

EXTRACTION OF HEMICELLULOSES PRIOR TO KRAFT COOKING: A STEP FOR AN INTEGRATED BIOREFINERY IN THE PULP MILL

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SUMMARY

Two treatments, an induced auto-hydrolysis and an acid-hydrolysis, were applied to *Eucalyptus globulus* wood chips prior to the cooking stage, to extract the hemicellulosic fraction that otherwise would be dissolved in the black liquor and burnt in the recovery boiler. The obtained hydrolysates, rich in xylose, were detoxified by overliming and used for ethanolic fermentation. Impacts of each wood pre-treatment on the kraft cooking process and on the quality of the produced pulp were evaluated. Both pre-treatments promoted an increase in the cooking rate but had a negative effect on pulp quality and overall yield. Auto-hydrolysis showed a less negative influence. However, auto-hydrolysates led to lower values of ethanol concentration, productivity and yield compared to the fermentation of acid-hydrolysates. To get more profit from the auto-hydrolysates they were also submitted to secondary-acid-hydrolysis and vacuum evaporation processes. Overliming followed by evaporation (with a concentration factor of 3) gave better results than the inverse method. This procedure raised the fermentable sugar content and led to the production of ethanol with a concentration of $\sim 10 \text{ g}_{\text{eth}} \text{ L}^{-1}$ (productivity of $0.23 \text{ g}_{\text{eth}} \text{ L}^{-1} \text{ h}^{-1}$ and yield of $0.50 \text{ g}_{\text{eth}} \text{ g}_{\text{xy}}^{-1} \text{ eq}^{-1}$) which compares well with the results obtained with the fermentation of acid-hydrolysates.

KEYWORDS: bioethanol, biorefinery, *Eucalyptus globulus*, hemicelluloses, hydrolysis.

INTRODUCTION

An Integrated Forest Biorefinery incorporates the lignocellulosic biomass conversion processes into fuels (e.g. bioethanol) and chemicals in addition to energy and traditional products manufacture (pulp and paper) of an existing chemical pulp mill, enabling competitive advantages [1]. Portuguese pulp and paper industry, which uses *Eucalyptus globulus* wood, produces high quality pulp and W&P paper, internationally recognized. In the majority of the mills, kraft liquors are used to dissolve and degrade the wood lignin. Along with lignin, nearly 50% of hemicelluloses are partly dissolved in the liquor [1-3]. Hemicelluloses degradation products, mainly a complex mixture of sugar acids, are difficult to separate and purify from the resulting black liquor. This stream is then conducted to a recovery boiler where it is burned to produce electricity and thermal energy. However, the calorific content of the hemicelluloses is lower than lignin and its energetic value could be better profited by introducing a pre-treatment to wood chips, prior to kraft pulping, to extract and use them as feedstock for sugar-based polyester polymers, chemicals or transportation fluids (e. g. bioethanol) produced by fermentation [1-3]. Nevertheless, the final pulp yield and quality should be preserved even with the hemicelluloses pre-extraction process. Therefore, this pre-treatment should solubilise the hemicellulosic fraction under optimized conditions, leaving a solid phase enriched mainly in cellulose but also in lignin which will be further processed in the kraft cooking stage and originating the final pulp [1-3]. Acid- and auto-hydrolysis are water-based methods that can be used to extract hemicelluloses from the wood chips [1-3], before the cooking stage. Acid-hydrolysis is usually carried out with inorganic acids at high temperatures, from seconds to minutes, originating high yields of soluble sugars, mostly monosaccharides [4]. Auto-hydrolysis is driven by temperature but the acetic acid released from xylan hydrolysis provides smooth acid conditions [4, 5]. To extract the eucalypt wood hemicelluloses prior to pulping these wood pre-treatments were used in our previous works:

acid-hydrolysis was carried out with sulphuric acid 0.4% (w/w) at 140 °C whereas auto-hydrolysis was performed at 150 °C, both for different reaction times [2, 6, 7]. Wood acid-hydrolysis promoted higher monosaccharides extraction yield favouring the ethanolic fermentation [2, 7]. However, the auto-hydrolysis showed, generally, a less negative influence on pulp quality and overall yield [2]. The resulting eucalypt hemicellulosic hydrolysates comprise C5 sugars, mainly xylose, and small amount of C6 sugars, like glucose, mannose, and galactose, besides some oligosaccharides. Since auto-hydrolysis is a milder pre-treatment than acid-hydrolysis, oligosaccharides are the major carbohydrates in auto-hydrolysates contrarily to acid-hydrolysates. In fact, a preliminary study [6] showed that auto-hydrolysates must be further hydrolysed to convert the oligosaccharides into monosaccharides before they can be used as carbon and energy source for the fermentation species [8]. A secondary hydrolysis was then carried out with sulphuric acid 4% (w/w) under reflux to increase the monosaccharides content [6]. During hydrolysis reactions, furfural, 5-hydroxymethyl furfural and formic acid may be formed from sugar degradation, and acid-soluble lignin derivatives are also extracted. Their presence may inhibit the microbial metabolism and, consequently, the fermentation of the hydrolysates shows a slow kinetics, with limited yield and productivity [8]. *Pichia stipitis* (optimal pH range 5.5-6.5) was the microorganism selected due to its ability to co-ferment a variety of sugars, particularly xylose, producing ethanol with high yields (up to 0.41 g g⁻¹) [9-11]. In our previous study [7], it was observed that lignin strongly inhibited the yeast metabolism. On the other hand, furfural up to 0.8 g L⁻¹ did not influence the yeast performance [7]. Several detoxification methods have been proposed to reduce the inhibitors concentration or transform them into inactive substances [12, 13]. Chemical detoxification processes comprise, among others, the precipitation of toxic compounds and ionization of some inhibitors under certain pH values [12], the adsorption on activated charcoal [13] or on ion-exchange resins [13]. Physical detoxification methods include vacuum evaporation process to reduce the concentration of volatile compounds, which also helps to increase the sugar content. However, it increases the concentration of non-volatile compounds as well (lignin derivatives) [5]. Two different chemical treatments based on pH value change (with different alkalis and methodology) were tested in earlier assays to detoxify the eucalypt hemicellulosic hydrolysates [2, 7]: i) Ca(OH)₂ or NaOH was added till pH 6.5 was reached; ii) Ca(OH)₂ or NaOH was added till pH 10 was achieved followed by sulphuric acid addition till pH 6.5, after precipitates removal. The treatment that enabled the best fermentation results obtained so far was the pH adjustment to 10 with Ