenter and the crystallizer (approximately 19% of total capital investment). In this scenario, 93.9 kg of xylitol could be obtained per ton of dry bagasse. The economic evaluation showed long recovery periods. However, optimization of different steps, as liquid to solid ratio of pretreatment, evaporation, or fermentation, could represent attractive and innovative alternatives to reduce the recovery periods of capital costs.

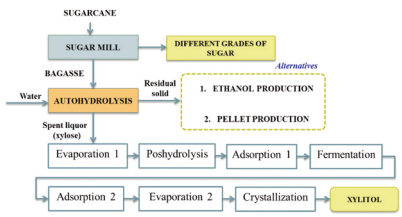

Fig.1 - Block flow diagram of the simplified process of xylitol production with two alternatives for the use of the residual solid

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CALCIUM CARBONATE IN PAPER PRODUCTION AND ITS POTENTIAL TO MITIGATE CLIMATE CHANGE.

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Calcium carbonate can comprise up to 20% of printing and writing paper. Usually the source is crushed rock or carbonate clays, that is, ground calcium carbonate (GCC), though precipitated calcium carbonate (PCC) is also used sometimes. Notably, the precipitation process can make use of pre-existing carbonate material as its source, or, alternately, carbonate that originated in atmospheric or oceanic processes. Current practices and future pathways for including calcium carbonate in paper production will be discussed. The potential for promoting carbon sequestration in the paper industry will be highlighted.

BLEACHING STUDY OF PULP OBTAINED FROM SUGARCANE BAGASSE – STEP 1: BLEACHING WITH H_2O_2

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Brazil is the largest producer of sugar cane. Sugarcane bagasse is a coproduct of the sugar and alcohol industries, it represents about 14% of the mass of the entire plant. It is highly available and inexpensive, so this biomass can be used in energy production by cogeneration, or have their value increased by processing. The purification of cellulose involves severe reactions, because the bagasse is comprised mainly of cellulose (35-45%), hemicellulose (20-30%) and lignin (20-30%) [1]. Several reagents have been studied in order to disaggregate this plant structure, but with less environmental impact and less invasively the cellulose chain, to not make it miss important features. Hydrogen peroxide (H_2O_2) has proven to be an effective bleaching agent, but when it reacts with lignin oxidizing it and removing it can also cause depolymerization of the cellulosic chain, requiring the use of additives that may act as chain protective agents [2].

In this study, were first separated hemicellulose and lignin fractions of the pulp by means of acidic and alkaline hydrolysis, respectively. Then, the delignified pulp obtained was subjected to bleaching tests H_2O_2. In an exploratory analysis was used an experimental design of fractional factorial type 25-1 with triplicate at the central point (19 experiments). The control variables were: H_2O_2 concentration 1 to 5%, pulp consistency 5 to 15%, $MgSO_4$ and $NaSiO_3$ concentrations 0.1 to 0.5% and temperature 50 to 70 °C. The response variables studied were kappa number, degree of whiteness and viscosity. The results obtained were studied statistically with the aid of software Minitab 17 ®.

As a result was possible to observe the variation of the kappa number, by means of Figures 1 (a) and (b), the H_2O_2 concentration was the only significant factor at 90% confidence. For this response variable, it is desired to obtain the lowest possible value, and these ranged from 29.63 to 38.88. The control variables $NaSiO_3$ e H_2O_2 concentrations to degree of whiteness reached were significant at a confidence level of 90% (Figures 1 (c) e (d)) and the values obtained for this response ranged from 24.23% to 35.30% ISO. In viscosity evaluation (Figures 1 (e) e (f)), was observed that these control variables didn't show a confidence level at 90%, however the most influential variables were the additives $NaSiO_3$ e $MgSO_4$. By means of figures, is noticed that the increase in $NaSiO_3$ concentration is beneficial, but the opposite happens with the second additives. To viscosity, the bigger values like response are desirables and ranged from 3.23 to 5.29 mPa.s. This study indicate that in futures steps of the treatment, the control variables temperature, consistency and $MgSO_4$ concentration may remain constants due to short influences the levels studied. In that case, it should be consider lower energy, water and reagent costs. In this study, there was an increasing trend of the degree of whiteness and viscosity with an increased of H_2O_2 and $NaSiO_3$ concentrations, this justifies further investigation with higher levels, to be established for them in order to optimize this step.

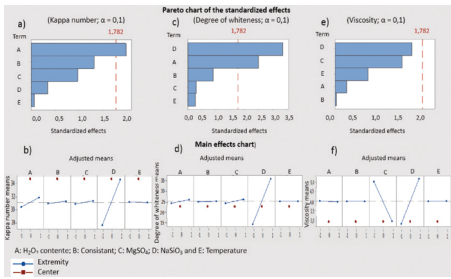

Fig.1 – Pareto charts of standardized effects for kappa number (a), degree of whiteness (c) and viscosity (e) and their individualized effects (b), (d) and (f).

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ENZYMATIC HYDROLYSIS OF PINE PRETREATED WITH ETHANOL AND SODIUM HYDROXIDE

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Pine sawdust, the most abundant and underutilized forest-industrial waste, can be used as raw material in a biorefinery. A possible product is bioethanol, obtained from cellulose present in this waste. The process involves three stages: pretreatment, enzymatic hydrolysis and fermentation. One of the most influencing factor in the hydrolysis is the chemical composition of the substrate, which is directly linked with the kind and conditions of the pretreatment to which the material was subjected. The aim of this work was to study the enzymatic digestibility of pine sawdust submitted to alkaline - ethanol pretreatments. The experiments were conducted under two alkaline charges (15 and 25% of sodium hydroxide on dried wood), two different times (60 and 90 minutes), liquor/wood ratio of 5/1, maximum temperature of 170ºC, and an alcohol/water ratio of 35/65. As reference, and to analyze synergic effects, the material was also treated separately with each reactive (soda and ethanol). The pretreated materials presented different chemical compositions, e.g., lignin contents varied from 3 to 22% on dried wood. NREL (National Renewable Energy Laboratory) standards were used for the characterization of the fibrous material. NREL standards were used also as guide to make the enzymatic activity and the hydrolysis. The enzymatic hydrolysis was performed at 2% consistency, pH 4.8 and 50ºC, with an enzymatic charge of 20 FPU/ g glucans and 40 IU/ g glucans. The reaction was monitored every 24 hours until 72 hours. DNS methodology was used to measure the percentage of reducing sugars after the enzymatic hydrolysis. The effect of alkaline charge on yields of reducing sugars was significant (p-value< 0.05), whereas the time did not show any effect. Ethanol and soda exhibited a positive synergy effect on the yield of reducing sugars. Results also suggest the existence of a negative correlation between the yield of reducing sugars and lignin content of the pretreated material. Highest yield from the enzymatic hydrolysis was obtained with alkali-ethanol pretreatment using a high charge of soda, this material have a content of lignin lower than 7%.

EXTRACTION AND CHARACTERIZATION OF XYLAN FROM SUGARCANE BAGASSE PRETREATED WITH ALKALINE SULFITE

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Hemicelluloses are the second most abundant renewable material produced by plants, and xylan is the major hemicellulose present in sugarcane bagasse. The objective of this study was to extract high molar mass xylan from pretreated sugarcane bagasse. Extraction procedures were performed with alkaline solution, assisted or not by xylanases. Structural features of the extracted xylan were also assessed samples. Sugarcane bagasse was chemithermomechanically pretreated with an alkaline/sulfite solution and washed (WB). A similar pretreatment procedure was performed without bagasse washing after pretreatment (UWB). The alkaline/sulfite treatment resulted in a considerable delignification (53% in WB and 43% in UWB) and minimal hemicellulose solubilization (24% in WB and 15% in UWB). This pretreatment increased xylan accessibility for the subsequent alkaline extraction. Two methods, based on xylan solubilization with alkali were carried out. In one of the methods, the sample was treated with sodium chlorite, before the alkaline extraction. Also, an enzymatic method of extraction was tested using xylanases and significantly lower alkali load. Recovery yields of the extracted xylan ranged from 21-79% and the lower yields were obtained with the enzymatic extraction method (21% in WB and 31% of the UWB). Chemical composition analysis of the xylans revealed the presence of residual lignin (7.2 to 9.8%). Xylan was the major carbohydrate fraction (80.7 to 85.2%), followed by arabinosil groups (9 to 11.7%) and glucan (5.8 to 7.6%). Washing or not of the pretreated bagasse and the type of xylan extraction procedure reflected in xylan-extraction yields and in the chemical composition of prepared xylan.

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DELIGNIFICATION OF OUTER AND INNER FRACTIONS OF SUGARCANE STALKS

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The production of first generation ethanol is already consolidated in Brazil and the growing need for increased production has moved researchers from academia and industry toward the development of technology for second generation ethanol from sugarcane bagasse and straw. The stalk of sugarcane consists of fibrovascular bundles dispersed in parenchymal tissue and involved in their outer part by the bark (or rind). Although concentrated in parenchymal tissue, the juice is extracted by crushing the whole stalk. This process requires the use of high pressures and provides a crushed material (bagasse) that is quite heterogeneous and difficults the separation of the fiber and pith fractions. In order to obtain more homogeneous fractions as well as facilitates the extraction process, this work proposes the previous separation of the external (rings) and internal fractions (core) of sugarcane and the subsequent treatment of the fractions by using delignification (pulping) processes. The production of the samples was made from stalks of raw sugarcane by cutting discs of about one centimeter thick, perpendicular to the direction of growth. The external layer (rings) were separated from the internal one (disks) by using punches with different diameters. Each of the rings / disks were crushed in a hydraulic press and the resulting fractions were submitted to two pulping processes (Soda and Organosolv). The juice obtained by crushing the different fractions was analyzed by determining the volume produced, sugar concentration and presence of extractives. The pressing of the disks allows the recovery of a lighter juice and the same volume as the obtained from the integral fraction (whole stalk). The bark fraction concentrates the extractives (mainly waxes) and has a low moisture content (around 40%) when compared to the integral and disk fractions. The soda pulping was carried out at 160 °C for 60 minutes and the organosolv pulping at 190 °C for 150 minutes. Under these conditions, soda pulping preserved in a greater extent the hemicelluloses and as a consequence, produced pulps with higher yield when compared to the organosolv process. Pulps obtained from the bark and core fractions showed, respectively, the higher and lower concentrations of residual lignin. The previous separation of the bark and core fractions can lead to savings in the process of obtaining first generation ethanol. The residue from the crushings (fractionated bagasse) may be more appropriate to valorize the fiber fraction (bark) and for production of second generation ethanol (from the core).

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THE CELLULOSIC FRACTION PURIFICATION OF SUBDIVISION OF DUST OF *Eucalyptus sp.* USING OZONE

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The lack of raw materials and declining availability of fossil fuels, have generated a wake-up call in the search for new alternatives to enhance the production of new energy sources. Thus the use of biomass is promoted, particularly lignocellulosic biomass, thanks to its wide availability as a renewable natural resource and its low-polluting nature. Its total use has generated the new Forest Biorefinery concept, which would ensure a plentiful supply of raw material as a solution to the problems mentioned above (Area & Vallejos, 2012).
The Forest Biorefinery concept is similar to oil refineries, but its main raw material is a renewable resource: lignocellulosic materials. From these can produce energy, fuels, chemicals, among others. The efficient use of lignocellulosic guarantee the enhancement of value-added products obtainable (Area & Vallejos, 2012).
Plant cells have unlike animal cell wall, thus most of all cells of fibrous lignocellulosic materials are cells. Structurally speaking the fibers have three major fractions: cellulose, hemicellulose and lignin, all bearing and fundamental to give rigidity to the plant (Mogollon, Garcia Hortal, & Leon, 2008).
Cellulose is the major component of lignocellulosic material, followed by finally hemicelluloses and lignin. Additionally there is a small percentage that is attributed to components of different chemical composition called extractives (Smook, 1990).
To achieve separate all these fractions is necessary to apply different methods of fractionation and purification. It is there when the application at first instance of a hydrothermal treatment to separate the hemicelluloses and extractives is proposed; secondly an alkaline cooking with soda anthraquinone inclusion to remove most of the lignin present in the material (Eucalyptus sawdust); and then oxidative treatments which guarantee the purification of the cellulosic fraction of the material, the main objective of the present investigation. The first process is an oxygen bleach that conditions of the material suitable for being subjected to different loads in ozone way different time periods. This stage is the research center and within all the processes mentioned above, is the varying conditions that will, this in order to evaluate the behavior of the material under ozone.
With this research is expected to be obtained from a mixture of sawdust of different types of eucalyptus pulp having characteristics that are within the ranges set to be classified as dissolving pulp for optimizing the cellulosic fraction of sawdust Eucalyptus sp. by oxidative methods involving the application of ozone (O_3), all under the Forest Biorefinery novel concept that allows us to further leverage other fractions of the material used.

SUGARCANE BAGASSE SODA PULPING ASSISTED BY STEAM EXPLOSION

Quintana, G., Marín-Sánchez, A., Gómez, S., Ramírez, C., Velásquez, J.,

Sugarcane bagasse was steam exploded to produce pulp in a soda pulping. Effect of the
pretreatment and pulping conditions on the physical–mechanical responses of pulp was evaluate by a screening experimental design. The factors studied and their respective variation ranges were pretreatment severity, 3.2–3.8; pulping time, 10-90 min, liquor bagasse ratio, 7–15, alkali dosage, 5–15%. Temperature pulping was fixed at 175°C. Design experiments with severities between 3.2 and 3.8 were obtained organic extractives content from 14.61% and 13.12%. The obtained pulps has a yield from 21 to 45%, with hydrolysis of the lignin of 91% and Canadian standard freeness (CSF) from 730 to 800 ml. The results suggest that the combination of steam explosion pretreatment followed by alkali pulping produce a good pulp with a low alkali concentration.
The raw material used was sugarcane bagasse dried at room temperature, milled at chips and sieving at 12 mesh to separate fines.
The steam explosion pretreatment was perform with 200 g charged of raw material in a stainless steel batch reactor of 15 l. After SE pretreatment, the material was delignified with different NaOH dosages and solid-liquid ratio, according to experimental design of 13 runs.

In the table 1 shows the overall results of the experiments carried out.

Table 1. Results of characterization pulps.

Sample	Humidity (%)	%LK (%)	CSF (ml)	Yield (%)
Sev 3,2	5,65	25,45	760	61,00
Sev 3,5	5,58	24,99	785	55,41
Sev 3,8	5,83	30,49	795	47,55
1	7,91	0,73	728	25,29
2	9,27	1,89	748	33,10
3	7,21	16,90	745	26,00
4	9,56	9,13	755	34,44
5	8,97	5,94	793	27,17
6	6,67	10,76	775	35,78
7	8,67	6,69	805	20,90
8	7,97	2,32	730	34,74
9	8,69	6,74	800	29,39
10	6,60	7,61	775	45,34
11	7,64	4,80	788	38,79
12	9,64	11,68	788	32,61
13	7,30	3,21	792	34,84

HIGH PRESSURE CO_2-H_2O MIXTURE – PROMISING TECHNOLOGY FOR DEVELOPMENT OF GREEN BIOREFINERY CONCEPT

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The development of green and economically benign processes for conversion of lignocellulosic biomass into fuels, chemicals and materials is a huge challenge in the context of biorefinery concept (Clark et al., 2009). The selective conversion of lignocellulosic constituents into desired products requires a pre-treatment step, in which each single component is optimally converted according to its chemical and physical proprieties.
High pressure CO_2-H_2O technology takes advantages of in-situ formed carbonic acid, which promotes acid-catalysed reactions resulting in an increase of dissolution of hemicellulosic fraction of biomass into xylooligosaccharides by 65% in comparison to CO_2-free technologies (Magalhães da Silva et al., 2014). Besides the hemicellulose hydrolysis, both chemical and physical effects of CO_2 on cellulose resulted in an improvement of enzymatic hydrolysis yield by 26% (Magalhães da Silva et al., 2014; Morais et al., 2014; van Walsum, 2001). These results demonstrated that without additional catalyst, high pressure CO_2 boost hydrothermal reactions giving higher total reducing sugars (as high as 84% in comparison to 67.4% with water-only reaction). Therefore, high-pressure CO_2-H_2O demonstrated to be an interesting alternative to conventional biomass processing technologies, being effective medium in fractionation and hydrolysis of lignocellulosic residues towards valuable products (Magalhães da Silva et al., 2014; Morais et al., 2015; Morais et al., 2014).

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TWO-STAGE CONVERSION OF AGRICULTURAL AND FOREST WASTES WITH SULFUROUS ACID AND CellicCTec2

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Conversion of plant's polysaccharides to monosaccharides solves important problems of processing and utilization of agricultural and forest wastes and promotes of new products production for the chemical industry and biotechnology. Wheat straw, birch sawdust and sugar beet pulp was used as sources of biomass for this study.
The feedstock was treated with diluted sulfurous acid, which can be regenerated in the process of hydrolysis. Hydrolysis of feedstock was carried out in sealed thermostatically controlled capsules in laboratory equipment of original design, allowing to perform chemical hydrolysis processes in the operating temperature range 100-250 °C under positive pressure 0-1,2 MPa (Fig. 1).

Fig.1 – Laboratory equipment for high temperature hudrolysis

Monosaccharide analysis of the hydrolysates was performed by HPLC on a column «CarboPacPA-1» (4x250 mm, «Dionex», USA) using pulsed amperometric detector PAD («Dionex»). Elution rate 1 ml/min. Column temperature 30 ° C. Buffers: A - 100 mM NaOH in 1 M AcNa, B - 15 mM NaOH.
The estimation of efficiency of wheat straw, birch sawdust and sugar beet pulp hydrolysis with sulfurous acid (0.6-2.5%) using different solid to liquid ratio (1:2, 1:3, 1:4, 1:5, 1:6) varying temperature 160-250 °C and time of treatment was carried out.
The maximum concentration of reducing substances reached during hydrolysis with sulfurous acid was 40 g/l, 77 g/l, and 85 g/l for wheat straw, birch sawdust and sugar beet pulp respectively.
Xylose, glucose and arabinose were predominant sugars in wheat straw hydrolisates (55,1 %, 20,5 % and 13,4 % of total monosaccharides respectively). In birch sawdust hydrolisates xylose and glucose were predominant sugars (up to 64 % and 24 % of total monosaccharides respectively). The content of arabinose, glucose and galactose in sugar beet pulp hydrolisates were 51,4 %, 32,9, and 7,3 % respectively. The total yield of carbohydrates during hydrolysis with sulfurous acid was 20,1%, 29,2%, 33,6 % for wheat straw, birch sawdust and sugar beet pulp respectively.
After acid hydrolysis the wheat straw, birch sawdust and sugar beet pulp were treated with enzymes CellicCTec2 (Novozymes) in order to obtain glucose. The pretreatment of wheat straw, birch sawdust and sugar beet pulp with sulfurous acid let to increase the glucose yield during enzymatic hydrolysis. The total glucose yield during two stage treatment of wheat straw, birch sawdust and sugar beet pulp was 80,2%, 75,14 % and 89,42 % respectively.
Hydrolysates of wheat straw, birch sawdust and sugar beet pulp can be used in production of valuable products such as ethanol, vitamin feed supplements, feed protein, furfural, cellulose, and organic acids.

LOW WATER CONSUMING ALKALINE H_2O_2 BIOREFINERY PLATFORM FOR SIMULTANEOUS GLUCOSE, XYLOSE, CELLULOSE, HEMICELLULOSE, LIGNIN AND ENZYMES PRODUCTION FROM WHEAT STRAW

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The chemical pretreatment of biomass was integrated with the enzyme production through the recycling of aqueous fractions. The alkaline/H_2O_2 delignification of wheat straw (WS) was performed to obtain a 75.1% w/w cellulose solid fraction, and to dissolve 94.4% and 83.5% of the original lignin and hemicelluloses respectively. Then a *Pleurotus ostreatus* native strain was left to grow for 120 hours in the resulting liquid fraction. After filtering the cells, the liquid medium was used alone or combined with the enzyme Accellerase 1500®. In order to reduce the chemicals and water used, the liquid fraction from the pretreatment was recycled to perform another one, with its corresponding pH, WS and hydrogen peroxide adjustment to the original conditions. Its further *Pleurotus o.* growing integrated with the hydrolysis was performed again. Every liquid fraction and samples from the fungal growing were analyzed for oxygen chemical demand (OCD), glucose (Glu), xylose (Xyl) and total reducing sugars measures (RS). Separately, in order to obtain valuable polymers from this integration process, solid hemicelluloses and lignin were isolated from the remaining liquid fractions trough a pH-polarity variation, then they were analyzed for its composition, by scanning electron microscopy (SEM), optical stereoscopic microscopy and by IR spectroscopy comparisons with commercial homologues. The maximum overall cellulose to glucose conversion from the pretreatment-hydrolysis integration was 7.7%, which represented 20% of the total conversion yield using only the commercial enzyme.

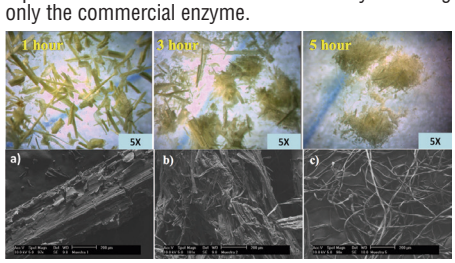

Fig.1 –First row, Optical Stereoscopic Microscope images of the Pretreated Wheat Straw at 1, 3 and 5 h of treatment; Second raw Scanning Electron Microscopy microphotographs at 100X of Untreated Wheat Straw (a), 5 h Pretreated Wheat Straw (b) and, 24 h PWS (c)

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EVALUATION OF THE EXTRACTION OF PHENOLIC COMPOUNDS FROM WOOD OF OLIVE PRUNING

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In Chile it is estimated that were generated 29600 tons of lignocellulosic biomass from the pruning of olive tree during the 2014. This byproduct is composed by leaves and wood and treated as waste and disposed in the farmlands through incineration.
The chemical composition of olive trees shows the presence of some phenolic compounds with antioxidant properties. The phenolics from olive leafs i.e., have proven effects preventing heart diseases and some cancers [1]. On the other hand, phenolics from olive pruning wood, difficult the fermentation processes to obtain biogas and sugars from this byproduct. The extraction of phenolic compounds from this resource appears as an opportunity to satisfy the growing demand for natural antioxidants in the pharmaceutical and food industry, and to facilitate the full utilization of this biomass.
This work focuses on the selection of the most suitable solvent to obtain extracts with potential antioxidant activity from olive pruning wood. For this, a battery of solvents with increasing polarity including hexane, dichloromethane, ethyl acetate, ethanol and methanol were evaluated. Higher extraction yields were obtained using ethanol and methanol. Furthermore the total polyphenol content (TPC) was quantified. The highest TPC was found in the methanol and ethanol extractions. The antioxidant activity was evaluated using the method of DPPH radical, where the highest bleaching percentage were obtained to the ethanol and methanol extracts.
The results reported suggest that the solvents with highest polarity values are those that showed the highest extraction yields, polyphenol content and antioxidant activity. In base of the results we recommend the use of ethanol for future extractions, considering too that is an environmentally friendly and an inexpensive solvent.

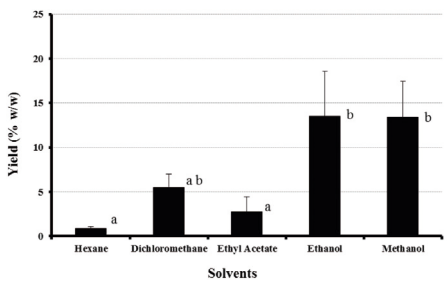

Fig.1 – Extraction yield as percentage weight for the different solvents used. Different letters (a or b) show significant differences (p = 0.05).

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SODA/ETHANOL-OXYGEN DELIGNIFICATION OF PINE SAWDUST FOR A BIOREFINERY

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The generation of large amounts of sawdust and other waste is a specific problem of the primary processing of wood. The objective of this work is to study an environmentally friendly delignifying strategy for a sequential fractionation, following a biorefinery concept. A *Pinus elliotti* and *Pinus taeda* sawdust mix from Misiones, Argentina, was tested as raw material for a soda-ethanol pulping. Three different conditions were performed in a 7 L pressurized reactor, with indirect heating. Cooking conditions were: liquor to wood ratio 5:1, maximum temperature 170ºC, time to maximum temperature 40 min, and time at maximum temperature 140 min. Experiences were performed as follows:
1) NaOH load of 27.6% w/w and a 35/65 ethanol to water ratio (%v/v).
2) NaOH load of 19.0% w/w and a 35/65 ethanol to water ratio (%v/v).
3) NaOH load of 27.6% w/w and a 10/90 ethanol to water ratio (%v/v).
Pulping yields and Kappa number were assessed in each experience. Pulps obtained from the different experiences showed the following results of total yield and Kappa number: 1) 41.4% and 25.74; 2) 47.24% and 59.40; and 3) 40.58% and 32.40. These pulps were subsequently subjected to a two-stage oxygen treatment, carried out in a multipurpose reactor with mechanical stirring and a glycerin-jacket heating. Oxygen treatments were performed at 10% consistency, 100°C of maximum temperature, 6 kg/cm^2 of oxygen pressure, NaOH load of 2% w/w, and 60 min of time at maximum temperature. $MgSO_4$ (0.1% w/w) was added in the first stage. Pulps were washed before each treatment. Kappa number dropped to 13.4, 38.8 and 15.4, for experiences 1, 2, and 3, respectively. Final Kappa numbers after the second oxygen stage were 8.2, 25.2 and 9.6. The highest drop in the Kappa number between oxygen stages corresponded to experience 2. Pulp viscosity was measured to evaluate the process selectivity. Pulp from experience 1 showed higher selectivity than the others. Yields of the two-stage oxygen treatment were 95.73%, 86.74% and 91.35% for experiences 1, 2 and 3, respectively. Finally, pulps were refined at 500 rpm in a laboratory pulp refiner (PFI). The highest difference between the initial and final ºSR was observed for experience 1.

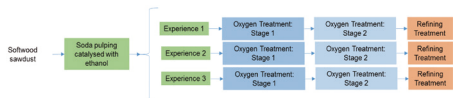

Fig.1 – Scheme of pine sawdust soda-ethanol-oxygen treatment

EXTRAÇÃO DE LIGNINA DE MADEIRA FUNGADA
LIGNIN EXTRACTION FROM WOOD ATTACKED BY FUNGI

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RESUMO
A lignina pode ser extraída dos resíduos florestais para ser usada como matéria-prima para produtos de alto valor agregado. Neste estudo, é verificada a possibilidade de extração da lignina a partir de madeira fungada, cujo destino provável seria a queima para geração de energia. O caule de um clone de Eucalyptus grandis x Eucalyptus dunni, apodrecido naturalmente por fungos de podridão branca, fornecidos por um fabricante de pasta celulósica, foi transformado em serragem para análises químicas (lignina Klason, lignina solúvel e cinzas) e calorimétrica e em cavacos para cozimento soda e organosolve (soda + etanol). As frações sólidas, e líquidas obtidas após o cozimento foram recolhidas e o rendimento determinado. Nas frações sólidas foram efetuados os mesmos ensaios do material de partida e foi verificado que os valores obtidos são próximos para todas as amostras. Nas frações líquida, foi efetuada a precipitação da lignina por meio de diminuição do pH, considerando a faixa de pH 4 a 1. O valor da absorbância do filtrado obtido, a 205 nm, foi usada para acompanhar a precipitação. Foi observado que quanto menor o pH menor o valor de absorbância e que abaixo de pH 2 a diminuição dos valores de absorbância podem ser considerados não significativos. O estudo mostrou que é possível extrair lignina de madeira fungada sem interferir de modo significativo nas propriedades calorimétricas da madeira.

Palavras chave: Madeira; Lignina; Biorrefinaria, Energia.

ABSTRACT
Lignin can be extracted from forest residues to be used as raw material for other high value-added products. This study investigates the possibility of extraction of lignin from wood attacked by fungi, whose destination would be likely burning for power generation. The stem of a clone of *Eucalyptus grandis* x *Eucalyptus dunni*, with white rot fungi, from a pulp manufacturer, was transformed into sawdust for chemical analysis (Klason lignin, soluble lignin and ash) and calorimetry analysis and into chips for soda and organosolve (soda + ethanol) pulping. The solid and the liquid fractions obtained after pulping were collected and the yield was determined. The solid fractions were submitted to the same tests done for the starting material and it was verified that all the results were similar. In the liquid fractions was carried out the precipitation of the lignin by decreasing the pH, considering a pH range of 4 to 1. The value of the absorbance of the filtrate at 205 nm was used to monitor the precipitation of the lignin. It was observed that the lower the pH the lower the absorbance values and that below pH 2 the decrease in the absorbance values are not significant. The study showed that it is possible to extract lignin from wood attacked by fungi without interfere significantly in the wood calorimetric properties.

KEY WORDS: Wood; Lignin; Biorefinery; Energy.

USE OF MINERAL SORBENTS FOR IMPROVED FERMENTATION POTENTIAL OF WILLOW WOOD HYDROLYSATES

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ABSTRACT
Lignocellulose is a carbon-rich source of renewable energy demonstrating a high energy potential, which can serve for second generation ethanol production. However, depending on the type of biomass as well as the method of pretreatment, production of ethanol generates a number of toxic by-products, including phenolic compounds, which have an adverse effect on enzymatic hydrolysis and fermentation. By making the right choice of a detoxification method, it is possible to improve the fermentation potential of hydrolysates and to increase the content of ethanol. This research has been designed to assess the usability of two mineral sorbents, zeolite and halloysite, for an improvement of the fermentation potential of willow wood (*Salix viminalis L.*) hydrolysates obtained from an acidic pre-treatment process including phosphoric(V) acid (H_3PO_4). The hydrolysates contained about 15.96 $g \cdot dm^{-3}$ of reducing sugars and approximately 3.69 $g \cdot dm^{-3}$ phenolic compounds. Detoxification with zeolite added in a 10.0wt% ratio and conducted for 60 minutes removed around 19.39% of phenolic compounds and resulted in a loss of over 11% of simple sugars. In turn, the use of halloysite for detoxification led to a decrease in the phenolic content by 28.30% while producing a very small effect on the content of simple sugars. The results suggest that halloysite is superior to zeolite as a sorbent used to improve the fermentation potential of lignocellulose hydrolysates.

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PRODUCTION OF DROP-IN BIOETHANOL FROM MICROALGAE: AN ENERGY-DRIVEN APPROACH UNDER A BIOREFINERY CONCEPT

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The fossil fuels depletion and climate change, in addition to the current global energy demands require the search for suitable substitutes, such as biofuels. Microalgae are an economic alternative for biofuels production, particularly bioethanol [1]. In the framework of the EU DEMA project (Direct Ethanol from MicroAlgae) we aim to cultivate autotrophically a cyanobacterial strain that directly produces drop-in advanced bioethanol at competitive costs and displaying a positive energy balance. Closed photo-bioreactors of different strains of GMO microalgae (*Synechocystis sp.*) are being cultivated in liquid nutritive medium supplemented with CO_2 for the direct synthesis of bioethanol from sunlight.
In this work we propose, on a LCA (Life Cycle Assessment) point-of-view, a large scale energy-driven scenario for the DEMA process, i.e., the production of fuel-grade ethanol, the biogas recovery from biomass digestion and the heat & power integration. This scenario was then modelled and optimized using SuperProDesigner v.8.5 (Intelligen Inc.) in order to perform preliminary mass and energy balances for an ethanol farm scale unit (1000 $_{Lethanol}$/day). Under this approach, the power generated from biogas burning is ca. 4 kWh, while the power consumed is ca. 2425 kWh. The ERoEI (Energy Return on Energy Invested) is, for these conditions, 2.3 MJ(Ethanol)/MJ(energy consumed). The ethanol separation and purification operational steps are the main energy consumers. Next goals to be attained are mainly: i) to improve the energetic balance, and ii) to increase of ethanol productivity of the DEMA strain, up to the limit of the strain ethanol tolerance.

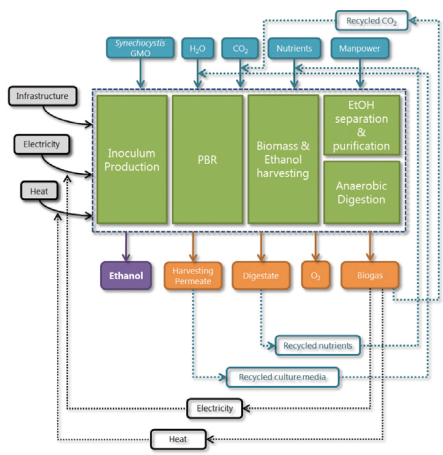

Fig.1 – DEMA energy-driven scenario flowsheet

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Acknowledgements
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EVALUATION OF ETHANOL PRODUCTION IN ETHANOL-TOLERANT THERMOPHILIC BACTERIA ISOLATED FROM GEOGRAPHIC MACROZONE NORTHERN CHILE.

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The increasing demand for sustainable biofuels is driving the need for feasible bio-refineries capable of utilizing highly renewable and environmentally friendly feedstock's to produce high-volume biofuels[1]. In the past decades various thermophilic microorganisms capable of producing low amounts of ethanol have been isolated and characterized from different environments with intention to evaluate their ability of fermentation and large scale ethanol production[2].

Previously, in the laboratory of Microorganismos Extremófilos, bacterias of the genus *Geobacillus sp.* were isolated from different places of the geographic macrozone Northern of Chile. Members of this genus have been described previously as etanol-tolerant and ethanologenic bacteria, highlighting *Geobacillus thermoglucosidasius* strain (M10EXG)[2], able to tolerate concentrations of ethanol up to 10% (v/v).
The aim of this work was to determine ethanol production in four bacterial strains isolated from the geographic northern macrozone of Chile, in addition to improving biomass production, determine byproducts of fermentation with the purpose to modify and enhance their productive capacities through their metabolic pathways for ethanol production.

The results indicate that the strains C3, T25, T23 and CJ1 have the capacity to tolerate up to 4% ethanol and grow at a temperature of 60°C, proliferating in the presence of xylose and glucose as the unique carbon source, in addition whit the ability to consume these sugars for biomass production in 12 hours, however the strain C3 was the only one with significant production of ethanol in a yield of 0.08 g ethanol/g glucose in 24 hours. The results of this work show that the C3 strain is a potential candidate for the production of bioethanol in thermophile bacteria by improving its metabolic pathways.

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PRODUCTION OF CELLULOSIC ETHANOL FROM STEAM-EXPLODED *Eucalyptus urograndis* AND CANE BAGASSE AT HIGH TOTAL SOLIDS AND LOW ENZYME LOADINGS

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Cellulosic ethanol is one of the most important biotechnological products to mitigate the consumption of fossil fuels and to increase the use of renewable sources for fuels and chemicals. Performing the enzymatic hydrolysis step in high consistency using high total solids (TS) and relatively low enzyme loadings can offer significant advantages to the cellulosic ethanol production process [1]. These advantages involve the reduction in capital cost for hydrolysis and distillation, particularly due to the fact that high sugar concentrations are produced in the substrate hydrolysate for fermentation [2].
In this work, different steam-exploded materials were used for enzymatic hydrolysis at high total solids (TS) and low enzyme loadings and the fermentability of their corresponding hydrolysates was tested using an industrial strain of *Saccharomyces cerevisiae*.
Pretreatment was carried out by autohydrolysis using *Eucalyptus urograndis* wood chips and sugarcane bagasse under conditions that were pre-optimized in earlier studies, 210 °C for 5 min and 195 °C for 7.5 min, respectively. Enzymatic hydrolyses were carried out in a Labfors 5 BioEtOH reactor (Infors-HT) during 72 h at 50 °C and 150 rpm, using 62.5 mg of Cellic CTec3/g of dry substrate in 50 mol/L acetate buffer at pH 5.2. The reactor was fed with pretreated material by adding 5 wt% TS at every 1.5 h, reaching 20 wt%. Fermentation was carried out with 1 g L^{-1} of Thermossac Dry (Lallemand) at 35 °C during 20 h.
The Figure 1A shows the logarithmic profile of glucose release during enzymatic hydrolysis of both pretreated materials. The final concentrations in glucose equivalents (GlcEq) were
103 g L^{-1} for bagasse and 125 g L^{-1} for eucalypt and the conversion values were approximately
95 %. The fermentation trials were performed with different initial concentration of glucose:
58 g L^{-1} for bagasse and 60 g L^{-1} for eucalypt as shown in Figure 1B. At the end of this process, different ethanol concentrations and yields were obtained: 25.65 g L^{-1} of ethanol and 86.7 % efficiency for bagasse and 27.31 g L^{-1} of ethanol and 95.0 % efficiency for eucalypt. Glucose consumption was complete after 12 h, suggesting that the process can be interrupted at this time without any loss in process efficiency.
Pretreatment by steam explosion resulted in high yields of enzymatic hydrolysis from both sugarcane bagasse and eucalypt chips, which were higher than 95 % after 72 h at high total solids and a relatively low enzyme loading. However, the glucose yield was higher for eucalypt while steam-treated bagasse gave yields 25% lower. With regard to fermentation, both hydrolysates were readily fermented in good yields using an industrial yeast strain, achieving and productivity values of 2.2 - 2.3 g L_{-1} h^{-1} after only 12 h of fermentation. Therefore, when comparing the results obtained in this study, the eucalypt was the biomass with the greatest potential for bioconversion, reaching around 37.3 L of ethanol more than bagasse per ton of biomass without considering the additional yields from C5 sugars.

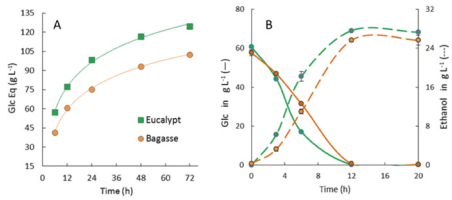

Fig.1. Release of fermentable sugars by enzymatic hydrolysis (A) and fermentation performance (B) of steam-treated materials and their corresponding enzymatic hydrolysates, respectively.

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ENZYMATIC SACCHARIFICATION BY ALKALINE-SULFITE PRE-TREATED SUGAR CANE BAGASSE

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In Brazil, during the processing of sugar cane, large amounts of bagasse (approximately 240 kg of bagasse with 50% moisture per ton of sugarcane) are simultaneously produced [1]. This raw material, due to its composition rich in cellulose, has been pointed as an excellent source of sugars for ethanol production. However, the conversion of cellulose to glucose by the enzymes is limited without the use of an efficient pre-treatment [2]. In this study, the bagasse was pre-treated with chemi-thermomechanical alkaline sulphite process (10 g Na_2SO_3/100 g of bagasse and 5 g of NaOH / 100g bagasse) at 120 ° C for 120 minutes, and then refined into a disk refiner [3]. At the end of the process, two materials were obtained, a washed bagasse (WB) and unwashed (UWB). In both samples, the total removal of acetyl group and the partial solubilization of the lignin (40%) was observed; while most of for the cellulose, was preserved, presenting a small removal around 6%. After pre-treatment, enzymatic saccharification with Cellubrix (Novozymes Denmark) commercial enzyme loads of 5, 10 and 15 FPU/g of biomass was carried out. To the Cellubrix extract, were added β-glucosidase (Novozyme 188) loads of 10, 20 and 30 IU/g of biomass and reaction was conducted at 45 °C, 100 rpm for 48 hours. For washed pre-treated bagasse, the maximal yields of cellulose conversion reached 56, 68 and 72% (Figure 1), whereas unwashed bagasse attained 51, 71 and 77% at loads of 5, 10 and 15 FPU/g biomass, after 24 h, respectively. Thus, increasing the enzyme loading from 10 to 15 FPU, the cellulose conversion of washed and unwashed bagasse was slightly enhanced (<10%). Based on these results, the hydrolysis can be provided at loading of 10 FPU/g of biomass until 24 hours of reaction; besides it was showed that the washing step after the pre-treatment is not necessary to obtain yields of about 70% in the conversion of cellulose.

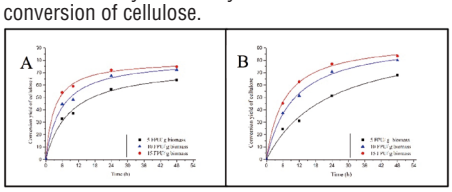

Fig. 1 - Cellulose yield conversion from washed (A) and unwashed (B) pre-treated bagasse

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OPTIMIZATION STUDIES FOR ENHANCING CELLULASES PRODUCTION BY CO-CULTIVATION OF TWO ENDOPHYTIC FUNGI AND USE OF THE ENZYMATIC EXTRACT FOR SUGARCANE BAGASSE SACCHARIFICATION

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The global use of fossil fuels has generated numerous negative environmental impacts [1] and ethanol has been one of the most important renewable fuels to overcome this problem [2]. The production of cellulosic ethanol (or bioethanol, or second generation ethanol – 2G ethanol) is an alternative, since it can be obtained from the fermentation of glucose released by the hydrolysis of cellulose in lignocellulosic materials [3]. There is a growing interest in low cost processes to produce enzymes that can be applied in the conversion of lignocellulosic residues into high value-added products, such as 2G ethanol. Endophytic fungi have ability to produce these enzymes and are underexplored in this context. Considering the above, the endophytic fungi *Botryosphaeria sp*. AM01 and *Saccharicola sp*. EJC04 were co-cultivated by solid state fermentation (SSF) on wheat bran and cottonseed meal (1:1 w/w), and some fermentation parameters were studied in optimization experiments for enhancing cellulases productions. The parameters evaluated were three sources of nitrogen (urea, ammonium sulfate and sodium nitrate); medium initial pH; inoculum concentration; moisture content; amount of Tween 80 and amount of substrate. A 2^{8-4} fractional factorial experimental design was performed, using two levels for each parameter. Results showed that highest endoglucanase and β-glucosidase productions were, respectively, 167.532, and 173.041 U/g of dry substrate. Maximum FPase production was 1.569 FPU. Among the 8 parameters studied only moisture content and amount of substrate showed significant effects on cellulases productions. It was verified that these two parameters had a negative effect, that is, with increasing of its levels there was a decrease in enzymes production. A new full factorial design was performed seeking the ascendancy to the maximum production of enzymes. The highest productions were 123.57 and 211.41 U/g of dry substrate for endoglucanase and β-glucosidase, respectively, and 0.074 FPU for FPase. The responses were not significantly different. The saccharification of sugarcane bagasse pretreated by steam explosion was carried out using the enzymatic extract obtained in the second factorial design (121.578 U/g of endoglucanase, 208.401 U/g of β-glucosidase and 0.074 FPU FPase). The highest yields in sugars were observed at 24 hours (0.640 mg/mL of glucose and 2.677 mg/mL of xylose). The data obtained can be considered promising and further experiments will be performed in order to optimize the saccharification of sugarcane bagasse, aiming to obtain glucose to be used for 2G ethanol production.

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DILUTE ACID HYDROLYSIS PRETREATMENT OF ORANGE PEELS

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An increase of demand for energy, depletion of traditional sources of fuel and concern about climate change around the world caused the upsurge of interest for plant materials as an alternative source of energy [1,2]. Lignocellulosic biomass is considered to be the most promising alternative to fossil fuels, since its rational use does not contribute to a net rise in the level of CO_2 in the atmosphere. Hydrolysis and fermentation are two important and major steps in bioethanol production from lignocellulosic materials [3]. One of the most frequent methods to analyze the lignocellulosic materials and to hydrolyze biomass in to sugar with capability of being fermented is acid hydrolysis (diluted and concentrated) [4]. In this study the effect of different concentrations of sulphuric acid (0.25, 0.5, 0.75 and 1.0 %v/v), time (10, 20 y 30 min) and temperature pretreatment (110 ºC, 115 ºC, 120 ºC and 125 ºC) on the release of reducing sugars from orange peels (OP) was investigated. Biomass and dilute acid were mixed at a solid/liquid ratio of 2.5% (w/v). After pretreatment the DNS (3.5-dinitrosalicylic acid) method developed by Miller [5] was used to determine total reducing sugars. Maximum concentration of reducing sugars, 0.13 $g \cdot g^{-1}$ of dry matter was achieved when reaction was carried out at 125 ºC for time period of 30 min with diluted acid maintained at 0.75% (v/v). According to proximate analysis, the three main componentes of OP are cellulose, hemicellulose and lignin. From the results reported in this study, it can be inferred that the dilute sulfuric acid hydrolysis can be an effective technique for the release of reducing sugars from orange peels. The amount of released sugars depends on acid concentration, temperature and pretreatment time. However, to increase the overall yield of the pretreatment process, an enzymatic hydrolysis step must be performed over the solid fraction obtained during the before pretreatment step. Therefore, this residue could be potential feedstock for ethanol production because of both their convenient low-cost and easy availability.

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FEASIBILITY ANALYSIS OF ETHANOL PRODUCTION FROM DIGESTED SLUDGE IN VACCINES FARMS

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Waste from livestock industries, such as manure can become a practical agronomic and economically viable management for a sustainable development. Its possible use as a source for biofuel is socioeconomically feasible. Compared to fossil fuels, the production and obtaining of biofuels such as bioethanol, biogas or biodiesel could be potentially economic and better to the environment and can be given from good use and treatment of livestock industries waste, contributing in two priority areas: environment and energy demand. In uncontrolled environments, this waste cause a great impact to the environment by its ease of producing biogas, recomposing mostly methane (CH4) and carbon dioxide (CO_2).

In this work, we carried out the optimization of the hydrolysis of manure from cows through different conditions and the study of its feasibility for bioethanol production and livestock within a community.

The sludge from vaccines (especially at tropical areas) by its composition has 12% of hemicellulose and 22% of cellulose, this represents an important energy potential by the amount of fermentable carbohydrates. Also by the large volume of generation and the hemicellulose and cellulose content, it can be hydrolyzed and generated fermentable sugars that can be converted to glucose and then ethanol.

It is important to mention that as precedent of this work, in a vaccine farm in the city of Silao, Guanajuato. México, this manure is used to produce biogas in an anaerobic digester and we consider as representatives the following samples: undigested sludge, digested sludge, and the liquid remain from the digestion process (effluent), those last two samples from the anaerobic digester located in the vaccine farm. We carried out the analysis of the content of sugars at those tree samples under different pretreatment and hydrolysis conditions as well as the feasibility study for bioethanol production in base on the results.

We concluded that by acid hydrolysis we obtained higher sugar concentrations than the enzymatic hydrolysis, although the sugar concentration in both cases does not exceed the 50%, by the amount of manure generated at the farm, bioethanol production is feasible.

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ENZYMATIC HEMICELLULOSE EXTRACTION FROM PRETREATED SUGARCANE BAGASSE.

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One of the most important agricultural activities in Brazil is the cultivation of sugarcane to produce sugar, ethanol and bagasse as lignocellulosic byproduct. Bagasse has a high carbohydrate content, but it is currently subtilized. In the present work, the bagasse was pretreated with a chemithermomechanically (CTM) process using alkaline/sulfite solution and disk refining with the aim of increasing cell wall accessibility for subsequent extraction of the hemicellulose fraction using the commercial xylanase "Luminase". Besides, bagasse was delignified with acidic sodium chlorite to produce a model with low lignin content and almost unalterated hemicellulose. The original chemical composition of sugarcane bagasse was 36.5% of cellulose, 26.6% of hemicellulose and 21.0% of lignin (g/100 g of original bagasse). When pretreated with 10% w/w Na_2SO_3 and 5% w/w NaOH, the chemical composition was 37.3%, 20.7%, 11.3% for cellulose, hemicellulose and lignin, respectively (g/100 g of original bagasse). Four-hour chlorite treatment resulted in a bagasse with 39.1%, 24.2%, 5.4% for cellulose, hemicellulose and lignin, respectively (g/100 g of original bagasse). CTM pretreated bagasse was treated with Luminase (5 UI/g bagasse) at pH 8, 50°C and solid:liquid ratio of 1:20. After 24 hours-reaction, the yield of hemicellulose extraction was 33%. Interestingly, increasing xylanase loading by 12 times (60 UI/g bagasse) the hemicellulose extraction was increased by only 7%. On the other hand, when the delignified bagasse was treated using the same conditions for the CTM pretreated bagasse, the yield of hemicellulose extraction with a xylanase loading of 5 UI/g bagasse was 47%. The increase of xylanase loading (12 times) enhanced the hydrolysis in 11%. The increase of xylanase loadings was not proportional with the extraction improvement, suggesting that lignin is not the only physical limitation. The literature suggests the existence of a hemicellulose fraction between highly packed cellulose microfibrils, supporting the low enhancement observed when the enzyme loading increased 12 times. Also, the results reported here show that CTM and delignification process cannot totally disrupt the strong interactions between cellulose on hemicellulose which will provide access to all hemicellulose fraction.

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SYNTHESIS AND SIMULTANEOUS OPTIMIZATION OF *Eucalyptus globulus* WOOD TREATMENT TO IMPROVE THE POLYSACCHARIDES EXTRACTION

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Global biofuels production was increased in recent decades by using raw materials like soybean, corn and sugarcane. This first-generation biofuels have been criticized for their competition for resources used for food [1]. For this reason, it has been recently promoted the development of processes to obtain ethanol from lignocellulosic materials, which is an alternative source of carbohydrates that can be subsequently converted to ethanol by fermentation. While there have been studies that show the technical feasibility of processing lignocellulosic materials, the successful implementation depends largely on production costs. It is important to maximize the conversion factor of the raw material into ethanol to keep the economy of the process competitive [2], [3]. In this work, it is proposed an optimization model for the synthesis of the pretreatment and post-hydrolysis steps with the objective of maximizing the conversion ratio of *Eucalyptus globulus* wood into fermentable sugars while are maintained acceptable levels of furfural and HMF, which are inhibitors of the fermentation process. A superstructure of the process is proposed which contains main alternatives of equipments and stream connections to be synthesized. This superstructures includes three alternative pretreatments and an acid post-hydrolysis step. The model is a General Disjunctive Program (GDP) and was implemented in General Algebraic Modeling System (GAMS). The results obtained are consistent with previous studies, it has been observed that the utilization of a post-hydrolysis treatment and medium severity conditions, allow a better conversion of wood's polysaccharides to fermentable sugars [4],[5],[6]. However it is noted that a configuration of dilute acid pretreatment without post-hydrolysis could get amounts of sugars very close to optimal values, by suitable choice of operating conditions.

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FERMENTATION OF HYDROLYSATES FROM LEAVES AND BUDS SUGARCANE USING A NATIVE YEAST, AVAILABLE TO CONSUME PENTOSES AND HEXOSES

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This research is into the framework of new biofuels generation under the bio-refineries concept, using sugarcane residuals as raw material to obtain, mainly, alcohol. Particularly, it was focused on the evaluation of two promising native yeast to ferment pentoses and hexoses, with the aim to integrate these results to the process that has been defined by the research group GRUBIOC, with such residuals. In our laboratories the yeast registered as AM45 and AM143 were identified within the Candida genus with ability to consume cellulose and hemicellulose.
Previously by GRUBIOC's research group the hydrolyzing conditions were found (Salcedo *et al,* 2011). The hydrolysates have been near 77, 46 g/L of total sugars (51, 60 glucose, 22, 00 xylose, 3, 86 arabinose in g/L). The fermentation target was looking for the best production alcohol yield by batch system. For this focus the pH and temperature effects for each strain were assessed, keeping the nutrients and vitamins concentration stable. Results (Figure 1 and 2), showed that AM45 strain (*Candida guilliermondii*), gave the better ethanol concentrations 23, 33 g/L, to achieve an efficiency of 50, 2 g/g (ethanol/ sugars), at a 31°C temperature and 4, 5pH. For AM143 strain (*Candida tropicalis*), the results were similar, a ethanol production of 23,04 g/L, but the efficiency was higher 61,5g/g (ethanol/sugars), at a 28°C temperature and a pH of 3.8, showing an uniform behavior but apparent regarding the operation ranks proposed by the different treatments (Figure 1 and 2). The two strains expressed higher yields than the theoretical (0,50g/g).
Also with these microorganisms individual consumption for the main sugars to determine the behavior of the fermentation was evaluated.

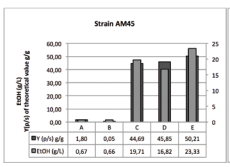

Figure 1. Concentration of ethanol produced (gray) and yield (black) AM45 in fermentations performed in the factorial experimental design 2^2.

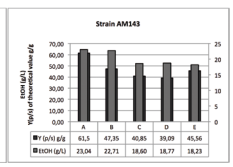

Figure 2. Concentration of ethanol produced (gray) and yield (black) AM143 in fermentations performed in the factorial experimental design 2^2.

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OBTENCIÓN DE BIOETANOL A PARTIR DE BIOMASA GENERADA EN UN CULTIVO ENERGÉTICO DE FORESTALES

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En la Facultad de Ciencias Agrarias de la Universidad Nacional de Cuyo, ubicada en la provincia de Mendoza, República Argentina, ha comenzado una línea de investigación que tiene como finalidad desarrollar técnicas que permitan la obtención de bioetanol a partir de materia prima (biomasa) originada por cultivos energéticos de Salicáceas y eucaliptos.
Las muestras de material lignocelulósico provienen de dos clones de álamo: Populus × canadensis '*Conti 12*' y *Populus × deltoides* 'Harvard'; dos clones de sauce, *Salix babylónica var. Sacramenta* 'Soveny americano' y *Salix babylónica × S. alba* 'Ragonese 131-27 INTA' y del Eucalyptus camaldulensis, todos de 2 años de edad, plantados en densidades de 10.000 y 20.000 plantas.ha^{-1}.
Pretratamiento: La biomasa cosechada se llevó a estufa a 40 °C hasta peso constante, para su secado; luego se molió en molino de martillos hasta aserrín fino. Posteriormente de las muestras molidas se colocaron en erlenmeyers en una solución acuosa dentro de un autoclave y se las sometió a una presión de 10atm, durante 10 minutos, descomprimiendo luego bruscamente en pocos segundos. Se filtró y la fase sólida se secó nuevamente a estufa a 40 °C.
Tratamiento Enzimático: Se colocó las muestras pretratadas en erlenmeyers, se adicionó buffer acetato de sodio, pH 4,8, se llevó a baño maría a 50 °C por 5min, posteriormente se agregó a cada erlenmeyer caldo enzimático (con enzimas de tipo celulasas y endoglucanasas) con una actividad de 30 FPA/g de muestra. Se dejó las muestras en baño maría durante 72horas.
Determinación de azúcares reductores liberados: se determinó su concentración en g.l^{-1} en espectrofotómetro a una absorbancia de 545nm. La biomasa proveniente del eucalipto y del 'Conti 12' fueron las que liberaron mayor cantidad de azúcares reductores.
Fermentación: las muestras de madera se colocaron en erlenmeyers, se les agregó levaduras (cepas locales de *Saccharomyces cerevisiae*), en una concentración de 1g/10ml de H_2O ; y se fermentaron en estufa a 25 °C durante 1 semana. Posteriormente se filtraron obteniéndose una mezcla hidroalcohólica en la cual se determinó su concentración de alcohol.
Determinación del alcohol producido: Los análisis se realizaron con espectrofotómetro de infrarrojo según el método OIV-MA-BS 02.
Producción de alcohol: En el clon Conti 12, se determinó una concentración de 0,7%± 0,04 (vol/vol), de alcohol en la mezcla. En el resto de las muestras analizadas de los diferentes clones y/o especies, la concentración de alcohol alcanzó valores de 0,4%± 0,04 (vol/vol).

Conclusiones: La metodología empleada hasta el momento ha permitido la obtención de alcohol a partir de biomasa proveniente de diferentes especies y/o clones de forestales. Se continúa ajustando los parámetros de cada uno de los pasos de la técnica a fin de alcanzar un rendimiento mayor en alcohol, que permita en el futuro la utilización de la misma primero a escala piloto y luego a escala industrial.

BATCH PERVORATIVE FERMENTATION WITH ADAPTED MEMBRANE: INFLUENCE IN ENERGY CONSUMPTIONS IN DISTILLATION STAGE

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Ethanol distillation to produce hydrated alcohol fuel is a high-energy demanding stage in overall alcohol fuel process. The distillation energy efficiency is strongly associated with the alcoholic fermentation performance in the process; therefore final ethanol concentration in the alcoholic wines has direct impact on consumption of thermal energy in alcohol separation. In this study, the ethanol production with a hybrid-simple batch membrane fermenter (H-SBMF) was modeled and simulated. In order to determinate the hybrid-fermentation influence on energy consumption in distillation, mainly on hot utilities (steam) and electrical energy needs in permeate recovery. A conventional fermenter was intensified with a poly-dimethylsiloxane (PDMS) pervaporation membrane module; hence, ethanol concentration in a fermentation medium remains lower than inhibition by products conditions, due a continuous ethanol pervaporation. An H-SBMF achieved a higher ethanol production, in order to 10-15%, concerning a batch fermenter without membrane and an increase in productivity of 150%. The distillation case was considered as a general arrangement used in Brazilian plants, consisting in two columns: an ethanol recovery column and a rectification column. It was considered the permeate recovery system (vacuum and compression stage), in order to determinate the unitary electrical energy supply, further the thermal energy. A decrease in steam consumption was evidenced with the adaption of membrane into fermenter. Higher energy efficiencies were achieved with larger membrane areas, obtaining almost 20% in steam reduction.

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KRAFT PULPING OF *Pinus radiata* AS A PRETREATMENT FOR BIOETHANOL PRODUCTION BY SIMULTANEOUS SACCHARIFICATION AND FERMENTATION

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Ethanol derived from lignocellulosic biomass (LCB) has long been recognized as a potential alternative for non-renewable transportation fuels. Due to LCB recalcitrance to enzymatic hydrolysis, owing to it rigid structure, the interaction between cellulose and hemicellulose plus the degree of lignification, a pretreatment process is necessary to disrupt the lignocellulosic matrix and increase its enzymatic digestibility. Various methods have been developed for pretreating LCB, this include biological, physical, chemical, and physicochemical processes. In this work, kraft pulping process was evaluated as a pretreatment of *Pinus radiata* for bioethanol production. Wood chips were pretreated under different pulping conditions (170°C, 14-26% alkali active (AA), H-factor 1200). Mean fiber length, fiber width, kink index, coarseness, and fines were determined using Fiber Tester. Simultaneous saccharification and fermentation fed-batch (SSF-fed batch) was conducted with Cellic CTec 3 cellulase from Novozymes dosed at 0.044g of enzyme/g pretreated material at 50°C. The experiment were started by adding *Saccharomyces cerevisae* suspension of 3 g/L. After the initial batch phase with 2% dry matter, pretreatment materials was added four times giving a total dry matter content of 10%. Results showed a total of 7 pulps were obtained, with pulps yields ranging from 48% to 64%, depending on the alkali active used in the delignificaction. Glucan remained in pulps were from 84% and 89%, while 60% of the hemicelluloses were solubilized. Lignin removal increased with increased with AA, reaching delignification of pulps between 60% to 91%. Changes in the morphology of the fiber is observed by increasing the load of AA, specially in kink index and coarseness that decrease when high charge of AA is used. The ethanol yields obtained from kraft pulps varied between 67 and 89% (wood basic). The maximum ethanol yields were 89% at H-factor 1200, 26% AA. New experiments are being performed in order to improve saccharification of substrates.

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BIOETHANOL FROM RESIDUES OF THE OLIVE OIL INDUSTRY

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Lignocellulosic materials represent the largest component of the residues generated by agricultural and agroindustrial activities in the world and are an abundant and reliable source of renewable energy. For this reason, the production of biofuels from them is of great interest [1]. Two phase olive mill waste is the semisolid residue from the two-phase olive oil production, mainly composed by cellulose, hemicelluloses, lignin and small quantities of other components such as pectins, proteins and ashes. In San Juan (Argentine), about 40,000 MG of two phase olive mill waste are annually produced. It is a highly polluting waste (COD: 230-240 g/Kg and BOD: 90 g/Kg, approximately), and its treatment and disposal is a concerning problem.
The process of converting biomass to ethanol comprises the basic steps of cellulose hydrolysis, to transform it into fermentable sugars, and the subsequent alcoholic fermentation of these compounds. The presence of lignin and hemicellulose materials hinder the accessibility of acids or enzymes to cellulose, reducing the efficiency of the hydrolysis. Therefore, the application of pretreatments is critical to assure high yields. Chemical pretreatment using diluted sulphuric acid is recognized to be one of the more advantageous methods because of its easy implementation, low cost and high efficiency [2]. However, it has not been clearly stated the importance of the conditions that may affect the efficacy of this treatment.
This work reports the results of studies carried out to assess the best conditions for ethanol production from two phase olive mill waste.
The relevant variables of the pretreatment with sulfuric acid were determined using a fractional factorial design (2^{k-1}), which were subsequently optimized using the Box Behnken method. The response variable used in both stages was the total sugars content. The significant variables of the pretreatment were temperature, acid concentration and solid / liquid ratio. The next step, enzymatic hydrolysis, was conducted at 45°C for 48 hours using cellulase and hemicellulase enzymes. The hydrolysates, with their corresponding synthetic liquors, were used as fermentation substrates for the production of ethanol by the yeast, Saccharomyces cerevisiae.
The results showed that working at the optimal pretreatment conditions, i.e. 95°C, sulfuric acid concentration of 10.1% w/w and a solid to liquid ratio of 0.17, total sugar content was increased by 68% and therefore the alcohol yield of the process.

Keywords: two-phase olive waste, diluted acid, ethanol

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BIOETHANOL PRODUCTION FROM MAIN CARBOHYDRATES OF BROWN ALGAE *Macrocystis pyrifera.*

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ABSTRACT
Valuable compounds derived from algae include proteins, carbohydrate, lipids, and a variety vitamins and antioxidants. Algae are an excellent source of raw material for bio-refinery processes. The idea of using macroalgae as a biofuel feedstock arose from the need for a sustainable biofuel with a low environmental impact. The brown algae studied in this work are *Macrocystis pyrifera* collected off the coasts of the South of Chile. The main carbohydrate components of algae are alginate (a linear polymer consisting of 1,4-linked βeta-D-mannuronic acid and alpha-L-guluronic acid) and cellulose (a polysaccharide consisting of a linear chain of βeta 1,4-linked D-glucose units). The carbohydrate content of M. pyrifera was determined via gas chromatography [1]. This alga was pretreated with water or dilute sulfuric acid, enzymatically saccharified with alginases or cellulases, and fermented into ethanol, by means of simultaneous saccharification and fermentation (SSF) using *Saccharomyces cerevisiae* or BAL1611 *Escherichia coli*. Glucose fermentation with *Saccharomyces cerevisiae* strain Ethanol Red®, dilute sulfuric acid/autoclaved and water/autoclaved pretreated *M. pyrifera* achieved 58.20 and 76.48 wt-% of the theorical yield for ethanol production. When non-pretreated algae residue was fermented, only 0.06 g of ethanol/g of glucose was obtained (corresponding to 2.75 wt-% of the theoretical maximum).
In terms of uronic acid fermentation with BAL1611 *Escherichia coli* (which was kindly provided by BAL Company), SSF of *M. pyrifera* residues pretreated with diluted sulfuric acid produced 0.095 g ethanol/g uronic acid, and SSF of M. pyrifera residues pretreated with water produced 0.028 g ethanol/g uronic acid. These values reflect 18.33 wt-% and 5.38 wt-% respectively of the theoretical yield of ethanol produced from uronic acids. The low production of ethanol could be due to the ratio between alginate, mannitol, and glucose in this alga, considering that *Escherichia coli* BAL1611 requires greater concentration of mannitol than is present in *M. pyrifera*. The uronic acid production was quantified according to the methods described by Milner & Avigad [2, 3], and ethanol content was analyzed using HPLC. To the best of our knowledge, this is the first report of simultaneous saccharification and fermentation conducted in parallel for both cellulose and alginate from brown algae. Future optimization of the operational conditions could further improve this process.

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OBTENCIÓN DE BIOETANOL A PARTIR DE ALMIDÓN DE AMARANTO (*Amaranthus Hiponcondriacus* L) POR EL MÉTODO DE SACARIFICACIÓN Y FERMENTACIÓN POR SEPARADO.

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Los biocombustibles han sido candidatos para sustituir los combustibles fósiles debido a que disminuye las emisiones de gases contaminantes a la atmosfera y es utilizado para elevar el octanaje de las gasolinas, es por ello que se han buscado diferentes fuentes (por ejemplo el almidón) y estrategias para la producción de bioetanol que puedan ser fáciles de utilizar y de procesar. Una de las estrategias usadas es la Sacarificación y Fermentación por Separado (SHF), la cual consiste en un hidrólisis enzimática y una fermentación en etapas separadas a sus temperaturas y pH óptimos de cada enzima para garantizar una completa atenuación de los azucares producidos en la hidrólisis enzimática. Por lo tanto, el objetivo de este trabajo fue la producción de bioetanol aplicando el método SHF usando almidón del grano de amaranto (1,2). La estrategia consistió en añadir α-amilasa a 100 mL de medio que contenía $(NH_4)_2SO_4$, KH_2PO_4, $MgSO_4$, $CaCl_2$, NaCl, extracto de levadura y almidón de amaranto aislado previamente. Se ajustó el pH a 4.2 con HCl y se calentó a 120°C por 15 min a 15 psi. Posteriormente, se dejó enfriar a temperatura ambiente hasta 60°C por agitación y se añadió amiloglucosidasa y pulalanasa y se incubo a 60°C por 30 min. Pasado ese tiempo se enfrió a 30°C y se añadió 5% de levadura *Saccharomyces cerevisiae*. Se dejó fermentar a 30°C por 72 horas, tomando muestra cada 12 h. Se cuantifico el etanol producido mediante cromatografía de gases. El contenido máximo de etanol producido usando SHF después de 72 horas fue de 17.45 g/L, el cual es bajo si se compara con otras fuentes de almidón como es el caso del maíz con el cual se obtiene hasta 56 g/L (3) usando la misma estrategia. Esto puede deberse a que haya una producción de azúcares que la levadura no puede metabolizar como es el caso de las maltotrealosas, dextrinas limite o isomaltosas. También puede deberse a que las enzimas desramificadoras no hidrolizan completamente las dextrinas producidas por la α-amilasa. Otro factor que pudiera estar afectando la fermentación de los azucares son los requerimientos nutricionales de la levadura no sean los adecuados y no estén ayudando a que se sinteticen algunas enzimas que ayuden a la degradación de azúcares mas grades como es el caso de las maltotriosas (4). Aunque los rendimientos de etanol son bajos, se puede considerar que el almidón de amaranto es una buena fuente para la obtención de bioetanol, pero se requiere de más investigación para obtener mejores resultados.

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PRELIMINARY STUDIES OF CELLULOSIC BIOETHANOL PRODUCTION FOR SIMULTANEOUS HYDROLYSIS AND FERMENTATION TESTING

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To cope with the exhaustion of fossil fuels,it has become necessary to find valid renewable sources that do not compete with food production at affordable costs and alternatives. Cellulosic biomass can be chemically or enzymatically hydrolyzed, and the obtained sugars can be fermented with yeasts to produce ethanol. The possibility of performing simultaneous hydrolysis and fermentation prevents enzymes inhibition, pollution risks and decreases production costs. The drawback is that the optimum temperature for the enzyme treatment is about 50°C; where as the corresponding fermentation is close to 32°C [1]. Rice husks (i.e. lignocellulosic waste of rice production), is an abundant resource in the NEA region of Argentina, and it is an excellent raw material for the bioethanol production because it has a high cellulose content. As preliminary tests for the application of simultaneous enzymatic hydrolysis and fermentation to rice husks, enzymatic hydrolysis on Whatman filter paper N°1 at temperatures below the optimum (32°C), and subsequent fermentation with four different yeast, *Saccharomyces cerevisiae*, *Candida tropycalis, Candida guilliermondii* and *Candida kéfir*, were performed at the same temperature. Yeast selection was based upon specific criteria. *Candida kéfir* can with stand temperatures up to 37°C, so it was taken as afeasible alternative for the simultaneous process at high temperatures [1]. *Candidas works* at 32°C, but they also have affinity for 5-carbon sugars such as xylose, present in small amounts if the material has been pretreated with acid to remove hemicelluloses. The filter paper was characterized through moisture and ash amounts. The enzymatic hydrolysis with celullases of *Trichoderma reseei* and celobiasses of *Aspergillus níger* was accomplished during 72 hours at 32°C in a rotary shaker.The amount of glucose obtained by the hydrolysis was determinate by High Performance Liquid Chromatography (HPLC) with refractive index (IR) detector.In the hydrolysis at 32°C the cellulose conversion to glucose was 81%. The supernatants of the hydrolysis were supplemented with nutrients, and were subjected to subsequent fermentation with the four yeasts listed above; stirring for 24 hours at 32 ° C. The ethanol generated in each case was determined by HPLC. Ethanol yields obtained were 66.2% for *Saccharomyces cerevisiae,* 61.47% for *Candida tropycalis*, 45% for *Candida kéfyr*, and 32.42% for *Candida guilliermondii*. Results showed that all yeasts generated ethanol, even when the fermentation time was only 24 hours. Based on these encouraging results (hydrolysis and fermentation at 32°C for 24 hours), the simultaneous processing ofrice hulks was accomplished in these conditions, obtaining up to 70% of ethanol when using *Saccharomyces cerevisiae*.

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PRODUCTION OF FERMENTABLE SUGARS FROM SUGARCANE STRAW

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ABSTRACT

Sugar cane straw is becoming an important source of lignocellulosic raw materials for producing second generation ethanol. In this way, the autohydrolysis process has been considered a simple, low-cost and environmental friendly technology for generation of sugars from biomass. In order to improve accessibility of enzymes during enzymatic hydrolysis as well as to allow the recovery of hemicellulose in the filtrate. In this study, it was used as raw material sugarcane straw, which was pretreated using autohydrolysis followed by a mechanical refining process aiming ethanol production. Two different autohydrolysis conditions were studied: 180 °C for 40 min, and 190 °C for 10 min. Autohydrolysis at 190 °C for 10 min provided the highest overall sugar (83.1%) with low enzyme application (10 FPU/g substrate) in subsequent hydrolysis process. The ethanol production from autohydrolysis of sugarcane straw seems to be a financial viable approach if a cost-efficient straw collection system is established.

EFFECT OF SEVERAL THERMAL AND MECHANICAL PRETREATMENTS ON SOLID COMPOSITION AND SACCHARIFICATION OF ORANGE PEEL WASTES

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Citrus fruits world production reaches each year more than 90 million tonnes. Half of it is the feedstock for the production of juices, and half this mass turns into a waste with a very high DBO5. This waste is scarcely useful, being only employed as an additive for cattle feed on a weight ratio of 20 to 30 kg per tonne feed, and only in some regions, as Florida. Therefore, there is a need for the development of new processes to valorise this waste. As with other biomass stocks, pretreatment is needed to get an open structure reactive to the action of cellulases, xylanases, estearases and other enzymes, as well as a way to remove, and recover, valuable volatile terpenes, polyphenols and a good part of the pectin.

In this work, blade milling alone or combined with hot water and steam pretreatments are studied, using the steam at atmospheric pressure (stripping) or at 3-6 atm absolute pressure with a sudden discharge to atmospheric pressure (steam explosion). The main variables for the pretreatments have been particle diameter, temperature, time and pressure (where applicable). Liquid fractions, or a citric buffer if needed, were used as reaction media for saccharification at 50 ºC, 300 rpm and pH 5.0 with a combination of enzymes (Celluclast 1.5L, Novozym 188, Pectinex Ultra-SP) kindly provided by Novozymes. Withdrawn samples were analysed by HPLC using a Rezex™ RPM-Monosaccharide Pb+2 column and water at 80ºC as eluent.

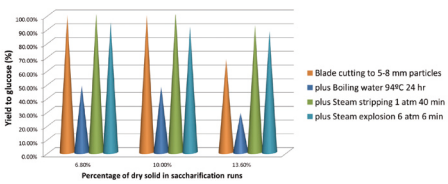

Fig.1 – Total yield to glucose in the saccharification of pretreated CPW of orange at 204 FPU/ dry g

Solid composition was analysed by NREL standard procedures and shows that steam explosion treatments increases the ratio of soluble polymers to insoluble polymers. Saccharification runs suggest good total and hydrolytic yields for glucose (figure 1) and fructose, reaching total sugar concentrations values as high as 100 g/L for a percentage of dry solid to liquid of 13.6%.

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TECHNO-ECONOMIC ASSESSMENT OF A BIOREFINERY FOR ETHANOL PRODUCTION AND VALUE-ADDED PRODUCTS USING AGAVE BAGASSE AS RAW MATERIAL

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Tequilana Weber agave is an endemic plant of the Mexican territory, its main use is the production of alcoholic beverages because of their high sugar content. Annually, an average of 495 594 MT of bagasse are produced as waste in the production process of Tequila [1]. Whereby the use of bagasse as raw material in a biorefinery platform is attractive because of its low cost and availability. Besides that recent studies have shown that ethanol from agave is a fuel with low environmental impact [2]. However, there are other possibilities for taking full advantage of this renewable source as the production of added value products [3].

In this work the design and the techno-economic assessment of two biorefinery schemes and three case studies for each one were done, using the design information provided by Aspen Plus v8.4, and the capital and operating costs calculated from Aspen Economic Analyzer. The first biorefinery scheme describes the production of ethanol, xylitol and sorbitol. Whereas the second biorefinery scheme considers the production of ethanol, xylitol and sorbitol integrated to a cogeneration system for producing bioenergy from the solid residues resulting from the mentioned processes, according to the Table I.

TABLE I. DESCRIPTION OF SCENARIOS

Scenario	Production			
	Ethanol [Mgal/ year]	Xylitol al 70% [Mlb/year]	Sorbitol al 70% [Mlb/year]	Electricity Cogen-eration [Kw/h]
Case 1[a]	61.34	-----	-----	-----
Case 2[a]	39.1	348.5	-----	-----
Case 3[a]	19.5	348.5	294.4	-----
Case 1[b]	61.34	-----	-----	6153
Case 2[b]	39.1	348.5	-----	6153
Case 3[b]	19.5	348.5	294.4	6153

[a]. First scheme production without cogeneration system
[b]. Second scheme production with cogeneration system

The main target of the economic assessment was to determine the minimum etanol selling price through a discounted cash flow analysis, as well as the variations that this one presents respect to the raw material cost and the opportunities to improve this price by the production of value added products and the use of cogeneration system. The above was done with the purpose to establish a rank of the ethanol-selling price in the country and the minimum etanol-selling price calculated, as well as the total production costs and revenue from product sales.

The results showed that in the first biorefinery scheme, for the case-study where only ethanol was produced, the minimum ethanol selling price was $1.3068/gal, while for the same case-study using the cogeneration system the minimum ethanol selling price decreased by 5.4% resulting in $1.2357/gal, these results allow establishing an ethanol selling price where a positive profit margin is obtained. The above is attributed to the very low cost of the raw material. However increasing the demand for this raw material its will increase value. Thereby the incorporation of added value products as xylitol and sorbitol shows that the profit margin can keep it without significant changes. In this sense, a biorefinery with a major number of products and low energy consumption is an important option for the development of the bioethanol industry.

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EVALUATION OF ETHANOL PRODUCTION IN ETHANOL-TOLERANT THERMOPHILIC BACTERIA ISOLATED FROM GEOGRAPHIC MACROZONE NORTHERN CHILE.

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The increasing demand for sustainable biofuels is driving the need for feasible bio-refineries capable of utilizing highly renewable and environmentally friendly feedstock's to produce high-volume biofuels[1]. In the past decades various thermophilic microorganisms capable of producing low amounts of ethanol have been isolated and characterized from different environments with intention to evaluate their ability of fermentation and large scale ethanol production[2].

Previously, in the laboratory of Microorganismos Extremófilos, bacterias of the genus *Geobacillus sp.* were isolated from different places of the geographic macrozone Northern of Chile. Members of this genus have been described previously as etanol-tolerant and ethanologenic bacteria, highlighting *Geobacillus thermoglucosidasius* strain (M10EXG)[2], able to tolerate concentrations of ethanol up to 10% (v/v).
The aim of this work was to determine ethanol production in four bacterial strains isolated from the geographic northern macrozone of Chile, in addition to improving biomass production, determine byproducts of fermentation with the purpose to modify and enhance their productive capacities through their metabolic pathways for ethanol production.

The results indicate that the strains C3, T25, T23 and CJ1 have the capacity to tolerate up to 4% ethanol and grow at a temperature of 60°C, proliferating in the presence of xylose and glucose as the unique carbon source, in addition whit the ability to consume these sugars for biomass production in 12 hours, however the strain C3 was the only one with significant production of ethanol in a yield of 0.08 g ethanol/g glucose in 24 hours. The results of this work show that the C3 strain is a potential candidate for the production of bioethanol in thermophile bacteria by improving its metabolic pathways.

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Cupressus lusitanica Mill. AS A BIOREFINERY FEEDSTOCK

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Cupressus lusitanica Mill. (cipreste-do-Buçaco) also commonly known as the cypress cedar of Goa, Mexican cypress, or Kenya cypress is a tree of medium height, fast growing, with a dense, conical crown and a trunk surrounded by a reddish-brown bark. It produces clear, light and low density wood, but with fine texture and high dimensional stability. It also produces small cones. It is considered a very adaptive species with respect to environmental conditions that enables it to grow on many locations, especially in humid climates. It has been used quite intensively in reforestation programs in Portugal in the last two decades, and its use as a biorefinery feedstock is currently being considered.

In this work, diverse fractions, namely wood chips, bark, leaves and cones, were chemically characterized, taking special interest on their extractives and hemicelluloses content and composition.

Essential oil was mainly found on leaves and cones, and its extraction was studied at lab and pilot scale. Soluble extractives found include monosaccharides and catechin derivatives.

Autohydrolysis was studied in detail for leaves taking special attention on the impact of the previous essential oils extraction. The process presented a high selectivity towards hemicelluloses, yielding oligosaccharides as the main products, and reaching yields close to 8.5 g OS /100 g of dry biomass, regardless if its leaves were pre-extracted or not.

The enzymatic digestibility of autohydrolyis treated materials was also evaluated and found to increase as a function of increased treatment severity, reaching 66 % for the severest condition.
Bioethanol production from pre-treated biomass was carried out and the results are discussed based on the overall mass balances, potential economical revenue, and the need for additional biomass pre-treatment processes, namely for lignin removal / production of lignin-derived products.

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ESTIMATION OF THE ENERGY POTENTIAL OF SOME FORESTRY RESIDUES FROM MEXICO: FOCUS ON TORREFACTION

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Abstract
The ¨Western Sierra Madre¨ is the largest mountain complex of Mexico, accounting for approximately 28.9 MM hectares of native forest. The 18% of this vast area is placed in Durango, where at about 33% of the national timber production is concentrated. The management and exploitation of timber in Durango generates huge quantities of residues (branches) which are actually wasted, due to the lack of a conscious strategy. This study aims to quantify the gross potential of those residues and to evaluate its energy valorization through torrefaction. With that end, we carried out (i) the identification and quantification of those species with higher exploitation rate, (ii) the characterization of residues from the selected species, through chemical and elemental analyses, (iii) the study of its thermal decomposition through TGA/GCMS and (iv) the evaluation of torrefaction as a thermal treatment to produce coal-like fuels. Main results indicates that *Quercus sideroxyla* (*Q. sideroxyla*) and *Pinus duranguensis* (*P. duranguensis*) are the species with higher rate of residues generation, with a joint gross yield of 10.75 tonne per hectare. Results from thermogravimetric decomposition of both *Q. sideroxyla* and *P. duranguensis* exhibited the typical performance of lignocellulosic materials, with three decomposition phases and two reaction zones (active and passive pyrolysis). The kinetics of these reaction zones was studied through the Ozawa-Flynn-Wall (OFW) and Starink isoconversional approaches. Values of activation energies for the hemicellulose degradation (0.1<<conversion>>0.25), obtained from both methods, were similar and ranged between 55 and 152 kJ mol^{-1} for *Q. sideroxyla* and (54 - 150 kJ mol^{-1}) for *P. duranguensis*, representing a multi-step decomposition process.

After chemical and thermal characterizations, both wood samples were torrefied in a custom designed lab-scale setup, varying the residence time (15-30 min) at four temperature levels (220-250-280-290 ºC). Effect of temperature on energy yield was higher than that of residence time, it produced an average yield drop from about 92% (at 220 °C) to 74% at 290 °C, which could be attributed to the exponential response of reaction kinetics. Main species detected in the liquid or condensable products from torrefaction, were Water>Carboxylic acids>Alcohols>Others (phenols, furfural, etc.). The formation of Levoglucosan in the case of *Q. sideroxyla* was marginal (<2.1%wt.), which could be due to the influence of its high content of inorganics (K, Li and Ca). The heating value of torrefied solids increased by a factor of 1.31 (*Q. sideroxyla*) and 1.22 (*P. duranguensis*), respectively. Considering the resources availability and the effect of torrefaction on its fuel properties, this is an interesting alternative to valorize the forest residues from Durango.

Keywords: Forest residues; Torrefaction; Kinetics, Energy yields, Volatile composition

STUDY OF BIO-OIL PRODUCTION FROM MICROALGAE SCENEDESMUS ALMERIENSIS DEPROTEINIZED: FOLLOWING A BIOREFINERY CONCEPT

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The effect of fast pyrolysis of deproteinized microalgal biomass was investigated. The production of microalgae without proteins (MWP) for pyrolysis was obtained by protein extraction from *S. ameriensis*. The first experiment was analyzing the effect on bio-oil yield and was carried out at 480 °C and 1.0 s of residence time for whole microalgae (WM) and MWP biomass in a batch scale-laboratory reactor. In order to determine the effect of preteins extraction in steam and non-condensable gases composition, a micropyrolyzer couplied to GC/MS was used. Like wise, the temperature effect was analized (450, 500 and 550°C) on the composition of steams and non-condensable gases generated. The results showed a decrease on nitrogen content of the biomass, due to the protein extraction by enzymatic hydrolysis. The bio-oil, biochar and non- condensable gases production on the organic fraction of WM, reached a 30%, 10% and 60% respectively and 15% of bio-oil, 3% biochar and 15% non-condensable gases for MWP. The higher protein content presence and carbohydrate, facilitates the formation of non-condensable gases at 480°C, due to their total decomposition is between 250° and 450°C. On the other hand, it is possible that the high ash content in WMP produces a significant increase of non-condensable steam generation, by its catalytic effect during the pyrolysis. Apart from requiring a lower residence time, which limited the consecutive fractionation of fumes generated during microalgal biomass desvolatilization, fostering the bio-oil production. The WM bio-oil has a wide variety of compound groups, which makes harder the interest compounds extraction, but from the biomass desproteinizacion, it allows to obtain a high Alkenes bio-oil, this process can be considerate as a previous process of refining of bio-oil. Finally, it is proposed to evaluate the characteristics of the pyrolytic products de MWP biomass to define their possible use.

ABLATIVE FLASH PYROLYSIS OF STRAW - CONVERSION PROCESS AND VALORIZATION OPTIONS FOR SOLID AND LIQUID PRODUCTS

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The Fraunhofer Innovation Cluster Bioenergy deals with the valorization of different types of residual biomass from farming as well as food/fodder production processes. For the conversion of dry, stalk-like biomass like cerial straw or hay, reed and miscanthus ablative flash pyrolysis was chosen, which gives pyrolysis char and pyrolysis condensates in the first step. Ablative pyrolysis was chosen, as there is no need for very fine particles with diameters below 1 mm in comparison to many other pyrolysis processes. Two test facilities in laboratory and pilot scale have been purchased. Product yield and composition was compared to well-described wood pyrolysis and significant differences were discovered (higher char yield, higher water content in pyrolysis condensates, instantaneous phase separation of pyrolysis condensates into a light aqueous phase and a heavy, sludgy organic phase).
An economic evaluation of a conversion unit operated close to agricultural land revealed the potential to deliver pyrolysis condensates to larger customers at roughly the same price as baled straw, as for baled straw 4/5 of the selling price comes from logistics, which can largely be reduced by direct conversion to intermediate liquid energy carrier like pyrolysis condensate.
The pyrolysis condensates have all the unfavorable properties like those from many other pyrolysis processes like high water content, high viscosity, high acid content and corrosivity, low heating value and additionally disintegrate into two separate phases. To improve the value of the liquid product, different upgrade options are tested.
Firstly, the esterification and acetalization of acids and ketones/aldehydes with alcohols (e.g. butanol) are studied. By using heterogeneous, solid acid catalysts, the properties of the liquids can be greatly increased: the water content could be reduced from 25 % to 3.5 %, the kinematic viscosity from 18 cSt to 6 cSt and the total acid number from 112 to 10. The esterified conden-sates can be utilized as substitute for heavy fuel oil applications in stationary boilers and as bunker fuel without further modification.
Secondly, a staged condensation process was developed, which gives 3 fractions with a rough separation of components according to their boiling point. The two high boiling fractions are nearly free of water and acids and might be used for the production of plastics (e.g. phenolic resins, rigid polyurethane foams) and the low boiling fraction as additional substrate in biogas.
Also for the char, several utilization options exist: it can be used as supplemental fuel in pulveri-zed coal power stations, as fertilizer in agriculture (PK-fertilizer as received and potentially upgraded to NPK-fertilizer after combination with digestate from biogas plant) and serve as carbon source for the production of activated carbon or carbon based catalysts.

BIODIESEL FROM BYPRODUCT OF RICINUS COMMUNIS PYROLYSIS

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Ricinus communis is a non-food plant that grows well in warm climates that can be used to restore degraded lands and then make full use of the plant. One of the main products of the plant are seeds of which castor oil is extracted. One of the possible uses of this oil is the production by pyrolysis of heptaldehyde and undecylenic acid. These products have a high added value.
A resinous substance that cannot be pyrolyze is formed during reaction. Therefore, castor oil conversion should not exceed 46%. This byproduct can be used to increase the castor oil pyrolysis profitability.
The byproduct was analyzed by infrared spectroscopy. It showed that the ester bond from the original triglyceride remains intact. Using an elemental analysis it was verified that the proportions of elements C, H and O remain in byproduct at similar values as the original castor oil. Therefore, the byproduct can be used as raw material for methanol transesterification.
This work aims to evaluate the methanol transesterification of the byproduct from the castor oil pyrolysis. The optimal conditions for the process will be determined using response surface methodology. The experimental factors are methanol/oil molar ratio, NaOH concentration, temperature, stirring speed and reaction time. The response variable will be the glycerin in aqueous phase after washing the biodiesel. Glycerin will be determined through enzymatic degradation to hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipirine and 4-chlorophenol forming a colored complex of quinoneimine. This complex is quantified using UV spectroscopy at 500 nm wavelength. On the other hand, the reaction kinetics is determined at obtained optimal conditions using standard techniques. Finally, an industrial plant for the production of biodiesel from this byproduct will be simulated including a financial evaluation.

GASIFICATION OF CORN PRODUCTION RESIDUES

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Energy costs associated to corn production account for about 30% of the total cost of corn production in Portugal. Thus, to improve the competitiveness of corn production sector it is fundamental to decrease energy costs in an environmental sustainable way. The objective of this work is to develop technology and assess the technical and economic viability of the use of corn by-products (cobs and straw) as raw materials for the production of renewable energy for drying corn itself and if possible to sell the excess energy to the power grid.

Corn biomass wastes were co-gasified and the gas produced was analysed to determine gasification conditions that favours the composition of the gas obtained and it suitability to be used for energy production. Different gasification conditions were tested to analyse corn biomass wastes behaviour during gasification, namely: reaction temperature, gasification medium and corn wastes composition. The gasifying/fluidising agent were mixtures of steam and air (or oxygen), which were fed through a gas distributor at the base of the reactor. Co-gasification temperatures from 750ºC to 900ºC were tested. Adjustments in steam/O_2 and in the gasification agent/solid fuel ratios were also performed.

Co-gasification tests were performed in a bench-scale installation. The reactor was a fluidized bed, made of a refractory steel and was circular in cross-section with an inside diameter of 0.08 m and with a height of 1.5 m. Gaseous main components: H_2, CO, CO_2, CH_4 and other heavier gaseous hydrocarbons were analysed by gas chromatography. Tar contents in gasification gas were determined by CEN/TS 15439:2006.

Co-gasification of corn wastes took place in a normal way and no changes in existing installation were needed. The rise of gasification temperature increased the gas yield and as there were no appreciable variations in its calorific value, the energy conversion of gasification process also increased. However, it is advisable to use a temperature not exceeding 850 ° C to reduce any bed agglomeration problems that may arise after long periods of gasification. The rise of air flow rate also reduced the tar content in the gas, since the combustion of tar was favoured by increasing the amount of air present during gasification. Though, a higher gas yield was obtained, the produced gas had a lower calorific value, due to nitrogen diluting effect. Thus, equivalent ratio should remain close to 0.2, not to drastically reduce the heating value of the gas, or the energy conversion process. The gas produced by the gasification of corn crop wastes can have a wide range of applications. However, it may be necessary to optimize the gasification process after defining the employment of the gasification gas produced.

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STUDY OF OPERATIONAL CONDITIONS AFFECTING PYROLYSIS CONVERSION PROCESS OF *Botryococcus braunii* SPENT-BIOMASS

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Fast pyrolysis of microalgal biomass was investigated. Bio-oil production yield was studied analyzing the critical variables particle size, temperature and residence time by means of sequential optimization. Production of microalgal biomass without proteins (MWP) for pyrolysis was obtained by protein extraction from B. braunii. The first experiment was to analyze the particle size effect on bio-oil yield and was carried out at 500 °C and 1.5 s of residence time for two fractions of MWP biomass. The first tested fraction presented a size range between 450 and 500 µm, whereas the second moved between 900 and 1,000 µm. As a result, it was found that there was no significant difference (α = 0.05) between the proportions of bio-oil, char and syngas obtained for each particle size distribution studied. Therefore, it was established to dismiss the effect of the factor 'particle size' in this research. To determine the effect of temperature, the production of bio-oil, char and syngas at 350, 400, 450, 500 and 550 °C was evaluated under a constant residence time (1.5s). Subsequently, to determine the effect of the residence time, the production of pyrolytic products at 1, 1.25, 1.5 and 1.75 s by using the best previously selected (480°) temperature conditions was evaluated.

The results show an increase in bio-oil production by increasing the reaction temperature, but simultaneously causing an increase in water content in bio-oil and syngas. The latter production can be controlled through a lower residence time, which limited the consecutive fractionation of fumes generated during microalgal biomass desvolatilization. Therefore, achieving a high desvolatilization of biomass allows the generation of a high fraction of gases from the biomass, which can favor a higher conversion to bio-oil under a low residence time. Additionally, the biorefinery concept was applied in this research; the extraction of proteins of B. braunni allows obtaining a high-added-value product and generates a residual biomass suitable for obtaining pyrolytic products of interest. Finally, it is proposed to evaluate the characteristics of the pyrolytic products to define their possible use.

STAR-UP OF A PILOT SCALE PYROLYSIS REACTOR FOR THE PRODUCTION OF BIO-OIL FROM MICROALGAE BIOMASS

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The star-up of a fast pyrolysis reactor for producing mostly bio-oil from microalgal biomass was carried out. The equipment was a new fast pyrolysis reactor recently implemented in La Frontera University (Fig. 1). Biomass from microalgae *Nannochloropsis gaditana* was used as raw material. The microalgae was selected due to *N. gaditana* can grow under saline conditions and have been widely investigated reaching good productivities in the North of Chile. The biomass was conditioned following successive steps. First, the biomass was dried in a drying tunnel for about 72 hours. Secondly, the biomass was ground in a manual mill and then sifted to get a particle size between 0.5 mm -1.0 mm. The biomass was characterized according to proximate analysis using a Thermogravimetric analyzer (TGA-STA DSC 6000 from Perkin Elmer) using the modified methodology from García et al. [1] to determinate moisture, volatile, fixed carbon and ash. The results of the biomass characterization showed a good performance of the tunnel dyer; where the biomass was concentrate from 91.9% of moisture until 4.4%. The other results of the proximate analysis were: volatile (55.5%), fixed carbon (9.1%) and ash (30.9%), where the high ash content seems to be a bad characteristic affecting negatively bio-oil production. The start-up of the pyrolysis reactor consisted in to check the state of seals, leaks in the nitrogen injection, verify cooling system, verify the temperature control and verify the feed systems. The performance of each system was tested separately. Additionally the inner diameter of the electrostatic precipitator was determined through an electric test. The reactor and the controller system worked in optimal condition. The heating rate of the oven (without biomass) was 40°C/min, this indicate that the operational temperature could be reached in a short time (500°C). The feed systems was tested with microalgae powder (1-1,4 mm diameter), reaching a maximun of 0.06 kg microalgae/h.

Figure 1. Photography of pyrolysis reactor

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MICROALGAE BIOREFINERY THROUGH PROTEIN EXTRACTION AND PYROLYSIS PROCESS

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Microalgae biomass is well known as a raw material for biofuel production. However, the harvesting and downstream processes have several drawbacks that difficult the implementation in the industry. In the case of biodiesel production, it needs several steps such as cell wall disruption, lipid extraction, products separation and biodiesel production among others. All these steps require high amount of energy, therefore energy balance is negative. As microalgae is composed mainly by protein, carbohydrates and less extent by lipids, is a good candidate for biorefinery process, in order to obtain high value products. In this sense, a new concept of microalgae biorefinery was proposed. This process consisted in the extraction of protein as a first step followed by a fast pyrolysis of deproteinized biomass. The proteins extracted were submitted to a hydrolysis process to produce a biofertilizer. From fast pyrolysis process was obtained bio-oil, biochar and syngas. The protein extraction reached a yield of 66% with a degree of hydrolysis of 2%. After, the deproteinized biomass was pyrolyzed, reaching a bio-oil with a less content in nitrogen compounds compared to the biomass without protein extraction. Additionally, chromatographic analysis indicates that the bio-oil obtained was fewer complexes that both bio-oil produced with whole microalgae biomass and lignocellulosic bio-oil. These indicate that the bio-oil produced under the new biorefinery concept proposed could be a good raw material to obtaining high value chemical due to its simple composition. Additionally, this work showed that microalgae biomass is a good raw material to obtaining several products with industrial interest.

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SCREENING OF ALTERNATIVE RAW MATERIALS SUITABLE TO BE USED AS ENERGETIC RESOURCE AFTER TORREFACTION PROCESS IN CHILEAN SOUTHERN CITIES

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Nowadays, there is a worldwide energy crisis that in Chile have been mostly related to people exigencies concerning to preserve the natural patrimonies. In this context, there is a complex problem related to stubble burning in southern cities, which have an important energy potential if an appropriate densification process is implement. Additionally, there are new alternatives raw materials that have become to be investigated with energy focus, such as microalgae biomass, olive kernel and so on, without satisfactory results at date. Along with the previous, there are necessities of solid biofuel that can to give an alternative to the southern cities in order to diminish the pollution for humid wood used as fuel. In spite of the advantages of the new alternatives raw materials, these have several drawbacks related to high moisture content, irregular shape and size, low bulk density, hydrophobicity, high transportation cost, and so on. An alternative to improve these drawbacks is the torrefaction technology. Torrefaction is a process to convert biomass feedstock into a homogeneous solid with significant higher energy density and calorific value. This pretreatment is a thermal process realized at low temperature range between 200 to 350°C and usually at inert atmosphere conditions. The aim of the present investigation is carried out a screening of alternative feedstock produced locally to propose suitable biomass to be used in residential combustion system after torrefaction process. Ten feedstock have been used in the study: vetch straw, hazelnut shell, oat straw, cherry solid waste, waste corn cobs, olive solid waste, grass, wheat straw, grape stalk and the microalgae *Nannochloropsis gaditana*. The biomass were characterized according to proximate analysis using a Thermogravimetric analyzer (TGA-STA DSC 6000 from Perkin Elmer) with the modified methodology from García et al. [1] to determinate moisture, volatile, fixed carbon and ash. Additionally, elemental analysis was carried out using an Elemental Eurovector EA 3000. Calorific value and inorganic content also have been analyzed. In spite of was expected to find high moisture content in the microalgal biomass, this had been previously centrifuged, therefore had only 47.9% of water content. Then, higher moisture content was found in waste corn cobs (85.2%) and grape stalk (56.4%). Related to calorific value, the high heating values obtained in MJ/Kg were: cherry solid waste 27.1 > *N. gaditana* 24.7 > olive solid waste 24.6 > hazelnut shell 24.3 > waste corn cobs 23.6 > grape stalk 21.3 > wheat straw 16.9 > vetch straw 16.3 > oat straw 15.9 > grass 15.9. Related to nitrogen content *N. gaditana* (8.4%), the grass (3.7%) and the waste corn cobs showed the higher content with 8.4, 3.7 and 2.0%, respectively. The results obtained at date will allow selecting potential biomass to be used in a torrefaction process such as cherry solid waste and olive solid waste.

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INFLUENCE OF THE CONDENSATION TEMPERATURE ON THE QUALITY AND QUANTITY OF FAST PYROLYSIS CONDENSATES

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The last decade has seen tremendous advances in the development of environmentally-friendly processes for the production of organic compounds derived from biomass as a replacement of fossil-based chemicals. Lignocellulosic materials can be efficiently and selectively converted into liquid through fast pyrolysis [1]. This bio-oil as a whole, consisting of all the condensed liquid fractions contains hundreds of oxygenated compounds and hydrocarbons [1]. As opposed to a one-stage condensation, a staged-fractional condensation of the pyrolysis volatiles through a gradual reduction of the condensation temperature can be used. This method aimed at increasing the quality of specific bio-oil fractions as a source of chemicals [2]. For example, Pollard et al. described a five stage condensing system consisting of a series of intensive coolers and electrostatic precipitators to separate individual substances (like acetic acid or phenols) [3].
On-going discussions on the nature of bio-oil led many researchers to investigate the state of bio-oil. Recent experimental evidences confirmed the co-occurrence of two main transport states for the pyrolysis volatiles, vapours and aerosols [4]. This result led to the development of more specific filtering (e.g., hot-gas filter, cyclone, ...) and condensation elements (e.g., jacketed condensers and electrostatic precipitator), whose efficiency seems to depend on the state and chemical composition of the transport phases, as well as on the operating conditions of the fast pyrolysis process. Therefore a more detailed investigation of the condensation process is necessary.
In this study, the condensation behavior of selected substances transported through vapours and/or aerosols was investigated as a function of the condensing temperature (between -13°C to 80°C). Pyrolysis volatiles are generated using a bubbling fluidized bed reactor with a nominal feedstock throughput of 100 g/h at UDT. Vapours and aerosols are respectively condensed via two cooled condensers and two electrostatic precipitators. Mass balances of fast pyrolysis runs at 540 °C using wheat straw (particle size of 0.3 – 0.6 mm) indicated that results were consistent with an average product distribution of 18 wt% of char, 52 wt% of total condensates and 30 wt% of losses and non-condensable gases determined by difference. In addition, a significant temperature dependence of the condensate yield was found. Both condensates yield and water content decreased with increasing condensation temperature. The results also indicate that the aerosols have a large share of the condensates (30 – 60%) and therefore play an important role in the condensation process. Furthermore the quantification of 15 individual main substances present in the condensates (i.e., vapours and aerosols) obtained at different condensation temperatures are being considered.
These results should contribute to a better understanding of the condensation process. An on-going and challenging study aiming at developing an improved multi-condensation model including the vapour and aerosol condensation will allow the optimization of staged- condensation method to isolate individual substances.

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THERMOECONOMIC ANALYSIS OF THREE BIOMASS UPGRADING PROCESSES INTEGRATED WITH A MUNICIPAL CHP PLANT

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Abstract:
The objective of this work is to assess the thermoeconomics of three biomass upgrading processes. The production processes under scrutiny are those for wood pellets, torrefied wood pellets and pyrolysis slurry (a mixture of pyrolysis char and oil). By process simulation, all processes were integrated with a communal combined heat and power plant. The work is based on work published earlier [1] and those results form the input for the calculation of the systems´exergy flows. The economic data for process assessement is extracted from the open literature and has been combined with the exergy data following the specific exergy costing approach in order to perform the thermoeconomic analysis. Highest exergy destruction rates were found for the combustion equipment. Contrarily, the upgrading processes appear highly efficient. The integration of pyrolysis slurry, torrefied wood pellets and wood pellets production increases the exergetic efficiency of the systems by 22%, 26% and 31%, respectively. This makes wood pellets the most efficient integration option. However, from an economic viewpoint, the integration of pyrolysis slurry production performs best under the projected price scenario. In addition it is also shown that pyrolysis slurry production also reacts more moderately on possible price fluctuations. Considering the future potential of pyrolysis products for transport fuel production and the commonly expected further price increase for fossil oil, pyrolysis slurry production constitutes as the best option to be integrated with the communal combined heat and power plant.

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CARBONIZATION OF BIOMASS AS A SUBSTITUTE OF COAL FOR THERMOELECTRIC POWER GENERATION – HTC

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The partial replacement of coal by biomass, through co-firing, is one of the simplest alternatives to implement in the short term, to introduce this renewable source of energy to the national electricity-generation system. Many technologies have been proposed to overcome the difficulties involving the incorporation of biomass in coal thermal power plants, within which, the pretreatment of hydrothermal carbonization stands out for its simplicity and innovation. Hydrothermal carbonization allows transforming biomass into a carbonaceous solid similar to sub-bituminous coal, through its decomposition in aqueous medium at moderate temperatures (180 - 300°C), obtaining mass yields greater than 50% and energy yields over 70%[1]. The reaction medium used has the advantage to use wet biomass waste, usually of little commercial value (for example, sludge from treatment plants, manure, etc.).
The University of Concepción, through its Technological Development Unit (UDT), carried out an investigation to assess this technology and its application in co-combustion by three biomass residues generated in the Region of Bio Bio: sawdust of pine, urban organic waste and sludge from water treatment plants. These raw materials were subjected to temperatures between 180 - 300° C and reaction times between 0.5 to 8h, generating an increase in the biomass calorific values between 40% and 50%, and energy yields of up to 80%. A greater repellency to water of the carbonaceous products is observed and a decrease in the content of inorganic matter, the latter due to the solubilization of alkaline compounds in water. However, in some of the cases studied was obtained significant increases in ash content, which can give rise to problems in the power generation. Co-firing assays with up to 50% of coal replaced by hydrothermally-carbonized biomass show decreases of more than 10% and 30% in the respective emissions of NOx and SO2, in comparison to coal combustion.

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REINFORCED NATURAL ADHESIVES USING CELLULOSE NANOFIBER.

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Manufacturing of reconstituted wood panels is one of the most important industry in Chile; significant production of PB (623,000 m³ / year), MDF (1,008,000 m³ / year), OSB (272,000 m³ / year), and plywood (1.403 million m³ / year) are key issues [1]. They are composites that keep many of the desired characteristics of wood (low density, insulation properties and beauty, among others), and overcome undesirable aspects (*e.g.* low homogeneity and geometric constraints). Regarding the mechanical properties of such composites two alternatives are proposed: 1) to expand the use of reconstituted wood panels in highest value applications or smaller thicknesses boards for conventional uses, 2) to reduce the consumption of resins and adhesives maintaining the mechanical performance. Resins and/or adhesives content represent near to one third of the production costs of reconstituted wood panels, so that a decrease of adhesive consumption has a strong impact on the total production cost. The present work shows the results obtained in the evaluation of tannin-based adhesives (TBA) reinforced with cellulose nanofiber (NFC) (Fig. 1) using Automated Bonding Evaluation System (ABES) Test. Preliminary results show that NFC increases the resistance strength of the adhesion bond parallel that the TBA content is decreased. NFC can be reduce adhesive consumption up to 20% according to ABES testing (Fig. 2).

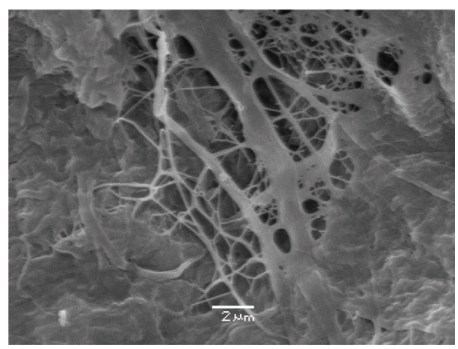

Fig. 1. SEM image of CNF obtained in UDT.

TBA content, %	20	18	18	16	16
NFC, %	0	0	1	0	1

Fig. 2. Comparative ABES analysis of tannin based adhesives TBA reinforced with CNF.

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Acknowledgment:

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CATALYST FOR NATURAL POLYPHENOLIC-BASED ADHESIVES USING FOR PLYWOOD AND MDF MANUFACTURE

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Adhesion phenomena are involved in several industrial processes but in forest industry, have an special focus based on manufacturing of wood-based panels as; oriented strain board (OSB), particle board (PB), medium density particleboard (MDP), medium density fiberboard (MDF), high pressure laminates (HPL) and plywood, as well as, in the surfacing of wood-based panels with wood veneer, melamine and phenolic impregnated paper or high pressure laminate. The present work shows the results obtained in tannin-based adhesives using a new additive for plywood and MDF manufacture. The evaluation of adhesives performance was carried out using automated bonding evaluation system (ABES) and board manufacturing in a pilot plant (MDF (15 mm) and plywood (five ply15 mm)). ABES is a powerful tool using for adhesives performance evaluation. [1]. The adhesion and mechanical properties of plywood; dry tensile strength, wood failure and modulus of elasticity values were determined according to EN 314 and EN 310, respectively. For MDF density, internal bond (I.B.), and swelling in thickness according to EN 323, EN 319 and EN 317, were determine respectively [2]. The results obtained for ABES evaluation show the behavior of tannin-, and activate tannin- based adhesives using hexamine (Figure 1).

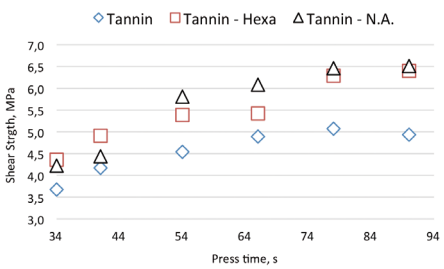

Fig.1 – ABES evaluation for different tannin-based adhesives with out and activated with hexamine and the new additive.

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Acnowledgement:

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SIZE EXCLUSION CHROMATOGRAPHY (SEC) AND THERMAL ANALYSIS (DSC) FOR NATURAL POLYPHENOLIC MATERIALS FROM FRESH AND AGED SOURCES.

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The present work shows results regarding the evaluation of different polyphenolic materials using Size exclusion chromatography (SEC) and Differential scanning calorimetry (DSC) in liquid and solid state respectively, by studying the effects of storage time, aging time, transitional temperatures, and relative humidity on the polyphenolic materials degradation. Samples were subjected to accelerated ageing under action of heat and moisture by increasing time lengths. The studies have proved that accelerated ageing causes a progressive decrease of the decomposition temperature, curing behavior and molecular weight distribution. The results can be interpreted as a partial reticulation under accelerated ageing, and change in composition . SEC results enables support DSC thermogram interpretation. Such studies can be useful for understanding the processes that take place on natural ageing of historical source of polyphenolic materials, as well as for establishing proper conditions of storage (Fig. 1).

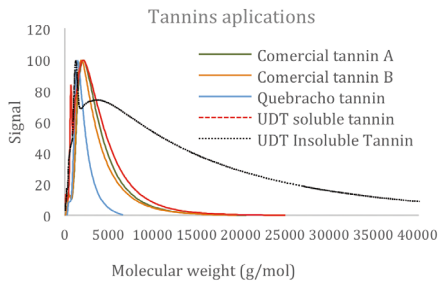

Fig.1 – Molecular weight distribution of different natural polyphenols without accelerated aged treatment.

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Acnowledgement:

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IMPROVED FLEXIBILITY OF POLYLACTIC ACID (PLA) BY TANNIN-BASED MELT-BLENDING

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The most important natural polymers in bioplastic engineering are aliphatic polyesters. Among others, polylactic acid (PLA) is an important macromolecule with similar physicochemical properties than petroleum-based polymers. However, PLA exhibit several limitations in polymer formulation such as low thermal stability and impact resistance [1]. The cited drawback limits plastic deformation at higher stress level. In order to tailor physicochemical and mechanical properties PLA was melt-blended with Pinus radiata modified and unmodified bark tannin (non-water soluble fraction). Polyethylene glycol (PEG-600) was used as conventional plastizicer. Rheological, morphological, structural, thermal, and mechanical properties were studied. The distribution of components was determined by fluorescence emission microscopy. Binary and ternary blends showed a good distribution of components on the PLA matrix (Fig. 1). PLA-melting temperature, thermal resistance and mechanical properties were affected by blend-composition. Tannin improved the blend processability in term of short-mixing, and induced PLA-crystallization. In addition, modified tannin enhanced the PLA-nucleation during cooling. A synergic effect between tannin and PEG-600 in PLA-crystallization behavior was observed. Tannin can be used successfully as nucleating agent in PLA-based thermoplastic systems.

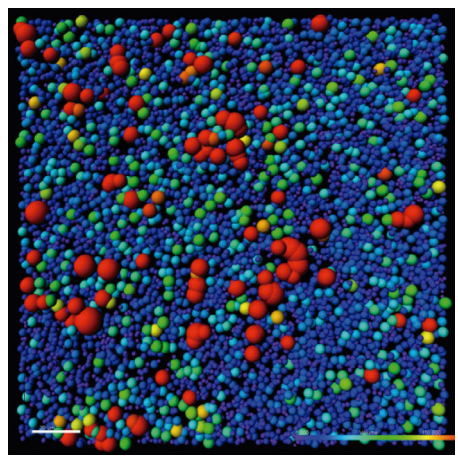

Fig. 1. Particle-size distribution of a ternary blend based on PLA/tannin/PEG. (Fluorescence emission microscopy image).

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Acknowledgments:

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A NOVEL POLYLACTIC ACID-DERIVED MATERIAL PREPARED BY TANNIN-BASED URETHANIZATION

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Biodegradable plastics from renewable resources promises an alternative solution for synthetic polymer disposal. Bioplastics such as polylactic acid (PLA), polycaprolactone (PCL), polyethylene oxide (PEO), poly(3-hydroxybutyrate) (PHB), and polyglycolic acid (PGA) are the most promising. Among alternatives PLA-derived materials have been gain interest [1]. On the other hand, tannin derivatives from bark of pine species are new macro building-block for polyurethane-based thermosetting and thermoplastic systems [2]. This work deals about characterization of novel materials obtained via co-polymerization of PLA (thermoplastic matrix), tannin (polyphenolic building-blocks), and PEG-600 (plasticizer) with MDI (diisocyanate) during melt-blending. Materials were characterized by rheological, morphological, structural, thermal, microscopic, and mechanical analysis. The new type of PLA-based material is expected to play an important role beyond the traditional food packaging applications, such as hot-melt adhesives, UV-filter for thermoplastics, antimicrobial block co-polymer, as well as high chemical resistance materials.

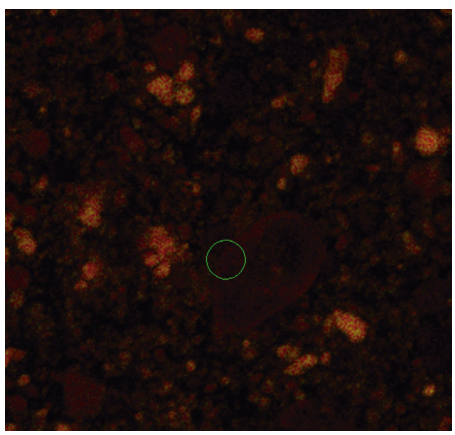

Fig. 1. Fluorescence emission pattern of a novel PLA-based material illustrating the urethane domains (spots in purple).

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Acknowledgments:

This work was supported by the Basal Proyect PFB27, UDT (2014).

Pinus radiata SEED OIL A NOVEL PLASTICIZER FOR POLY(LACTIC) ACID-BASED BIOMATERIALS

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The use of natural compounds as functional agent for conventional plastics is an important issue today [1]. Specifically, the combination of plasticizing agents onto thermoplastic polymers are a convenient strategy in order to tailor selected properties. However, most current plasticizers are fossil-based chemicals [2]. In order to study the effect of renewable sources as alternative plasticizer an experiment was carried out. *Pinus radiata* seed oil (ASP) was used as plasticizing phytochemical of poly-(lactic) acid (PLA) at different loads (2 to 8 wt. %). In addition, other oily-liquids such as poly-ethyleneglycol-600, silicone-1000 oil, glycerol, triethyl acetate citrate, and pyrolytic oil from PP and LDPE polymers were utilized in order to reference the ASP´s behaviour. Binary blends were obtained for melt-blending in a torque rheometer at 170 °C. Samples were characterized by rheology, melt-flow index (MFI), FT-IR, thermal analysis (DSC and TGA), and mechanical properties. The results showed that the ASP exhibits similar properties than suitable PLA´s plasticizer. The advantages in terms of processability, thermal stability, and mechanical properties of blends foresees ASP's applications in materials engineering, particularly those requiring plastic deformation at high stress level.

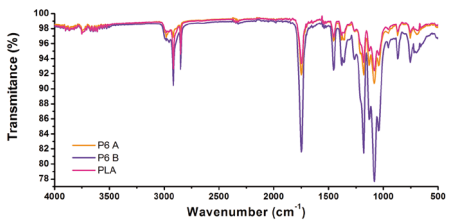

Fig. 1. FT-IR spectrum of neat PLA and PLA/ASP blends.

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Acknowledgments:

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HYDROXYPROPYLATION AT ROOM TEMPERATURE OF CONDENSED TANNIN OBTAINED UNDER PILOT-PLANT SCALE

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Condensed tannins (CTs) are polyphenolic compounds widely distributed in the plant kingdom. Beyond traditional uses CTs have been utilized in leather industry, traditional medicine, and as adhesives component [1]. Despite current applications CTs have been undervalorized as a building-block co-polymer [2]. In order to diversify properties in view of envisaged applications CTs from *Pinus radiata*, mimosa (*Acacia mearnsii*), and Quebracho (*Schinopsis lorenzii*) were modified using propylene oxide. The hydroxypropylation to three different degrees of substitution (DS) produced polyphenolic derivatives in a reasonable yield (60-90%). The isolated derivatives were characterized by spectroscopic methods (FT-IR, UV-vis, ^{1}H-NMR) and by molecular and physical characteristics (solubility, molecular weight (GPC), and thermal behaviour -DSC, TGA-). The DS determination allowed stablishing a wide range of reaction efficiency associated to the chemical structure. Chemical modification affect the solubility pattern and the thermal behavior. Hydroxypropyl tannin derivatives mainly for radiata pine are promising biomacromolecular building-blocks for tannin-derived materials design.

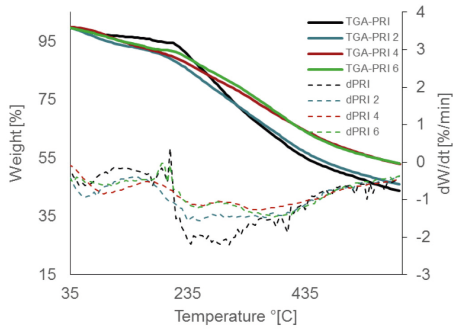

Fig. 1. Thermogram of *P. radiata* bark tannin (non-water soluble fraction) and tannin-derivatives at 5 °Cmin^{-1} in N_2 atmosphere.

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CHEMO-ENZYMATIC EPOXIDATION OF ORGANOSOLV LIGNIN WITH LIPASE B

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Lignin modification holds the potential to yield highly functionalized, monomeric, oligomeric or polymeric products that can be useful as starting pre-polymers for different processes in the chemical or biotechnology industries. Epoxy resins are the one of the most important classes of thermosetting resins, and are widely used in fiber-reinforced materials, additive for paints, adhesives and surface coating and other applications. As a group, epoxides are highly reactive, versatile, polyfunctional chemical intermediates used in the production of chiral compounds. Most of these thermosets are industrially manufactured from Bisphenol A (BPA), a compound that was initially synthesized as a chemical estrogen. BPA is one of the highest volume chemicals. A novel chemo-enzymatic treatment for functionalizing oxirane rings using lignin is proposed for the synthesis of epoxy resin adhesive. *Candida antarctica* lipase B (Novozyme 435; CalB) has demonstrated that it is capable of catalyzing the formation of peroxyacids from fatty acids in the presence of hydrogen peroxide. The peroxyacid generated can react with unsaturated vegetable oils to form oxirane rings via epoxidation of the carbon-carbon double bonds in the molecule. The chemo-enzymatic epoxidation of organosolv lignins from *Eucalyptus globulus* and commercial vanillic acid with CalB/hydrogen peroxide in the presence of caprylic acid was investigated. The lignin fractions were characterized through ^{31}P NMR and GPC to determine the quantity of phenolic-OH group and the molecular weight, respectively. The system for chemo-enzymatic functionalization to generate oxirane rings was successfully applied to the phenolic compounds. The oxirane ring content of the all experiments exhibited a maximum at an intermediate reaction time, followed by a decline. The results of the lignin fractions system suggested that a polymerization mechanism may be occurring during epoxidation, and dimerization in the system using vanillic acid. Vanillic acid epoxide content only reached approximately 50% of theoretical before dimerization occurred, while for the lignin samples, epoxidation was nearly 100% of theoretical prior to the onset of crosslinking. The functionalization of lignin has potential applications in the production of epoxy adhesive resins such as other biomaterials of added value, and chemo-enzymatic synthesis represents a greener pathway to the synthesis of epoxide structures.

INFLUENCE OF BIOCHAR CHARACTERISTICS UPON THE PHOTODEGRADATION OF METHYLENE BLUE UNDER UV-VISIBLE IRRADIATION

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One of the most important challenge of the XXI sustainable society is to develop efficient methodologies to solve the problem of the polluted water. Up to day, more than a million children dead per year by insufficient conditions of water [1]. In this sense, the photochemical activity of different nanoporous biochars prepared from the sawdust of a soft wood (*Apamate, tabebuia pentaphylla*) was studied under UV-Vis irradiation conditions for the degradation of methylene blue (MB) as a common pollutant form textile industry [2]. Biochars were prepared by physical activation under CO_2 flow (800ºC, 1h), by pyrolysis under N_2 flow (1000ºC, 1h), and by chemical activation after impregnation with $ZnCl_2$ and H_3PO_4 (5% wt%.) following carbonization under N_2 flow (450ºC, 1h). The kinetics of adsorption in the dark of MB following its photodegradation were studied on the biochars. TiO_2 P-25 was used as commercial photocatalyst for comparative purposes and to verify synergistic effects between both solids. The kinetics of MB photodegradation was performed under UV-Visible irradiation to verify the scaling-up to real solar irradiation conditions of Biochar-TiO_2 binary materials. It was verified that biochars were intrinsically photoactive, and a synergy effect between both solids was detected and estimated from the pseudo first-order apparent rate constants in the photodegradation of MB. This effect enhances the photoactivity of TiO_2 up to a factor about 9 under visible irradiation and it was associated to the surface properties of carbons mainly by the formation of photochemically oxygen-containing reactive species. It can be concluded that heteroatoms-doped biochars are photochemically active under UV-Visible irradiation.

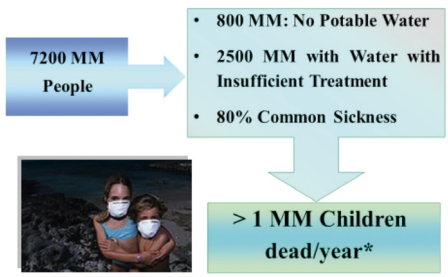

Figure 1. *Polluted Water: the first problem to be solved for the sustainable society.

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NANOSTRUCTURED HYBRID C-TiO_2 PHOTOCATALYSTS FOR THE PHENOL PHOTODEGRADATION

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An important quantity of the wide world production of pharmaceutical industry use phenol derivatives in any manner during the reaction-steps. Unfortunately, the associated waste water of these process became in an important environmental hazard. Different technologies exist for the removal of these type of organic molecules but photocatalysis and adsorption with biochar-based photocatalysts have received an increase attention. Our group has showed [1,2] that functionalization of biochars surface plays an important role upon the photoactivity of TiO_2. In this work, different biomass-derived molecules such as furfural, chitosan and saccharose have been used to prepare hierarchically nanostructured and mesoporous hybrid materials C-TiO_2 by solvothermal synthesis. The kinetic studies of adsorption in the dark and photodegradation of phenol under UV-visible irradiation have been performed and results compared against that obtained on a commercial standard semiconductor. The influence of the origin of carbon was verified by using furfural, chitosan and saccharose as biomass precursors. Characterization was performed by N_2 adsorption isotherms, FTIR, XRD, DR/UV-vis and SEM. XRD patterns showed that carbon precursor affects not only textural parameters such as surface area and pore size distribution but also clearly affects the crystalline framework of TiO_2. In spite of the photocatalytic activity of C-TiO_2 was slightly lower than that of TiO_2-P25, the present results suggested that these materials can be potentially used as eco-friendly and low-cost photocatalysts.

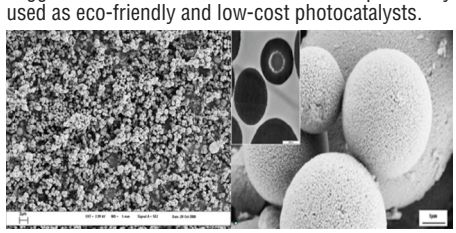

Figure 1. SEM images of Fu-TiO2-C. Inset showed the TEM image of a microtome sample.

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VARIABILITY OF NANOMETRIC POROSITY ON CELLULOSES PULPS

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The biomass derived from plants is an important source of renewable materials as the main product or as a residue of industrial processes. The cellulose present in biomass is interesting material with many applications, as biofuel production like second generation ethanol or new materials like nanocelluloses. Because of this there are many studies in literature describing extraction and conversion of the cellulose present in biomass looking to improve these processes and making them more competitive at industrial level. In this context the study of the variability of nanostructure of cellulose pulps is an important contribution to the conversion processes of cellulose. This work explores the nanostructure variability of celluloses looking at the accessibility of different sources of cellulose for chemical reagents and enzymes, by measuring the nanometric porosity. We select six types of cellulose sources, sugarcane rind and pith, eucalyptus and pine sawdust, sisal and coconut fibres. The hydrothermal treatment, alkaline (sodium hydroxide) and acetosolv (acetic acid) delignification were selected as process to create differences in nanometric porosity in these materials. The treatment conditions were 180°C for hydrothermal treatment, 160°C for alkaline and 110°C for acetosolv delignifications. The reaction times were 60 minutes for hydrothermal and alkaline delignification and 180 minutes for acetosolv delignification, with solid-to-liquid ratio of 1:10 for hydrothermal and alkaline delignification and 1:20 for acetosolv delignification. The bleaching of the pulps were performed by using a solution of 3% of sodium chlorine at 70°C and ratio solid-to-liquid of 1:3 for 180 minutes. The nanoscale porosities were determined by calorimetric thermoporometry [1]. Ours finding reveals the nanoscale porosity increased as a consequence of chemical treatment and the direct alkaline and acetosolv delignification produce the materials with the higher porosities. Thus, these processes are candidates to promote materials with more accessibility that can improve subsequent cellulose conversion processes. Another important result of this work relates to the nanometric porosity observed after the hydrothermal treatment of the different sources of cellulose. All the material submitted to hydrothermal treatment showed the lowest nanometric porosity, which is even lower than the observed for bleached raw material. These results indicate that, despite the advantage of hydrothermal treatments as they employ only water as reagent, the obtained treated materials have a drawback to present only a small gain in the improvement of nanoscale porosity.

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EXTRACTION AND CHARACTERIZATION OF XYLANS FROM BLEACHED *Eucalyptus globulus* KRAFT PULP FOR BIOMATERIALS APPLICATIONS

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There is a growing interest in the development of biorefineries which has promoted the use of biopolymers from wood as a renewable raw material to produce different types of chemicals, including those which are based on the use of polysaccharides such as the hemicelluloses fraction. Hemicelluloses are defined as a group of heteropolysaccharides consisting of xylose, mannose, arabinose, glucose, galactose, and residues of acetyl groups and methyl-D-glucuronic acids. Xylans are the major hemicellulose of hardwood species comprising between 20% to 35% by weight and its generally reported use is as additives in papermaking, films, thickeners, hydrogels, emulsifiers, adhesives, functional polymers, among others. However, the current use of xylans is very limited because of the challenges in the extraction process, recovery, and efficient conversion in to higher value products. In this work, xylans from bleached *Eucalyptus globulus* kraft pulps were isolated by alkaline extraction with 2% and 4% NaOH for 60 and 120 min at room temperature. The extracted material was then precipitated in successive washes with ethanol 95% and the recovery was accomplished by ultrafiltration and lyophilization procedures. Preliminary results showed that between 80% and 90% xylans present in the bleached pulps can be recovered. The recovered xylans were characterized by acid hydrolysis, acid methanolysis, FTIR and molecular weight distribution. With the obtained material, a chemical functionalization will be performed (sulfation) to give antimicrobial properties and evaluated as a additive for special paper production.

DEVELOPMENT OF BIODEGRADABLE COMPOSITES IN FOREST INDUSTRY

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The use of biodegradable polymers became a common topic in the last few years, attending the demand to replace petroleum-based polymers. Indeed, conventional synthetic polymers are not easily degraded due to their high molecular mass and hydrophobic character [1]; thus generating disposal problems and representing a significant source of environmental pollution [2]. The production of these new biomaterials will help to solve the increasing demand of these one-time use biopolymers required in various areas such as food packaging, manufacturing, biomedical, agricultural and lately in the forestry area [3]. Poly(lactic acid) (PLA) is a renewable biocompatible and biodegradable polymer, which is widely used as degradable bioplastic [4]. This thermoplastic polymer known for its excellent mechanical properties has been selected to develop new composites mechanically resistant but also degradable in shorter period than pure PLA. In this study, biodegradable composites combining degradable PLA polymer, a commercial resin and natural polymers (starch), were prepared to have similar mechanical properties of polymers required in forestry industry and also specifications allowing them to be processed by injection and extrusion. In addition to their good mechanical and rheological properties, these resulting composites displayed remarkable biodegradable characteristics (Fig.1) and no phytotoxicity effects. When compared to pure commercial biodegradable polymers (X and Y), the new biocomposite produced showed a better biodegradation ability, with a weight loss up to 44.7 wt.% after 90 days of incubation against -1.78 wt.% for the commercial biopolymer. The biodegradability of these new biocomposites was found to linearly depend on the mixture ratio between commercial biopolymers and starch (Fig.1).

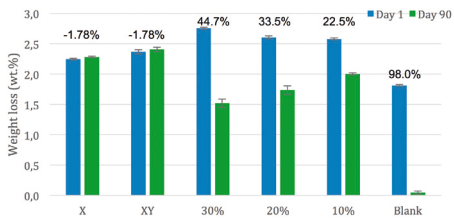

Fig.1 – Test of biodegradability

The plates were placed in compost optimal for biodegradation, the high presence of fungus and bacteria in soil promote the degradation of the organic matter. The new biocomposites have an addition of 30%, 20% and 10% from natural polymer respectively. The phytotoxicity test reveal an innocuous effect from the new biocomposites, analysis parameters as germination (98%), relative radicular grow (97%) and germination index (95.06%).

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NATURAL INSULATION MATERIALS BASED ON *Eucalyptus globulus* BARK FIBRES

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The second most important species of the Chilean forest industry is *Eucalyptus globulus* with an annual roundwood production (bark- free) of 11.5 Mio m3 per year. The bark of *Eucalyptus globulus* makes up about 15 % (2.5 Mio m3/ year) [3] of the volume of its wood and is only used for energy production or even stored in dump. Figure 1 draws the idea of a life cycle assessment of eucalyptus bark. Instead of no usage or low- value utilization, the bark can be processed and used as a building material. Towards the end of its life cycle, it is still possible to use it in incineration sites and after that even as fertilizer in the forest industry.
Bark material shows natural resistance towards biologic activities, is characteristically lightweight and due to its low- value utilization a cheap resource, which makes it a very interesting raw material for the production of thermal insulation boards. Due to its fibrous geometry, eucalyptus bark is easy to decompose. In turn, the fibres tend to mechanically crosslink with each other and facilitate the adhesion within a future insulation board.

A production process with dry stream incorporation [1] allows a fast production process of less than 5 min and an appropriate panel thickness for insulation issues. The mechanical crosslinking of the fibres allows a fabrication with a very low incorporation of resin, while natural resins can likely be used. The strong hydrophilic behaviour can be reduced by 20 % with the incorporation of a low percentage of commercially available paraffin or black liquor, a by- product of the cellulose industry. The weakness of the material is the low fire resistance, whereas the material glows slowly and in a predictable way.
The mandatory application of thermal insulation on buildings in Chile from 2016 onwards provides a big market for the new designed environmentally friendly product [2].
A cheap raw material, interesting, competitive product characteristics and a regional availability of eucalyptus bark together with a high market request promise prosperous specific results with regard of a future industrial use.

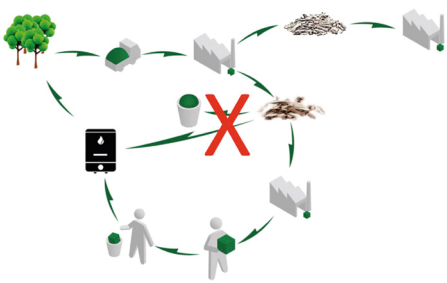

Fig.1 – Life Cycle Assessment (LCA) for eucalyptus bark insulation boards

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NATURAL POLYPHENOLIC FOAMS- AN INNOVATIVE LIGHTWEIGHT MATERIAL

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Mentioned in literature the first time in 1994 [5], polyphenolic foams based on natural polymers and additives were intensively investigated with regard at their polymerisation mechanism and material properties.
The present research was focused on an economisation of the process, using extractives of regional available plant material, as well as an industrial application of the product. The raw material was obtained by solid- liquid extraction of forestry and agricultural by- products like pine bark or wheat straw [1]. Polyphenolic- based rigid foams are produced with 100 % natural resources, using pine bark and mimosa tannin, as well as pine- bark and wheat straw lignin. Based on the chemical composition of the polymer material, presented by Link, et al. [4], the formulations were adapted to the new raw material and their foreseen field of application as insulation material.
The formation of the foams takes place in wooden moulds, where the polymer material achieves a force-fitting crosslinking with the decking layers parallel to the foaming process. Therefore, an integrated production of a structural element is achieved.
The materials underwent normative tests, evaluating their mechanical strength, hydrophobic properties and its behaviour towards fire and thermal conductivity. Polyphenolic- furanic foams show a high correlation of all characteristics towards its density [6], wherefore the formulations described where designed to a target density of 200 kg/m3. With the integration of lignin in the tannin- furanic polymer, the mechanical resistance can be increased significantly in comparison to foams with a similar production process under the influence of an external energy source [3]. Furthermore, the incorporation of lignin decreases the extremely hydrophilic behaviour of the polymer and achieves a further increase of the dimensional stability. Foams based on pine tannin presents a water uptake of more than 350 weight- % within 2 h with dimensional changes of less than 5 %, whereas the incorporation of lignin takes up about 100 weight- % of water with a swelling factor of less than 1 %.
Independent of the usage of different raw materials and production processes, the resistance towards fire is at a similar level like presented by Celzard, et al. [2], describing tannin foams as outstandingly high fire retardants. With regard to its designed area of application, the most important characteristic is the thermal conductivity. Figure 1 presents the increase of temperature (starting temperature app. 24 °C) after 5 min on the opposite side of a heat source of panels with a diameter of 2.5 cm. The thermal heat flow of an empty production mould (left), a reference sample a mould filled with polyurethane (centre) and a tannin- based rigid foam is presented, showing significate elevation of temperature with the empty mould ,whereas the samples of the foams keep nearly unchanged.

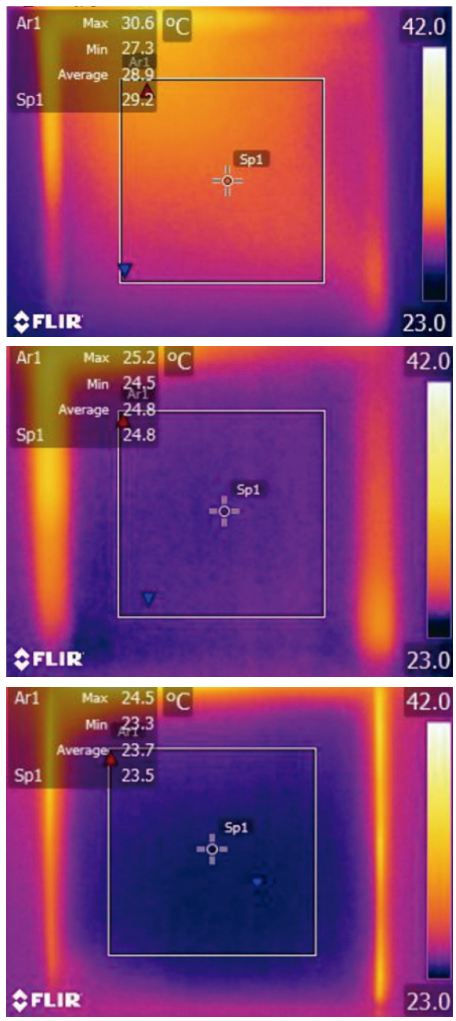

Fig.1 – Heat flow analysis of empty mould, polyurethane foam and a tannin foam (from up to down)

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PHYSICAL PROPERTIES OF BAMBOO-PLASTIC COMPOSITES

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In the present study, the water absorption and colorimetry of bamboo-plastic composites (BPCs) have been investigated. BPCs are made of particulate bamboo (PB[2]) with three different kinds of particulate (-30/+40, -50/+60, -60/+80) and polypropylene[3], and the same mix ratio (PB 30/ PP 70). BPCs were produced by compression molding. The water absorption of the composites was determined according to the American Society for Testing and Materials ASTM D 570-98 (2010) and the colorimetry by the Technical Association of the Pulp and Paper Industry TAPPI 527 om-02.
The water absorption of the three bamboo-plastic composites is shown in Fig.1a. It is observed that the specimen with particles -30/+40 (blue line) presents the highest water absorption of the cases. Meanwhile, the resulting tendency lines for the specimens with particles -50/+60 y -60/+80 are fairly similar. Wolcott and Englund points that adding wood to a thermoplastic matrix will inevitably cause an increase in moisture uptake [1]. As a result, it might be said that the particle size has an inverse relationship with density and water absorption: smaller particle size, higher composite density and absorption.
Fig.1b shows the color variation of BPCs expressed by the *L** value; no significant statistical differences between all composites are observed. Similar results were obtained in each composite, therefore the *L** value is not significantly affected by the particulate size. These specimens have not yet been subjected to durability and exposure processes.

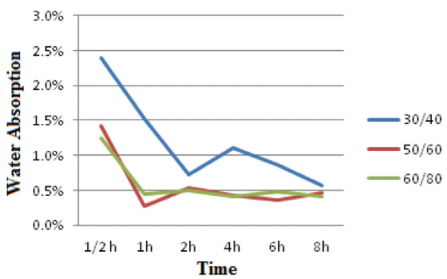

Fig.1a. – Water absorption by time period

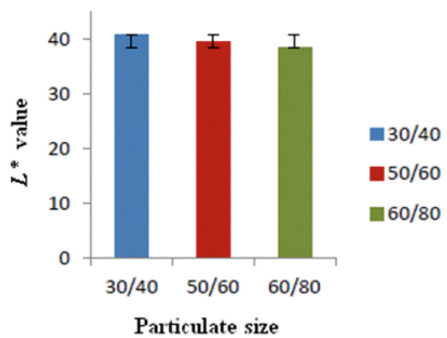

Fig.1b. – *L** Values by particulate size

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RECYCLED LIGNOCELLULOSIC MATERIALS FOR OBTAINING MOLDED PRODUCTS. PART I: EVALUATION OF PHYSICAL AND MECHANICAL PROPERTIES

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This paper presents a study of the evaluation of physical and mechanical properties of recycled lignocellulosic materials with the ultimate objective of their use for the production of molded pulps. It is desired select the components that optimize the variables analyzed to use in the production of products moldings suitable for plants growing.
These products, flower pots, allow replace the plastics materials, which are contaminants and hardly biodegradable. Their function is to contain the substrate and provide physical support to the plant during their stay in the nursery; enable mechanized production operations, and maintain adequate levels of moisture and aeration of the substrate, so they must have good strength properties to maintain the structural integrity and good permeability to control the temperature, the contained moisture and aeration of the set pot-substrate.
For the development of the research, were used, two types of recycled lignocellulosic materials: Newspaper, office paper and old corrugated containers pulps and pine sawdust.
The pulping were performed at medium consistency, and later cleaned. The sawdust was fractionated by manual screening and for experiences, was selected the fraction: "Pass-Retained 40-60 mesh". As of an experimental design type mixture of extreme vertices, were prepared, standard laboratory sheets weighing 150 g / m^2. The pulp content is between 0 and 100%, and the content of sawdust between 0 and 40% by weight. Were evaluated, physical-mechanical properties (density, bulk, tensile, tear, burst, stiffness, compression ring and porosity), in accordance with TAPPI standards.
It was observed that, with an increase in the percentage of office pulps, it is obtained, higher density value lower specific volume and consequently, higher value of porosity, and higher value of tensile, burst, and compression; with an increase in the percentage of old corrugated containers pulps are obtained, higher tear resistance properties, and, newspaper pulps higher stiffness properties. In all cases, an increase in the content of sawdust decreases resistance properties corresponding to a maximum of 20% in weight percentage of the possible use.
Therefore it is concluded that, in the design of molded products, a higher content of pulps office papers improves most properties of strength and porosity, except tear and stiffness that is achieved to improve with the use of pulps old corrugated containers and pulps newspapers. As to the sawdust, it is possible to incorporate up to 20% of the mix of different pulps; higher percentages cause a marked decrease in the properties. The second part of this study, are used, mixtures that benefit, the development of compressive strength and stiffness of molded pulp. The results will form the basis for the reuse of other lignocellulosic materials from the farm in the production of products molded.

FEEDSTOCK FLEXIBLE SUPPLY APPROACH AS AN ALTERNATIVE FOR OPERATING MEDIUM-LARGE LIGNOCELLULOSIC BIOREFINERIES IN SOUTHERN EUROPE

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Lignocellulosic biomass is abundant and renewable resource that can be used such as feedstock within the biorefinery framework for the production of biofuels, chemicals and value-added products. In order to meet the requirement of biomass supply for the successful implementation of a commercial scale biorefinery in Southern Europe, the use of feedstock mixtures was proposed in this work as an alternative to the use of individual feedstock. Eucalyptus residues (ER), wheat straw (WS) and olive tree pruning (OP) were chosen as representative feedstock from this region due availability and the balancing seasonality. This further potentiates their use in a multi-feedstock biorefinery under continuous operation throughout the year. Moreover, the high polysaccharides content of these lignocellulosic materials, constitute potential source of sugars that can be used as substrates for bioconversions. In this framework, lignocellulosic biomass fractionation processes are an absolute requirement. The hydrothermal treatment is an efficient process for the selective hemicellulose solubilization of these materials, leaving the cellulose and lignin fractions mostly intact in the solid phase.
In this work, ER, WS and OP were subjected at hydrothermal treatment (autohydrolysis) separately and in three different combinations among them. The study of the pretreatment of each individual feedstock allowed the understanding of the specific characteristics of each one and their impact in the different mixtures composition and processing. In each mixture an individual feedstock was set at 50% of the total weight and the other two contributed with 25% each (50ER, 50WS and 50OP). The hydrothermal pre-treatments were performed under non-isothermal conditions, at different temperature ranges (195-230ºC for individual feedstock and 150-230ºC for the feedstock mixtures), using a liquid-to-solid ratio of 7 (w/w). The liquors obtained from autohydroysis were characterized in terms of oligosaccharides, monosaccharides, and degradation products, as well as solid residues in terms of polysaccharides and lignin content.
The results showed that the different feedstock mixtures (50ER, 50WS and 50OP) generated very similar pretreated materials (liquid and solid fractions), although the content of each fraction slightly varied according to the contribution of the predominant feedstock. A range of pretreatment temperatures (205 220ºC) was identified as providing high saccharides recovery from the three different feedstock mixtures, but at 210ºC a maximal was found, which coincides with the optimal temperature for saccharide recovery from individual feedstocks in the liquid fraction. Both fractions recovered after autohydrolysis, liquid and solid, can be applied in several bioconversion processes with the advantage of having more similar composition than the simple use of different materials.

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MANUFACTURING AND CHARACTERIZATION OF NANOFIBERS OF CARBOXYMETHYL CELLULOSE AT DIFFERENT DEGREE OF SUBSTITUTION: RHEOLOGICAL MODIFIER ADDITIVE

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The Nanofibers of Cellulose (NFC) are basic structures on the wall of plant cells. Morphologically, these fibers are filaments composed by a crystalline and amorphous region of cellulose with a diameter from 5 to 30 nm, and a length of several microns. Turbak *et al.* [1] and Herrick *et al.* [2] achieved the isolation of this fibers through mechanics treatments on fibers of cellulose. Nowadays, scientific research is still needed to develop their production, and potential application to be the material of tomorrow. Its large specific surface allows them to interact intimately in the liquid phase where is located, and to be at the same time a much more viscous solution. Therefore, the nanofiber of cellulose have been proposed as rheological modifiers in several applications, such as foodstuffs, cosmetics, paints, among others [3]. However, the factors to define the characteristics of the NFC as potential rheological modifiers still need to be developed. This fact is due the lack of research, and therefore it make necessary new ways to explore this area of interest. This study evaluated the potential use of the carboxymethylated NFC as rheological modifiers agents. The effect of the change on the degree of substitution (DS) of this NFC has been studied in the disruption of the fiber in the homogenization process, and also in the rheological properties of their suspensions.

The manufacturing of carboxymethylated NFC was carried out in three stages: (i) Refinement of *Eucalyptus sp.* bleach kraft pulp at 10% (w/w) of consistency, and 10.000 RPM, (ii) Chemical modifications of the fibers at different DS, using $ClCH_2COOH$ and NaOH in an organic phase, and (iii) Defibrillation of the modified fibers through a homogenization in aqueous phase a high pressure (600 bar). The NFC are characterized by their thickening performance, morphology and energy consumption on the defibrillation stage. The results were compared with commercial carboxymethyl cellulose (CMC) at high and low viscosity, and also with NFC free of chemical modification.

Figure 1 has shown that all sampled suspensions of the carboxymethylated NFC revealed a higher rheological response than the solutions of commercial CMC. The increasing in the DS promotes a more efficient process of disruption on the fibers (Figure 2); therefore, the energy consumption was reduced in a 96.7% regarding the NFC production free of chemical treatment. In conclusion, the homogenization process of pulps is facilitated at a high DS, and the products have presented better morphological characteristics. Besides, these characteristics also promote the potential as rheological modifiers additive on the carboxymethylated NFC.

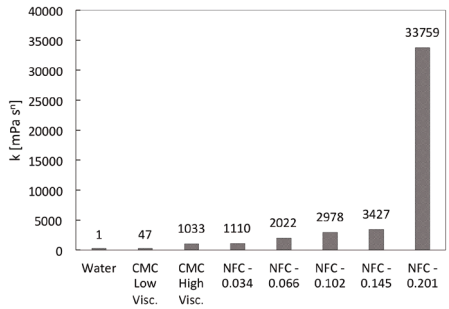

Figure 1: Consistency Index, k, between CMC and carboxymethylated NFC at 2 % p/p and 1 pass throughout the homogenizer.

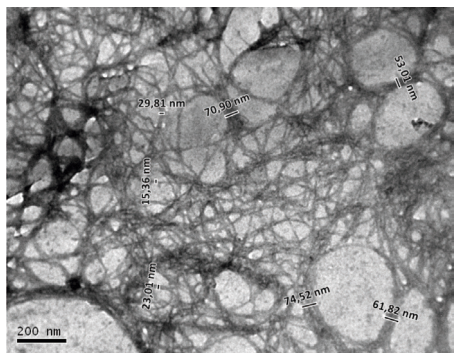

Figure 2: TEM image of NFC with carboximethylation modification.

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PRODUCTION OF NANOFIBRILLATED CELLULOSE (NFC) FROM THE FINE FRACTION OF RECYCLED PAPER

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Nanofibrillated cellulose (NFC) is an amazing nanomaterial obtained through mechanical disintegration of cellulose fibers. It consists in a bundle of elementary fibers, with a diameter less than 100nm and some micrometers of length. In recent years, this material has been attracted the attention of many researchers because its high aspect ratio, specific superficial area, crystallinity and mechanical properties [1]. However, efficient production of NFC is still challenging with respect to energy consumption. A typical process demands 27000 kWh/ton [2]. Although some pre-treatments (chemical and enzymatic) allow to decrease the energy consumption, which means an increase in the cost of reagents, and adding the pulp cost the process is still not feasible.

Recycled pulp is an interesting alternative for NFC production. Because of the recycling process the fibers are damaged, with a consequent buildup of fines [3]. Even though this is a problem for the paper recycling it could be an advantage for the production of nanofibrillated cellulose. A weaker structure will be easier to delaminate, compared to virgin fibers. In addition, recycled pulp has a low cost and high availability.

The aim of this work is use the fines derived from an industrial recycled pulp, of a testliner paper factory, as raw material to produce NFC. The fines were obtained by sieving the recycled fibers on a 28 mesh screen. Then, the fines (2% consistency) were disintegrated in a GEA Niro Soavi Panda Plus 2000 homogenizer for 5, 10, and 15 passes through the equipment, without any chemical or enzymatic pretreatment. The resulted material was characterized with TEM microscopy, and a total of five images for each condition were analyzed with a free software (GIMP) to determine the average diameter of the NFC. Also, the rheological properties, superficial area, crystallinity, were measured for every sample.

The Figure 1 shows representative images for the resulting NFC to different levels of fibrillation. The NFC with 15 passes through the homogenizer has a high degree of fibrillation with diameters in the range of 7-81 nm. With a lesser number of passes it is also possible to get cellulose nanofibers, obtaining diameters from 7 up to 208nm, proving that the fines derived from recycled pulp is a suitable low cost raw material. All the samples presented a pseudoplastic behavior, characteristic of NFC derived from other raw materials. The crystallinity of the fines showed a reduction with the number of passes, from 69.4 to 53.0%, while the superficial specific area rose from 72 m^2/g to 97 m^2/g.

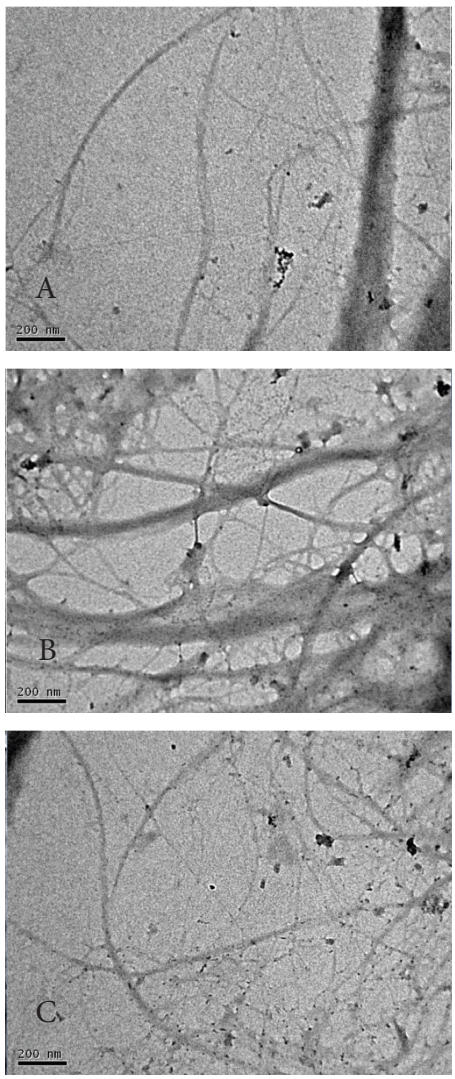

Fig.1 – TEM images of NFC obtained from recycled pulp fines. A) 5 passes B) 10 passes and C) 15 passes through homogenizer

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EPOXIDATION OF LIPID AS ROUTE FOR OBTAIN POLYMERIC RESIN: PRELIMINAR STUDY

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Abstract:
Vegetable oils represent a promising route to develop renewable chemicals and polymers due to their ready availability, inherent biodegradability and low toxicity[1]. The vegetables oils can be modified to produce materials that can act as replacement for materials derived from petroleum[2]. Thus, during the last decade, a variety of vegetable oil-based polymer has been developed. In this, vegetable oil-based polymer most developed has been the epoxy resin production with epoxidized vegetable oils[3, 4]. In this study the epoxidation of lipid from oleagineous crops such as rapeseed oil using a heterogeneous and homogeneous catalyst were evaluated. Two heterogeneous catalysts (Amberlyts-15 and Dowex 50WX8) and sulphuric acid as homogenous catalyst were used.
The epoxidation process of oils was carried out by using in situ generated performic acid to produce epoxidized lipids. Performic acid was produced by mixing of formic acid (HCOOH) as oxygen carrier and hydrogen peroxide (H_2O_2) as oxygen donor. The effect of lipid: HCOOH: H_2O_2 molar ratio, temperature and catalyst type were studied. The presence of oxirane ring of epoxidized lipids was characterised by fourier transformation infrared. The evaluation of conversion and selectivity is now in process (data not shown in this abstract)
The results showed the presence of oxirane ring of epoxidized lipids using the two heterogeneous catalysts (Amberlyts-15 and Dowex 50WX8), where the main advantage of the use of heterogenoeus catalyst is its possible reuse. Besides, the sulphuric acid used widely as catalyst in this process, can be replaced by an acid solid catalyst. So, the disposal of salts formed during the neutralization of catalyst and all problems associated with their use can be eliminated, adding that can be reused in the reaction. Further, the presence of oxirane ring of epoxidized lipids was observed at high molar ratio of lipid: HCOOH: H_2O_2 and low temperature of reaction due at decrease of stability of the oxirane ring.

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BIOTECHNOLOGICAL PRODUCTION OF IMPORTANT PLATFORM ORGANIC ACIDS USING CAROB BY-PRODUCTS DERIVED MEDIA

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Carob tree is widely cultivated in the Mediterranean area where it is considered to be an important economic crop. Carob pod is the fruit of the carob tree and its two main constituents are (by weight): pulp (90%) and seed (10%). The seed is used industrially for the production of several products, most noteworthy locust bean gum. Conversely, the pulp does not have any significant industrial application, even though it has a high content of sugars, that can reach a total above 45% (w/w). Therefore, an upgrade strategy for this material is clearly needed.
A possible alternative is the production of organic acids, such as succinic acid that can be used as a precursor of many industrially important chemicals in food, chemical and pharmaceutical industries. The industrial viability of succinic acid production is dependent on the utilization of low-cost renewable resources and the development of a biorefinery concept leading to the production of value-added co-products. In this respect, the utilization of carob pulp as a raw material for succinic acid production could lead to a sustainable and cost-competitive process.
In order to evaluate the potential of succinic acid production using hydrolysed carob syrup [1] as sole carbon source, fermentations were carried out using the bacterial strain *Basfia succiniciproducens* JF4016 (DSMZ 22022), which is a recent isolate consider as a potential industrial succinic acid producer. Cultures were carried out with or without CO_2 sparging and an initial total sugar concentration of 20 to 40 g/L.
Under no CO_2 sparging, carob syrup was effectively consumed, and efficient bacterial growth was observed, presenting a biomass productivity of 0.1 g/(L.h). Lactic acid was the main metabolic product found. Under continuous CO_2 sparging, *B. succiniciproducens* grown on three different carob syrup concentrations presented the highest yield of succinic acid (0.44 g/g) at 30 g/L of total sugars with a corresponding productivity of 0.27 g/(L.h). It is noticeable that no growth was observed at 40 g/L initial sugar concentration, indicating the presence of inhibitory compounds. The phenolic compounds present in the carob syrup are putatively indicated as the main factor responsible for inhibition, but this must be studied further.

Acknowledgement

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UTILIZATION OF DISACCHARIDES AS NUTRITIONAL SUPPLEMENTATION OF SUGARCANE STRAW HEMICELLULOSIC HYDROLYSATE FOR XYLITOL PRODUCTION BY *Candida Guilliermondii* FTI 20037

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Xylitol is a sugar-alcohol with important applications on food, odontological and pharmaceutical industries, whose market is having rapid and continuous growth. It was already suggested that this polyol is one of the high-value chemicals that can support economically the production of low-value biofuels in an integrated biorefinery. Xylitol is commercially produced by an expensive and non-environmentally friendly process of chemical catalysis. Biotechnological production of xylitol is not only an alternative for replace the chemical process but also an attractive route for valorization of hemicellulosic fraction of lignocellulosic biomass, such as sugarcane straw, whose availability is increasing in Brazil. The aim of the present work was to study the effect of supplementation of the disaccharides maltose, sucrose or cellobiose (5.0, 10.0 or 15.0 gL^{-1}) to sugarcane straw hemicellulosic hydrolysate (xylose 57gL^{-1}) on xylitol production by *Candida guilliermondii* FTI 20037. Experiments were performed at initial pH 5.5, 30ºC, 200rpm and 72h, in 125mL Erlenmeyer flasks with 50mL of medium. The best performance of the yeast was achieved with sucrose supplementation (10gL^{-1}), condition in which were obtained the highest values of xylitol production (41.36gL^{-1}), volumetric productivity (0.61 gL-1h^{-1}) and efficiency (75.70%). These values represented increments of 9%, 6% and 5%, respectively, regarding the medium without disaccharides addition (37.93gL^{-1}, 0.57$gL^{-1}h^{-1}$ and 72.09% respectively). In the case of maltose, values of xylitol production (39.78gL^{-1}), volumetric productivity (0.60$gL^{-1}h^{-1}$) and efficiency (74.15%) slightly higher than those in the medium absent of disaccharides were obtained in the lowest evaluated concentration (5.0gL^{-1}). Maltose concentrations above 5.0gL^{-1} (10.0 and 15.0gL^{-1}) caused reductions of 6.5% and 8.4% on xylitol production. Cellobiose supplementation resulted in similar values in all the evaluated concentrations of xylitol production (32.72gL^{-1}), volumetric productivity (0.50gL-1h^{-1}) and efficiency (70.03%), which were lower than those achieved without disaccharides addition. Under the experimental conditions here employed, both sucrose and maltose can be used as nutritional supplementation of sugarcane straw hemicellulosic hydrolysate for xylitol production. Based on these results, it can be suggested the possibility of using complex sources of disaccharides, mainly sucrose and maltose, such as by-products or co-products of agro-industrial processes, as nutrients for xylitol production from sugarcane straw hemicellulosic hydrolysate, allowing not only to favor the yeast performance but also to replace more expensive nutritional sources and to reduce the cost of this bioprocess.

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ENZYMATIC TREATMENT TO VALORISE LIGNIN AND PHENOLIC EXTRACTIVES

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Lignin is the main by-product of the pulp industry, which is mostly used as fuel for energy production. However lignin could be a source of high added value products like dispersants, carbon fibres or additive in phenolic resins [1]. In nature lignin works as an adhesive that gives trees, grasses and straw their strength and flexibility. Thus, lignin is a perfect candidate to replace synthetic adhesives, such as formaldehyde which is a toxic and carcinogenic substance.

Laccase is an oxidoreductase enzyme which is involved in the natural lignification of lignocellulosic biomass. This enzyme has the ability to oxidize phenolic compounds, leading to a free radical polymerization [2]. Hence, laccase is an interesting biocatalyst to produce polymers from lignin which could be used as bioadhesives in fibreboard production or coatings of lignocellulosic materials [3].

Wood extractives are a complex family of substances, including phenolic compounds, which are present in bark, leaves, fruits and hardwood of trees. These compounds have a wide range of applications since they have antibacterial, antifungal, antioxidant, anti-inflammatory or analgesic properties. Nevertheless, these compounds are not valorise in pulp mills and they are destroyed in the digesters or burnt in the recovery boiler. Moreover, they are involved in the consumption of cooking liquor during the wood cooking and also in the formation of paper spotting in the pulp and paper process production, also known as *Pitch* problem. Thus, the recovery of these compounds before the wood cooking could solve important problems of the pulp production process and at the same time, it could open new markets for the complete valorisation of lignocellulosic biomass.

A treatment involving phenolic wood extractives and laccase enzymes can modify the physical and biological characteristics of lignocellulosic materials, conferring them antibacterial, antifungal or hydrophobic properties [4]. For instance, we have increased the hydrophobicity of beech veneers three fold by a treatment with laccase and *Pinus pinaster* sawdust extractives. These extractives have also antifungal properties against wood decay fungi, so that an enzymatic treatment of wood with *Pinus pinaster* extractives is a promising tool to provide antifungal and hydrophobic properties to wood fibres.

The phenolic structure of most of the pulp mill by-products leads to use the laccase enzyme as the right tool to treat lignocellulosic materials with lignin and phenolic extractives. By means of the physical and biological properties of these compounds, and their covalent bond to lignocellulosic fibres, it is possible to modify wood materials easily, effectively and by environmentally-friendly pathways.

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FOREST-BASED BIOREFINERY: DEVELOPING A SUGAR PLATFORM FOR THE PULP AND PAPER INDUSTRY

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Industrial production of pulp and paper is very demanding in terms of energy and raw material consumption, and thus producers make every effort to optimise its use in a sustainable manner. The ultimate ambition is to achieve zero-waste mills within a forest-based biorefinery concept, in order to maximise overall process economics and minimise negative environmental impact. Hence, all waste streams and by-products should be valorised. Indeed, these lignocellulosic residues constitute an inexpensive and renewable resource for bio-based marketable products.
The present work deals with the biological upgrading of eucalyptus residues from pulp and paper mills, i.e. fines (wood shavings) and bark. The conversion of some fast-growing energy crops widely available as native forest species, such as acacia and miscanthus, was also assessed. These lignocellulosic materials were evaluated under a sugar-based biorefinery concept.
Taking advantage of the pulp and paper industrial know-how and equipment, kraft pulping was applied as an innovative alkaline-based pretreatment. Kraft cooking, generating a solid virtually free of lignin with partial hemicelluloses removal, was compared with hydrothermal processing, which promotes an extensive solubilisation of hemicelluloses. A biorefining strategy integrating kraft pulping and enzymatic hydrolysis was very effective for the conversion of the large majority of the lignocellulosic materials tested into concentrated sugar solutions (> 100 g/L). Indeed, kraft pulp from eucalyptus fines was converted into glucose with approx. 97%-yield, similar to the one obtained with hydrothermal processing and to industrial kraft pulping. Glucan conversion of kraft pulps from acacia and miscanthus chips was also effectively accomplished, with 88% and 72%-yields, respectively. Bark was inefficiently processed by kraft cooking, but with hydrothermal pretreatment enzymatic hydrolysis was above 55%-yield.
When coupling a fermentation step, maximum ethanol overall yield (82% of maximal theoretical on glucan basis) was obtained from kraft-processed eucalyptus fines (10% w/v solids). This result is comparable to the yield achieved with industrial kraft pulp and hydrothermally-processed fines. Maximal concentration of 68 g/L was obtained from 20% (w/v) solids of kraft-processed eucalyptus fines (77%-yield). Kraft-processed acacia and miscanthus (20% w/v solids) generated 64 and 52 g/L of ethanol (69 and 64%-yields), respectively. Eucalyptus fines, acacia and miscanthus (20% w/v solids) yielded, respectively, 138, 125 and 107 g/L of lactic acid, corresponding to 78, 68 and 67% of maximal theoretical yield for glucan conversion. Considering the maximal overall yields, obtained for conversion of eucalyptus fines, the developed processes provided 235 kg of ethanol or 364 kg of lactic acid per metric tonne (dry basis) of lignocellulosic biomass.
Thereby, the biorefining potential, through a sugar platform, for the production of biofuels (e.g. ethanol) and other bio-based products (e.g. lactic acid) was demonstrated for all the forest biomass sources tested. In a biorefinery based on lactic acid and ethanol fermentation, ethanol is used as fuel for transportation and lactic acid is polymerised to form polylactate, which is used as a plastic. Lactic acid and ethanol might be esterified to produce ethyl lactate, which is used as a biodegradable solvent.

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XYLITOL BIOPRODUCTION FROM SUGARCANE BAGASSE: DETOXIFICATION AND FERMENTATION STRATEGIES

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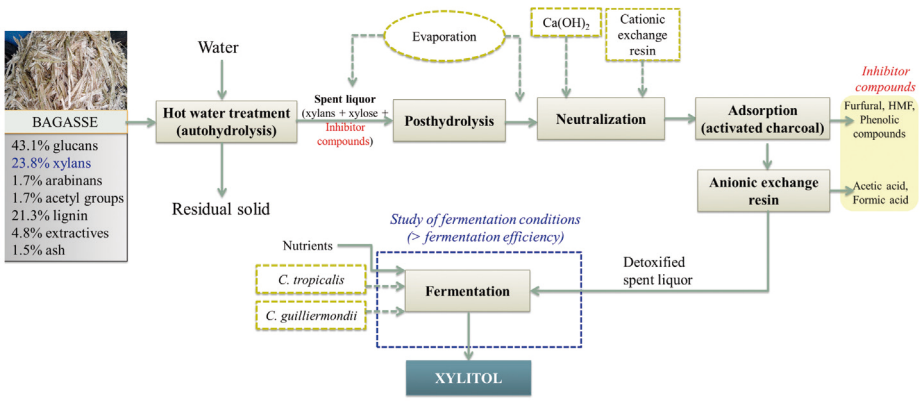
Hemicelluloses from bagasse are mainly pentosans (xylans) which can be depolymerized into sugar (xylose) as primary carbon source for the bioproduction of xylitol, ethanol, and others. Xylitol is used as a food additive and sweetening agent, and is industrially produced by expensive chemical processes. It can also be produced by the fermentation of xylose extracted from hemicelluloses. Improvements in biomass treatment, detoxification and fermentation processes are needed to make xylitol production cost-effective, opening new markets and creating new applications for it. The aim of this study was to evaluate detoxification and fermentation strategies for spent liquors from the autohydrolysis of sugarcane bagasse, for xylitol biotechnological production. Hemicelluloses were removed from bagasse by autohydrolysis treatment. Different sequences of treatment for spent liquor detoxification were accomplished, and their effect on sugars loss, inhibitors removal, and xylitol production were evaluated. Spent liquor was concentrated under vacuum before and after posthydrolysis, and previous to activated charcoal treatment. The xylans were converted to xylose by posthydrolysis of the spent liquor in 1% H2SO4, and the acid was removed by precipitation with $Ca(OH)_2$ to pH 10 (gypsum formation). Two methods were applied to adjust spent liquor to pH 5: (i) phosphoric acid, and (ii) anionic and cationic exchange resins. Spent liquor was subsequently treated with activated charcoal (3%, 100 rpm, 60°C for 1 h) to remove main inhibitors compounds. Acetic acid was removed by anionic exchange resins (Figure 1). Various experiences of fermentation were performed with commercial xylose to select the yeast (*C. guilliermondii, C. tropicalis*), and the fermentation conditions (nutrients, concentration of yeast cells). Conditions of detoxified spent liquors fermentations were obtained from these experiences. Sugars and organics acids were quantified by HPLC chromatography. Total phenolic content was determined by the Folin-Ciocalteu method, and main degradation products of lignin were identified by HPLC chromatography. Yeast cells concentrations were measured by turbidimetry. HMF and furfural were completely removed by evaporation under vacuum. Total HMF, furfural, and phenolic contents decreased more than 95% after activated charcoal treatment. Acetic acid was almost completely removed by anionic and cationic exchange resins. Evaporation after posthydrolisys of spent liquor produced the highest loss of sugars (50%), due to entrainment of liquor produced by gypsum precipitation after the addition of $Ca(OH)_2$. C.tropicalis behaved best in all fermentations. Samples of liquor rich in xylose from each detoxification stage were used as culture medium for xylitol production, and the results were compared with a sample of commercial xylose. Xylitol concentration of 12.5 gL-1 (fermentation efficiency of 36%) was achieved with the following fermentation conditions: 40 gL^{-1} of initial xylose, 2.4 gL^{-1} of yeast cells, 30°C, and 120 rpm. This concentration was 27% lower than that obtained from the sample of commercial xylose, fermented in the same conditions. Other fermentation experiences at high initial xylose concentration were performed, increasing fermentation efficiency.

Figure 1. Sequence of treatments for xylitol production (dotted line: alternatives).



A BIOLOGICAL PRE-TREATMENT OF *Eucalyptus dunnii* BARK TO INCREASE CELLULOSE ACCESSIBILITY

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In Uruguay forest industry has grown in the last 25 years. Planted area has reached one million hectares, and the zone destined for *Eucalyptus* plantations raised to 726.323 ha in 2013 (1). *Eucalyptus* wood is used to produce cellulose pulp, and significant amounts of bark are generated as byproduct. *Eucalyptus* bark, as well as most other lignocellulosic materials, is composed by cellulose, lignin, hemicelluloses and extractives.

In particular, *Eucalyptus dunnii* bark is composed primarily by cellulose (37%), hemicellulose (9.8%), lignin (27 %) and extractives (14%) For this reason bark could be a suitable option to produce biofuels such as second generation ethanol from the glucose obtained from these lignocellulosic materials (2).

The lignin and hemicellulose together form a binding layer that sticks to the cellulose (Figure 1) preventing saccharification. The use of biomass sugars entails pre-treatment to breakdown the lignin-carbohydrate complex and exposing the carbohydrates to enzymes.

Our research group has studied the bark lignin degradation by *Dichostereum sordulentum* isolated from *Eucalyptus* decaying wood. In optimized conditions a delignification of 30% was achieved increasing the carbohydrate content.

In order to get a further increase the saccharification of the remaining material, a further step using xylanases might be performed (3).

An efficient strategy in the search for new xylanases activities consists in the isolation of microorganisms from natural habitats where the polymer is present. Our group obtained a set of endophytic and epiphytic microorganisms with xylanase activity from *E.globulus* branches. In this work we present hemicellulose degradation of previously delignified, *E. dunnii* bark using commercial xylanase and crude enzyme extracts from the selected microorganisms.

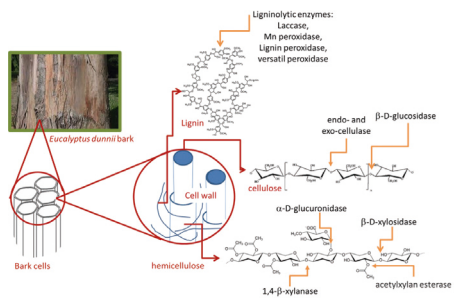

Fig.1 – Bark composition and lignocellulolytic enzymes

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EFFECT OF AIREATION IN THE PERFORMANCE OF THE SOLID SUBSTRATE FERMENTATION OF AGRO-WASTE BY NATIVE FUNGI OF ROT-WOOD

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The lignocellulosic residual biomass is a sustainable feedstock for the production of biocatalysts, biomaterials and biofuels [1]. In order to achieve the bioconversión levels of the structural polymers that comprise it which ensure the feasibility of the process, has been necessary overcome the operational complexity of the solid substrate fermentation. One of the main operational limitations of this method of cultivation is the generation of heat during the fermentative process, which if not be effectively dissipated may cause harmful thermal profiles for the viability of the microorganisms. The control of temperature and humidity is achieved by a circulation system humid air through the solid substrate so that, the moisture and flow used must meet the needs of heat and mass transfer in the bioreactor. The filamentous fungi are the organisms that present the best option for solid substrate fermentation because of its tolerance to low water activity and, to high osmotic pressure as well as for their colonization ability and assimilation of nutrients available in the solid using their system of extracellular enzymes [2]. According to these background it has been postulated that is possible to improve the of the fungal bioconversion performance of the agricultural waste, favoring the production of ligninolytic enzymes and therefore the delignification of biomass, using a reactor system implemented with a feed continuous of moist air. This research was conducted at the Biological Treatment Laboratory of Chemical Engineering Department - Usach, for the purpose to study in a lab scale packed bed bioreactor, the effect of forced aeration in the performance of the solid substrate fermentation of agro-waste by Inonotus sp. SP2 a native fungi of wood rot from southern forest of Chile [3]. The spent tea leaves were used as substrate, this residue which was generated in a process of production of instant drinks infusion, serves as support growth and source of nutrients for the fungus.

Static cultures were performed in flasks without forced aeration to produce enzyme extracts and establish the corresponding profiles of ligninolytic activity, besides of obtaining the partially degraded residual biomass. To analyze the effect of aeration was implemented a bioreactor of column at laboratory scale of 23 mm internal diameter , packed with a fixed bed of 30 mm high substrate, which is operated with a continuous flow of moist air provided by a mini-blower (Figure 1). In static cultures in flask it was detected that higher ligninolytic activity corresponds to Lignin peroxidase (LiP) reached on 30th day 2 ± 1 (U / g ss); while in the culture aerated in fixed bed with a flow of 0.06 LPM was obtained 55.95 (U / g ss). Furthermore the presence of cellulolytic enzymes was established indirectly, by sugar production, reaching 6.7 ± 0.2 (mg / g ss), value that turned out to be 8.9% greater than that obtained in static culture.
In conclusion, is observed a favorable effect of the forced aeration on the degradation of biomass as well as, both production enzyme and sugar.

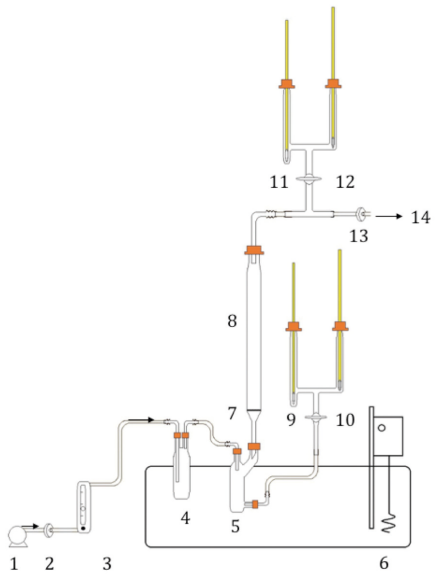

Figure 1: Experimental device used for solid substrate fermentation (1) air blower, (2) (13) air filter, (3) flow meter, (4) humidifier, (5) drop eliminator, (6) water bath, (7) air distributor, (8) cultivation bed, (9) (11) wet bulb sensor, (10) (12) dry bulb sensor, (14) gas outlet.

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BIOREFINERY SCHEMES FOR BREWERY AND DISTILLERY WASTE STREAMS

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Food processing is one of the strongest industrial activities. For example in Germany and Mexico the primary energy demand shares about 20% of industries' total primary energy demand. With respect to trade volume of food processing industries the meat processing industry is the biggest one followed by the dairy industry in Germany. On the fourth place with a turnover of 12.2 billion Euros is the production of alcoholic beverages.
These industries are mostly found as small and medium-sized enterprises. Their business challenges are best met by an increase in energy efficiency and the reduction of wastes by intelligent management of secondary streams. Schemes may differ for type and scale of production facilities. This study reports about the integration of an anaerobic digester for treating liquid waste streams of a small scale brewery and vine distillery (90 000 hL/a) as well as the production of biochar from spent grains. In this respect it represents form of on-site biorefinery. The anaerobic digestion treats both high and low strength brewery waste water (COD: 4 - 0,2 g /L) as well as seasonal distillery residues (COD: 40 - 45 g/L) in a joint process. The brewery shows an output of 90 000 hectoliters (hL) of beer per year with a specific footprint of 5 liters of liquid waste stream per liter of beverage. Operation of the anaerobic filter during one year under stable conditions with an organic loading rate of 6 kg COD m-3 d-1 resulted in an average removing rate of 75% with a production of 0.7 m 3 Biogas per kg of removed COD. Spent grains are transformed into tailored biochars by hydrothermal carbonization (HTC) or low temperature conversion (LTC) for special applications.

EVALUATION OF ENZYME ACTIVITY AND KINETIC PARAMETERS FOR COMMERCIAL THERMOSTABLE β-GALACTOSIDASES AT MODERATELY HIGH TEMPERATURES

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Galactooligosaccharides (GOS) are carbohydrates with a glucose molecule linked to 2-5 molecules of galactose. GOS are prebiotics with better technical characteristics like thermal and acidity resistances compared with other prebiotics like inulin and fructooligosaccharides. GOS are industrially obtained by simultaneous enzymatic reactions of hydrolysis and transgalactosylation. The source of GOS might be lactose or cheese whey, with a lactose content higher than 70%.

Enzymatic reactions depends on pH, enzyme concentration, substrate concentration and temperature. However, substrate concentration is closely related to GOS production. The higher the substrate concentration, the higher yield is reached by the enzymatic reaction. Substrate concentration is limited by its solubility, which depends on temperature, and it is determined by the thermostable capacity of the enzyme.

In this work, two commercial thermostable enzymes were evaluated in the production of galactooligosaccharides (GOS) capacity at optimum temperature near to 60°C. At this temperature the lactose solubility is twice the solubility at 40°C, which is a common temperature used for GOS production.
The parameters evaluated were hydrolitic activity with lactose and ONPG, transgalactosylation activity, kinetic of reactions, yield and purity of the product. All these variables are based on GOS concentration, it was determined by HPLC with RI detector. The protein content was evaluated by Bradford Method.
The enzyme 1 had higher hydrolytic activity, while enzyme 2 had higher transgalactosylation activity. Were determined the kinetic parameters and were adjusted kinetic models in both enzymes. Michaelis-Menten model was applied, but it hadn't got good agreement, for that reason was necessary to apply a Michaelis-Menten modified model.

FERMENTATIVE HYDROGEN PRODUCTION FROM ALKALINE AND ALKALINE HYDROGEN PEROXIDE PRETREATMENT

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Introduction: Energy crises, as well as the current increase in the cost of oil, emphasize the need for alternative renewal sources of energy. In specific, molecular hydrogen (H_2) has attracted much interest as a promising alternative source of energy because exhibits the highest heating value per mass of all chemical fuels (142 MJ Kg^{-1}) is regenerative and environmentally friendly because only water vapor is released when it undergoes combustion [1–3]. Hydrogen can be produced from biomass by thermochemical or biological conversion. Production of hydrogen by biologic means is receiving more attention because it is a sustainable and clean technology.
In the other hand, among the agricultural residues, wheat straw is one of the most available biomass in our country. This lignocellulosic material can be used for obtain different kind of energy vectors as methane, ethanol, molecular hydrogen among others [4]. For the process success, a physic-chemical pretreatment is needed to make their crystalline organization more accessible to enzymes to degrade the structural polysaccharides into monomeric sugars [5].

Goal: The aim of the present work was obtain a comparison among two different pretreatment methods and their influence in biological molecular hydrogen generation.

Methodology: To achieve the aim, a clinical isolated strain of *E. aerogenes* was used for biological conversion. Chemical pretreatments include alkaline (NaOH: 0.5%; 1%; 2%) and alkaline hydrogen peroxide (H_2O_2: 1.5%; 2%; 2.5% at pH 12.5). Wheat straw composition was evaluated before and after pretreatment by HPLC and the reducing sugar liberated with enzymatic saccharification was measured by the dinitrosalicylic acid (DNS) method. Batch fermentation configuration was carrying out for microbiological growth and molecular hydrogen production.

Results: Alkaline pretreatment exhibit the highest total sugar values (over 80% w/w), among them 1% NaOH show 68.7 % w/w glucose content. Furthermore, all alkaline pretreatment analyzed showing highest reducing sugar release in comparison with alkaline hydrogen peroxide. This result is coherent with molecular hydrogen production, because more sugar is available for fermentation process.
Conclusions: Biological systems and lignocellulosic biomass utilization have significant potential as an environmentally friendly means of producing hydrogen. In particular, wheat straw is a suitable resource for this mean. Alkaline pretreatment with NaOH show best results for molecular hydrogen production under the conditions tested.

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IMPACT OF DANDELION ROOT RESIDUES ON BIOMETHANATION AS THE FINAL STEP IN A BIOREFINERY SCHEME

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Introduction: Inulin is the main sugar present in dandelion (Taraxacum sp) roots, and is currently used as an intestinal modulator in in animals, in order to control CH4 emissions to the atmosphere. Ruminal modulation has been widely stated for inulin, but there is no information regarding the impact of this fiber against wastewater treatment plant (WWTP) methanogenic population. The main objective was to evaluate the effect of different inulin concentrations, as found in dandelion`s residues, on a biomethanation process under a biorefinery approach.

Materials and Methods: Commercial inulin (Terrium) was used in the experiments. Total solids (TS) and ash were quantified (AOAC 2012). Inulin was digested in 100 mL vials at concentrations of 0.5, 1.0 and 1.5% ST and maintained at 37 (±2)°C. Digested sludge of a traditional WWTP at 1.5g SV/L was used as inoculum. The volume of methane produced was measured by liquid displacement, the final proportion of methane was quantified by GC-TCD.

Results: Methane production was correlated with the amount of initial TS as presented in Fig. 1. Methane yield was in the range of 1.8 to 2.3 mL CH4/g ST and the specific methane yield between 122.8 to 154.4 ml CH4/g SV-g ST being both inversely correlated to TS. Maximum velocity was achieved in the first 3 days and decreasing constantly afterwards, reaching the 80% of the total methane production at 20th of process. Considering the production of methane by the microorganisms, methane yield obtained was 772.2, 1321.3 and 1842.7 ml CH4/g SV for 0.5, 1.0 and 1.5%, respectively. GC-TCD analysis at the end of the period showed percentages of methane in the biogas between 35 to 48% not being correlated to the percentage of inulin in the medium.
Reports indicate that the presence of this fiber in the fodder can affect methane production in [1]. In the case of WTTP sludge, no inhibition was observed even at 1.5% ST, in which case high COD concentration (10.5 g COD/L) was present in the medium. Methane production was observed from the start of the process with no significant adaptation period of the microorganisms to the fiber content.

Conclusion: Inulin present in dandelion roots is a fiber that can be successfully converted into biogas showing no inhibition activity at a concentration of 1.5% of ST.

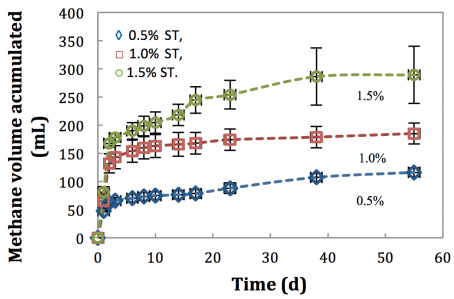

Fig.1 – Methane accumulated in time for the inulin degradation at different concentrations and under mesophilic conditions.

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In silico ANALYSIS OF HYDROGEN PRODUCTION FROM GLUCOSE/XYLOSE MIXTURES BY A DEGENERATED VARIANT OF *Clostridium acetobutylicum* ATCC 824

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The increasing demand for energy, environmental concerns related to fossil fuels use and depletion, together with incentives for biofuels production have led to intensive research in this field. Among the proposed alternatives, hydrogen has been identified as a potential candidate for the replacement of fossil fuels, being its null CO_2 emission during combustion a key advantage. Moreover, H_2 can be biologically produced from a wide range of substrates, which include the main components of lignocellulose, making it a second-generation biofuel.

Despite significant advances, low yield is still the main barrier for the industrial production of biogenic H_2. Microorganisms produce H_2 in combination with volatile fatty acids and/or solvents, which compete for reducing equivalents. Experimental results have shown that a high H_2 yield is associated with the production of acetate and butyrate, while the opposite is true with the production of lactate and solvents. The maximum theoretical yield achievable in biological systems has been calculated to be 4 mol of H_2 per mol of glucose or 2 mol of H_2 per mol of glucose when acetate or butyrate are the only subproducts, respectively [1]. However values between 1.9 y 2.4 mol mol^{-1} are usually obtained in fermentation systems.

The biological production of H_2 has been mainly studied using consortia, being usually *Clostridium spp.* the predominant microorganisms. However its specific contribution to hydrogen production has not been studied in detail [2]. Most studies using axenic cultures of *Clostridium spp.* are focussed on exploiting its solventogenic capabilities instead of the production of H_2. In this regard, it should be noted that solventogenic Clostridium spp. might not be an appropriated platform for the production of H_2 because reducing equivalents can be deviated towards the production of solvents through NADH oxidation pathways. However, the genes responsible for solvent (acetate, ethanol, butanol) production and sporulation in *Clostridium acetobutylicum* reside in a plasmid (pSOL1) [3], which is lost during successive subculturing and continuous culture if appropriate precautions are not taken. Because the production of solvents and sporulation are detrimental for the production of H_2, the use of a degenerate strain lacking the pSOL1 plasmid is therefore an interesting case study for this purpose. Moreover, the genome of *Clostrdium acetobutylicum* ATCC 824 has been sequenced and annotated, and a number of genome-scale metabolic models have been developed [4-8], which allows the study of its metabolism from a systems point of view.

The aim of this work is to carry out a systematic *in silico* evaluation of the metabolic capabilities of a degenerated variant of *C. acetobutylicum* ATCC 824, which lacks the genes responsible for solvent production present in the pSOL1 plasmid, for the production of H_2 using glucose/xylose mixtures as the carbon source. This analysis is carried out through a constraint-based approach using the Constraint Based Reconstruction and Analysis (COBRA) toolbox.

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AN ADVANCED BIOREFINERY FOR BUTANOL, ACETONE AND ELECTRICITY COPRODUCTION UNDER A CONSOLIDATED BIOPROCESSING SCHEME

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The conceptual design for an advanced biorefinery under a Consolidated Bioprocessing (CBP) scheme is presented for acetone-butanol-ethanol (ABE) fermentation. Wheat straw is employed as feedstock. Biogases (hydrogen and methane) produced at the dark fermentation and methanogenic production stages are considered for electricity cogeneration. Separation of butanol and acetone from the fermentation broth is achieved by a novel extraction-distillation stage. A parametric analysis of butanol total production cost (TPC) was carried out for distinct plant capacities, including plant capacity regarding WS availability in west Mexico. A wide range of plant capacities (100, 250, 500, 1,000, 1,500 and 2,000 ton/day) and feedstock purchasing price depending on its polysaccharide content (PC) with values of 45, 50, 55 and 60% w/w were analyzed. WS purchasing prices were considered from 6.00 USD/ton for composted WS (45% PC) to 75.00 USD/ton for new WS (60% PC). Surplus of electricity and acetone are sold as byproducts. Energy integration and end-use energy integration are analyzed to determine the plants resourcefulness and impact. The best case scenario resulted using a plant capacity of 2,000 ton/day and 45% PC with a butanol TPC of 0.53 USD/L. A feasible plant capacity according to the regions WS availability corresponds to 500 ton/day, achieving a TPC of 0.79 USD/L with 45% PC.

REFINING TREATMENTS FOR OBTAINING FERMENTABLE SUGARS AND NANOFIBRILLATED CELLULOSE FROM SUGARCANE BAGASSE IN A GREEN APPROACH

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The present work is a contribution for the sugarcane bagasse biorefinery, and proposes a route of environmentally non-aggressive methods for obtaining fermentable sugars and nanocellulose structures, mainly from non-bleached pulps. In order to improve the cellulose accessibility without using chemical reagents, this paper proposes the use of high shear refining for the modification of the ultrastructure of three different organosolv pulps (lignin content between 3 and 9%). Refining methods were chosen because its delamination effect modifies the cell wall organization and increases the active surface area for hydrolytic agents, however with no change in chemical composition. The effects of physico-mechanical treatments on the morphology and physical structure of lignocellulosic fibers were studied in terms of fiber dimensions, porosity and crystallinity. After the refining, the treated pulps were submitted to different conditions of enzymatic hydrolysis in order to produce, in separated essays, glucose and nanofibrillated cellulose (NFC). The total yield of each process was calculated considering the losses of mass in the pretreatments, the total sugar content in enzymatic liquors and the obtained mass of NFC. The characteristics of the NFC were established from the results of size determinations (measured by AFM images), XRD, TGA, water sorption and tensile strength of NFC films. It was found improvements up to 104% in cellulose digestibility after refining that could be related to increases up to 32% in accessible pores area and decreases up to 63% in fibers length, followed by increases up to three times in fines content. The higher glucose yield obtained in this study was 90% for the refined organosolv pulp with 3% of lignin. The NFC obtained from unbleached pulps showed adequate characteristics for further applications. This integrated view of process contributes for the improvement in the cellulosic ethanol industry and nano-based high value-added materials production.

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CULTIVO DE *Spirulina platensis* EN CUATRO COLUMNAS BURBUJEADAS DE 200 L EN CONDICIONES AMBIENTALES

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Resumen
En este trabajo se aborda la producción masiva de la microalga en cuatro bio-reactores tipo columna burbujeada de 200 L. El cultivo se realizó al exterior, bajo condiciones ambientales por 14 días, en un medio constituido por NaCl, Na_2HCO_3 y una mezcla de nutrientes que incluye N, P, Mg, K y otros iones requeridos para el cultivo. El desarrollo del cultivo se siguió por la lectura de su densidad óptica. Las variables que se midieron a lo largo del proceso fueron biomasa, pH, conductividad, temperatura exterior, así como la irradiancia en µmoles de fotones/m^2s. La cosecha del alga se efectuó filtrando 180 L de los 200 L. El material así producido se secó en charolas a 60oC, se molió y se guardó para su posterior análisis proximal. Se caracterizó una *Spirulina* comercial PronatUltra (Abastecedora de productos naturales SA de CV, Mexico) con fines de comparación.

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A la Compañía ENERVIVA por la autorización para usar su cepa de *Spirulina* y por el apoyo técnico recibido. Se agradece a G Ruiz, S. Alejandre y Y. Lopez (UPIBI-IPN) por su participación en el trabajo experimental.

PHYTOHORMONE EFFECTS ON THE KINETICS OF GROWTH OF MICROALGAE FOR PRODUCING BIODIESEL

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Algae or commonly called microalgae (Chlorophyta, Rhodophyta and Charophyta) share common ancestors with the upper floors, which is why over the years has seen some of the molecular signals that they thought were unique to plants also will find in organisms that are further down the evolutionary chain as bacteria, fungi and algae. Some of these molecular signals that have been widely studied in higher plants, are phytohormones. These compounds can produce large effects at low concentrations, however it is not yet known with certainty what the role of these signals in microalgae. Recent research suggests that unicellular algae in the presence of low concentrations of phytohormones may respond to an increase in the overall biomass or increased growth rate [1–3]. In this context the use of bio-stimulators in the process of cultivation of microalgae in the industry of biofuels, born as an alternative to genetic methods proposals which attempts to increase the productivity of total biomass.

In this study we analyzed the effect on cultivation of microalgae *Dunaliella tertiolecta* (DT) and *Tetraselmis sp.* (Tsp.) in presence of varying concentrations of 3-indole acetic acid, 6-benzylaminopurine and 3-indole butyric acid on growth curves, showing a positive effect on growth in the case of DT.

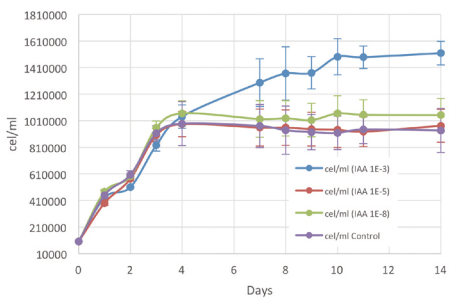

Fig. 1 Growth curves of *Dunaliella tertiolecta* and varying concentrations in the presence of 3- indole acetic (IAA)

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IMPROVEMENT OF PHLOROTANNIN AND PROTEIN EXTRACTION FROM *Macrocystis pyrifera*

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Seaweeds are a good source of chemicals and building block materials that can be tailored through proper biorefining processes (Algal biorefineries). Compounds of particular commercial interest include pigments, lipids and fatty acids, proteins, polysaccharides and polyphenolics. Phlorotannins are a type of polyphenol that is found exclusively in brown seaweeds and exhibit a variety of different biological properties, including antioxidant [1], anti-inflammatory [2], antiallergic [3] and neuroprotective [4]. On the other hand, all brown seaweeds have essential amino acids similar to those presented in animal proteins. The objective of this work was to improve the extraction of phlorotannins and proteins from brown seaweed *Macrocystis pyrifera*. To improve the extraction of phlorotannins and proteins three extraction parameters (temperature, time and ratio solid/liquid) were evaluated by Taguchi´s experimental design. After determining the best extraction condition, a pretreatment using oligo alginate and alginate lyase enzymes in different proportions was incorporated. For the liquid fraction obtained in each dot (extracts) the total concentration of phlorotannins was determined by the Folin-Coicateu method [5], soluble protein concentration by the Bradford method [6], and antioxidant activity by the method of radical DPPH [7].

The parameters that have the greatest influence on the extraction of proteins and phlorotannins are the solid/liquid ratio followed by temperature, both contributing to the extraction of compounds by 72.08 and 84.21%, respectively; obtaining an extraction yield of phlorotannins and proteins of 2.2 and 6.1%, respectively. Considering the contribution of these parameters and the optimal values of each parameter predicted by the experimental design, it suggests that the best extraction condition is the solid/liquid ratio of 15, and extraction time of 180 minutes at a temperature of 120°C. The parameters influencing on the antioxidant activity extracts are temperature followed by extraction time with a 37% and 27%, respectively.
Finally, the increasing enzyme concentration of oligo alginate lyase, 0.045% v/v, leads to better yield from phlorotannin extraction by 54%. A 6% increase was also observed in the amount of phlorotannins when the time of pretreatment was increased by 2 days.

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PRODUCCIÓN BIOMASA, PIGMENTOS Y LÍPIDOS POR *Chlorella vulgaris*, ACOPLADA A ELIMINACIÓN N Y P DE LAS AGUAS RESIDUALES

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Resumen
La producción de biomasa de microalgas genera productos de alto valor económico tales como proteínas, hidratos de carbono, pigmentos, vitaminas, lípidos y otros productos químicos finos. Por otro lado, las microalgas son capaces de eliminar fósforo P y nitrógeno N de las aguas residuales municipales durante el crecimiento autotrófico. El objetivo de este trabajo es el desarrollo de una tecnología para la producción de biomasa, pigmentos y lípidos, junto a las aguas residuales de tratamiento terciario (es decir, la eliminación de N y P).

La cepa *Chlorella vulgaris* se cultivó durante 15 días en cuatro aguas residuales con y sin tratamiento (es decir, ARNT aguas residuales no tratadas, ARNT/E esterilizados aguas residuales no tratadas, ARNT/FE esteriliza y se filtra las aguas residuales no tratadas, y las aguas residuales tratadas ART) y el medio de BBM definido (que contiene nitratos como fuente de N). Filtración consistió en pasar el ARNT por un filtro de 1,2 µm. La esterilización se llevó a cabo durante 15 minutos. Las aguas residuales se tomaron muestras de la forma del San Juan Ixhuatepec, Edo. de México. Los cultivos se desarrollaron en matraces de 1000 ml Erlenmeyer, agitados a 150 rpm. Se emplearon períodos oscuridad/iluminacion 12:12 y la intensidad de iluminancion de 100 µmoles fotones/m2s. La temperatura se controló en 22+/-oC. La biomasa se midió usando un peso en seco. Los pigmentos se determinaron utilizando los valores de absorbencia, específicos para ciertos pigmentos. Los lípidos se midieron usando la técnica de disolvente caliente. Nitrógeno como fracción N-orgánico se midió de acuerdo a la norma mexicana NMX-AA-026-SCFI-2010, mientras que P como ortofosfatos se midió como se describe por Taussky y Shorr (1953). Las aguas residuales tratadasy no tratadas se caracterizaron en términos de algunos de los siguientes parámetros: pH, conductividad, DQO, sólidos sedimentables, PO_4, NO_3, $N_{orgánicp}$, N_{NH4} y $N_{tot\ Kjeldal}$. Al final, se evaluó el efecto del pH inicial de las aguas residuales sobre la producción de microalgas y de lípidos. Se evaluaron los pH iniciales de 6,25, 7,25 (original), 8,25 y 9,25.

Las aguas residuales mostraron marcadas diferencias en cuanto a la materia y nitrógeno orgánicos contenidos en las diferentes formas señaladas. *Chlorella vulgaris* creció en todas las muestras tratadas y no tratadas. Como se muestra en la figura 1, el valor más alto de la biomasa fue para el agua ARNT, seguido por ARNT/E y ARNT/FE y ART, se observó el menor crecimiento cuando se utilizo el medio BBM. Las productividades de biomasa estuvieron en el rango de 40,1 y 50,7 mg/L.dia para BBM y ART, respectivamente. La producción de lípidos fue diferente para cada medio evaluado. Para estas evaluaciones, los lípidos tenían una concentración de entre 15 (BBM) y 111,7 (ARNT/E) mg/L.

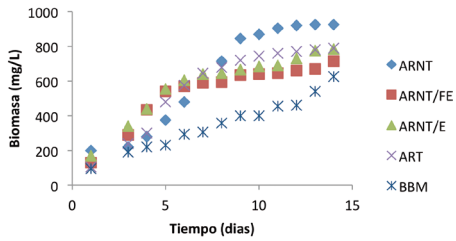

Figura 1. Producción de Biomasa por Chlorella vulgaris en diferentes medios.

En cuanto a la eliminación de N, *Chlorella* creciendo en ARNT y aguas residuales ART eliminó hasta el 91 y 80,4% de NKjeldal total a partir de la concentración original en comparación con el experimento en el que se utilizó BBM (eliminación de 33,5%). Por último, Chlorella creciendo en las aguas residuales ART fue capaz de eliminar el 82% del PO_4 originales presentes en el medio. El valor de pH inicial ART tuvo un efecto sobre el crecimiento de la microalga y lípidos producción.

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OIL EXTRACTION PROCESS AS PRE-TREATMENT PROCESS FOR MESOPHILIC AND THERMOPHILIC ANAEROBIC DIGESTION OF MICROALGAE *N.gaditana*

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Most of the efforts to take advantage of microalgae as a source of bio-energy have been directed to biodiesel production. Despite the advantages above mentioned, there is concern related to a potentially low energetic yield in the biodiesel-from-microalgae production process using current technologies [1]. Indeed, some authors have calculated a negative energetic balance, with the largest production costs associated with harvesting and drying steps [2].
In this scenario, anaerobic digestion of the residual biomass (spent biomass) seems to be a promising strategy, due to the energy recovery in the form of biogas, the potential re-use of the released nutrients in the microalgae culture and the fact that anaerobic digestion can be used to stabilize the waste biomass and avoid other costs related to its disposal and management [3]. Few studies have evaluated the energetic contribution of anaerobic digestion in the biodiesel production process from microalgae. However, these studies have indicated that a considerable part of total energy contained in the biomass can be recovered if anaerobic digestion of spent microalgae is applied [4].
In relation to anaerobic digestion of microalgae, the main drawback indicated in reports was associated with degradability of microalgae. Even, many efforts have been directed to evaluate different pre-treatments in order to improve anaerobic digestion. To date, some authors have indicate that lipid oil extraction could acts as pre-treatment improving microalgae degradability in anaerobic digestion. This research evaluated the effect of oil extraction in anaerobic digestion considering physical effects on microalgae structure.
The effect of lipid extraction on anaerobic digestion was evaluated considering microalgae *N.gaditana*. Anaerobic digestion of total and spent microalgae was carried-out under mesophilic and thermophilic conditions. Results indicates that anaerobic degradability increased when spent microalgae was used as substrate for both anaerobic conditions. Thus, lipid extraction acts as pre-treatment increasing anaerobic degradability and hence, biogas production. In order to evaluate physical effect of lipid extraction on microalgae, observation in scanning electronic microscopy was carried-out showing that lipid extraction affects cell structure (weakness) and no cell lysis was evidenced. Thus, lipid extraction increase anaerobic degradability and hence, biogas production.

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MIXOTROPHIC CULTURE OF THE COLOMBIAN NATIVE MICROALGAE *Chlorella sp.* AS ENHANCER OF THE BIOMASS PRODUCTIVITY AND THE NUTRIENTS REMOVAL IN WASTEWATER

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At present time, biodiesel is the principal substitute of diesel. This biofuel is produced by the transesterification process, mainly from lipids of oilseeds, and microalgae. This microorganism has a productivity that can surpass other terrestrial feedstock used in biodiesel production, besides of being promising for other applications under the biorefinery concept [1]. It also has the capacity to remove nutrients from wastewater (WW) as it is rich in phosphate, nitrate and COD. For these reasons, the microalgae can be used with a double purpose, for bioremediation and to obtain biomass and lipids to produce biodiesel. But the efficiency and production of biomass using this growth media depends on its composition and the treatments applied. Therefore, this project evaluates the growing of the Colombian native microalgae *Chlorella sp* in Medium Basal Bold (MBB) and Sorokin & Krauss (S & K), to compare it with its growing in the wastewater coming from the secondary treatment of the PTAR of the Universidad Autónoma de Occidente. The *Chlorella* was inoculated at 0,05 g/L in 500 ml Boeco bottles that contained 350ml of culture media (WW). The air was supplied by bubble and the microalgae was set to grow with a 12:12 light/dark cycle, at 25 ± 1°C and at a light intensity of 650 lux. During 14 consecutive days, 1 ml samples were taken each day to track the biomass, and nitrate, phosphate and COD removal. Biomass was quantified by spectrophotometry at 760 nm from a calibration curve made in deionized water. Each of the trials was realized three times each. A physicochemical characterization (17 parameters) was made in the WW by Hach [2] protocols. To evaluate if carbon was a limiting factor to microalgae growth, both an inorganic ($NaHCO_3$) and organic (glucose) carbon source was added into each culture media, in different doses: 0.5 and 0.1 g/l.
As a result, it was found that the WW had a lower concentration of macronutrients in relation to the values obtained in other synthetic media, such as 6 mg/L of nitrates and phosphates, and 8 mg/L of sulfates. The concentration of orthophosphates was 50 times lower than the obtained in synthetic mediums. The prevailing form of nitrogen was ammoniac with a value of 50 mg/L. Micronutrients were found in a concentration 100 times higher than those obtained in the MBB, showing the following concentrations: 1,9 mg/L of total iron, 0,7 mg/L of total manganese and 0,16 of total copper. In spite of the differences in the composition it was found that the microalgae in WW had a productivity 26.8% higher than S &K media and 11.1% higher than BBM. A higher availability of micronutrients and nitrogen in ammoniac form [3] [4] might have influenced the results. In turn, the microalgae served to bioremediate the WW, removing 65% of nitrate and 26.3% of phosphate on the third day of culture. The presence of organic and inorganic carbon increased the biomass productivity by 40% and favored the removal of the nutrients to the lowest dose of 0.5 g/l, demonstrating that native *Chlorella sp* can grow mixotrofically.

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PRODUCCIÓN DE LÍPIDOS PARA BIODIESEL EN MICROALGAS (*Neochloris oleoabundans*) USANDO RESIDUOS LÍQUIDOS DE LA INDUSTRIA CERVECERA COMO MEDIO NUTRITIVO

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La amenaza del agotamiento de las reservas de combustibles fósiles en el mundo, ha establecido la necesidad de buscar nuevas fuentes energéticas renovables. En este trabajo se estudió el efecto de la intensidad de luz y concentración de CO_2 en el crecimiento y acumulación de lípidos en microalgas oleaginosas *Neochloris oleoabundans*, para evaluar su factibilidad en la elaboración de biocombustibles. Se utilizaron Residuos Industriales Líquidos (RILes), provenientes de una planta elaboradora de cerveza como medio nutritivo para cultivo. El cultivo (inóculo al 20% peso) se realizó en mangas de polietileno transparente (10L) y fue sometido a 2 tratamientos en duplicado: T1 (350µmol $s^{-1}m^{-2}$ y 5 % vol CO_2) y T2 (100 µmol $s^{-1}m^{-2}$ y 0,3% vol CO_2). El nivel de luz PAR fue monitoreado mediante un medidor de Radiación Fotosintéticamente Activa (Spectrum). Un flujo de alimentación controlado por flujómetro consistió de CO2/aire (0,5 vvm) y la temperatura de la sala de cultivo se mantuvo a 24°C±1°C.

A. Crecimiento de la biomasa | B Montaje experimento | C. Acumulacion de lípidos

El crecimiento de biomasa fue monitoreado cada 24 h mediante espectrofotometría UV/Vis (600 nm) durante 10 días. La acumulación de lípidos se cuantificó por colorimétría Sulfo-fosfo-vainillina. Se cuantificaron lípidos totales mediante extracción Bligh & Dyer y un posterior fraccionamiento en lípidos neutros, glicolípidos y fosfolípidos. Los perfiles grasos respectivos se identificaron mediante GC/MS. El tratamiento T1 permitió un mayor rendimiento de biomasa alcanzando 0,57 mg L^{-1} mientras T2 sólo alcanzó un promedio de 0,37 mg L^{-1} tras 10 días de cultivo. Por su parte, al igual que la máxima producción de biomasa, la acumulación de lípidos alcanzo su máximo (55% peso) con T1, mientras el T2 sólo logró un 39%. En ambos los casos las mayores acumulaciones de lípidos se logran entre el día 9 y 10 de cultivo.T1con mayor generación de lípidos presentó un perfil con 40% de ácidos grasos poli insaturados con un 21% de C18:2 en la fracción útil para biodiesel (61,3% lípidos neutros). De acuerdo al estándar EN 14214 [1], el límite máximo de C18:2 es de 12%. Bajo este punto de vista, el aceite obtenido a partir de estas microalgas supera la norma y no cumple los requerimientos para la elaboración de biodiesel, Por otra parte, el estudio sí demostró, que a partir de un experimento sencillo, la microalga puede crecer y acumular lípidos utilizando RIL de cervecería, presentando una ventaja al utilizar un residuo, tener un impacto positivo en la economía del proceso y perfilarse como una alternativa de tratamiento para residuos industriales líquidos.

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DESIGN AND FABRICATION OF A PY-REACTOR FOR PRODUCING BIO-OIL FROM MICROALGAL BIOMASS

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Fast pyrolysis process consists in a thermo-chemical degradation of biomass in absence of oxygen. This process occur at low residence time (lower than 2 s) and at temperatures in the range of 300-550°C. The main product of this process is bio-oil, and less extent biochar and syngas. Bio-oil is a dark brown liquid with a high energy density that can be used as biofuel. Microalgae biomass is an interesting raw material to produce bio-oil, biochar and syngas. Indeed the bio-oil from microalgal biomass is characterized by a higher hydrogen content and lower oxygen content compared to lignocellulosic bio-oil, favoring higher calorific value. In spite to the previous, in our research group, the performance of the conventional pyrolysis reactor implemented have not allowed getting high bio-oils yields due mainly to the poor performance of the pyrolysis reactor. In fact, the high syngas production obtained in our previous studies (until 54%) could be related with a poor Electrostatic precipitator (ESP) performance, being important quantities of the condensate fractions released to the syngas fraction. Therefore, considering that there is not equipment designed specially to fast pyrolysis process of microalgal biomass, the aim of this works consisted in design and implement a fast pyrolysis reactor for producing mostly bio-oil from microalgal biomass. The details of the reactor are presented in Fig. 1. The dimensions of the whole equipment were 2.3 m in height, 2.4 m in length and 0.65 m in width. The reactor was made of steel with a preheated zone or oven (1510 mm in length and I.D 440 mm) and a pyrolysis zone (930 mm in length and I.D. of 40 mm). The oven was made with two concentric tubes. The equipment is a semi-continuous reactor that can works until 700°C. Comprise a feed system to the continuous injection of microalgal biomass using N2 as gas carrier to inject the biomass. Additionally, the gas carrier allows regulate the residence time of the vapors and gases inside to the reactor. The equipment includes a biochar storing systems. To avoid the drawbacks of the previous reactor, the new pyrolysis reactor included two condenser which will allow to catch the mostly the bio-oil produced. In addition, an ESP with a power supply of 40 volts will allow to increase the residence time of the aerosols commonly produced in high quantities when microalgal biomass is pyrolyzed.

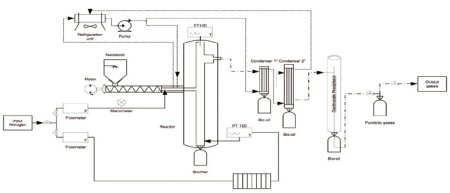

Fig. 1: Schematic fast pyrolysis reactor

Acknowledgements
Proyecto IDEA FONDEF CA13I10145.Thermalconversion of depleted microalgal biomass for the production of bio -oil, syngas and biochar by product after extracting a high value

ROLE OF PROCESS SYSTEM ENGINEERING FOR INTEGRATED BIOREFINERY RISK ANALYSIS

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When performing techno -economical assessment of biorefineries, there is a high uncertainty in all parameterizations and process assumptions. The state of the art of bioconversions is not a matured knowledge, so a novel risk analysis approach is needed. Risks to be taken into account are: the feedstock availability risk, technological risk, products market risk, sustainability risk, and financial risk. Except for the sustainability issues, all the other feedstock, products and financial investment risks are, in some sense, dynamically money valued risks. The current status of the methodology for selecting routes and technologies are briefly described here. After the step of the process selection, the risks are described from the methodology view of point. Each one of the risks analysis associated with the methodology has his own associated uncertainty. Finally the published works on biorefinery plants risk analysis is reviewed. This would be the open contribution of Process System Engineering. The present paper claims that the modern third generation biorefinery, that is, highly integrated bio-materials, bio-chemicals and bio-energy production is very complex for conventional techno-economic analysis and it is an opportunity to research methodology from process engineering and financial analysis areas. This extensive and comprehensive review of published works is expected to be one of the roadmaps of project engineers and investment analysts.

In vitro PREPARATION OF SELF-ASSEMBLED SUPER-SWOLLEN HYDROGELS FROM SOLUTIONS OF LIGNOCELLULOSE IN N,N-DIMETHYLACETAMIDE/ LITHIUM CHLORIDE

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Super-swollen stable hydrogels have been prepared from lignocelluloses of various origins via in vitro direct dissolving in N,N-dimethylacetamide/lithium chloride solution (DMAc/LiCl) and subsequent spontaneous regeneration from the solutions. The main physico-chemical properties of the hydrogels such as equilibrium solvent content, swelling, porosity and dye uptake have been determined. They revealed that the hydrogels retained a large amount of water (up to 2500 wt. %), had high values of the porosity and the dye uptake and developed specific surface areas. The formation of the hydrogels occured via spontaneous self-assembly from the solutions due to reconstruction of a new hydrogen bond web in the cellulose chains.

EVALUATION OF WILD CARDOON AS A SOURCE OF BIOMASS IN CHILE

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Cardoon (*Cynara cardunculus*) is a perennial very deep-rooted herb of Mediterranean origin that although it is not cultivated in Chile, it is widely distributed in the territory as a weed. The potential of cardoon as an energy crop mainly lies in its application as solid biofuel. The main characteristics of the crop that suggest this application are: low crop inputs, large biomass productivity (14-20 t dry biomass ha^{-1} $year^{-1}$), low moisture content (14%) of the biomass at harvest, biomass composition mainly lignocellulosic, and higher heating value (16 MJ kg^{-1}). Cardoon germinates whenever the soil moisture and temperature are favorable. Hence, the sowing time can be autumn or spring in the Mediterranean climates. For energy purposes, mechanical harvest is similar to cereals. The aim of this work was to carry out the characterization of cardoon stems growing wild in Chile and to evaluate the pellets as solid fuels produced from this biomass. Measured responses were pellet bulk density, pellet durability and amount of fines. A proximate analysis was performed according to the ASTM standards. In particular, the percentage of volatile matters varies from 72 to 74 wt%, while the fixed carbon content ranges between 12.8–14.7 wt%. A higher ash content compared to other solid biofuels is observed, which accounts for 7.8 – 8.3 wt%, which contained high levels of Na, K, P, Ca, and Si. However, the high ash values are characteristic for cardoon biomass, since this varies between 4% and 17% on dry basis. Additionally, the samples analyzed showed comparable results with other studies made in Europe in the nitrogen and sulphur content. More specifically, nitrogen is lower than 1.7 wt% on dry basis, while sulphur equals to 0.1 wt% on dry basis. The higher calorific value (HHV) was approximately 16.7 MJ/kg. On the other hand, durability is related to the effectiveness of densification. Pellets should endure different efforts during shipping, loading and transportation to the final destination. Otherwise, considerable amounts of dust might be generated, going into the combustion chamber without burning and increasing emissions and wastes at home, which could imply health risks (explosive atmospheres, respiratory diseases, etc.). Cardoon pellets had mechanical durability values above 95%, and showed values of bulk density and amount of fines of 685 kg m^{-3} and <0.5% respectively. This indicates that wild cardoon from Chile can be utilized as an acceptable energy source. However, future works are necessary in order to improve cardoon biomass quality and to evaluate the possibility of using it in blends with other biomass sources.

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SOLID FUELS PRODUCED FROM OLIVE POMACE

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Chilean olive industry has had a significant and sustained growth over the last decade and each year new hectares of olive orchards are added to the production of virgin olive oil and new facilities for the extraction process are installed. High growth of this industry has resulted in significant generation of organic residues, which are called olive pomace. This residue is the solid by-product obtained from the extraction of olive oil. It consists of pieces of skin, pulp and stones. In Chile, more than 50,000 tons per year of olive pomace is generated, therefore the management and utilization of this residue is a major concern of all olive oil producing plant. Because of the high heating value of olive pomace, which is equivalent to 4,500 kcal/kg for dry stone and 4,100 kcal/kg for dehydrated olive pomace (pulp, skin and stone), it presents an interesting potential for generating densified solid biofuels (pellets). In this study the use of olive pomace was evaluated for obtaining densified solid biofuels. The results showed a low moisture content of the olive pomace pellets, which complies with the DIN 51731 standard. The pelletized olive pomace reaches a bulk density in the range of 1.06 to 1.13 g/cm^3. This reduces the volume of material and facilitates its handling, storage and transport. Olive pomace pellets have a significant energy potential due to the high calorific value obtained in the different treatments, very similar to those commonly reported for pellets from forest residues and well above the values required by DIN 51731 standards. This is attributed to the high lipid content of this residue. The sulfur and chlorine content are low in all the treatments, thus avoiding the generation of sulfur oxides during combustion and excessive corrosion of the combustion equipment. The chlorine content is an important quality parameter of biofuel because high chlorine content can create problems of corrosion and fouling in combustion equipment. The treatments evaluated showed low chlorine content, which are close to the limit set by the DIN standard. The ash content of the olive pomace pellets exceeded the maximum value set by DIN standards in all treatments evaluated. However, these values are within expected for densified fuels made from agricultural and agro-industrial biomass. The high ash content of olive pomace pellets could probably lead to ash slagging for burning temperatures 900-1000°C. Further experiments are needed to find the degree of sintering of the ash deposits.

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History

Iberoamerican Congress on Biorefineries

The Iberoamerican Congress on Biorefineries (CIAB) is the main scientific event of SIADEB – The Iberoamerican Society for the Development of Biorefineries. There have been two prior events:

1-CIAB
1er. CONGRESO IBEROAMERICANO SOBRE BIORREFINERIAS

CIAB2012: **1st Iberoamerican Congress on Biorefineries.** From October 24 to 26, 2012, San Jose de Los Cabos (BC), Mexico. Organizers: University of Guanajuato, Cinvestav, Universidad Michoacana de San Nicolás de Hidalgo (University) and SIADEB.

2-CIAB
2°CONGRESO IBEROAMERICANO SOBRE BIORREFINERIAS

CIAB2013: **2nd Iberoamerican Congress on Biorefineries**. From April 10 to 12, 2013, Jaén, Spain. Organizers: University of Jaén, IFEJA and SIADEB.

Latin American Congress on Biorefineries

To date, there have been three versions of the Latin American Congress on Biorefineries in Chile. These events have been a platform for information and discussion of latest trends in the development of processes and products from agricultural, forest and algal biomass.

Primer Congreso Latinoamericano
Biorrefinerías
Oportunidades de innovación para el sector forestal
21 y 22 de noviembre, Concepción / Chile

First Latin American Congress on Biorefineries, Innovation Opportunities for the Forest Industry. From November 21 to 22, 2006, Concepción – Chile. Organizers: Technological Development Unit of the University of Concepción and Fraunhofer Institut Umsicht, Germany.

II CONGRESO LATINOAMERICANO
Bio refinerías
Materiales y Energía
4, 5 y 6 de mayo de 2009. Termas de Chillán, Chile.

Second Latin American Congress on Biorefineries, Materials and Energy. From May 4 to 6, 2009, Chillán – Chile. Organizers: Technological Development Unit of the University of Concepción and Technical Association of Pulp and Paper Professionals, Chile.

III Congreso Latinoamericano
Biorrefinerías
Ideas para un mundo sustentable
19 al 21 de noviembre de 2012, Pucón, Chile

Third Latin American Congress on Biorefineries, Ideas for a Sustainable World. From November 19 to 21, 2012, Pucón – Chile. Organizers: Technological Development Unit of the University of Concepción and Center for Biotechnology, University of Concepción and the Scientific and Technological Bioresource Nucleus of the University of La Frontera.

International Symposium on Lignocellulosic Materials

The 1st International Symposium on Lignocellulosic Materials was held on August 20 and 21, 2013, together with the 13th Congress on SAM-Conamet Materials. The event took place at the Convention Center of the Amerian Portal Hotel in Iguazu, Argentina, and was organized by the *Instituto de Materiales de Misiones* (Institute) (IMAM, UNaM-CONICET), the Net Value Added Products from agricultural and forest-industrial waste (PROVALOR, CYTED), the Iberoamerican Network for Teaching and Research on Pulp and Paper (RIADICYP) and the VTT Technical Research Centre in Finland.

In addition to invited lectures given by experts from Austria, Brazil, Spain, Finland and Mexico, the program included some 100 papers between lectures and posters of outstanding researchers from 18 countries in America, Europe and Asia. The topics ranged from biorefinery based on lignocellulosic materials to cell wall deconstruction; from nanotechnology to micro- and nano-cellulose and their potential applications; composite materials and nanocomposites; polymers from renewable resources; bio-natural adhesives; advanced analytical techniques; advanced materials from paper and cardboard; and recycling of complex lignocellulosic materials.

The papers presented at the 1st International Symposium on Lignocellulosic Materials can be downloaded from the RIADICYP website (www.riadicyp.org).

3rd Iberoamerican Congress
4th Latin American Congress
2nd International Symposium on Lignocellulosic Materials

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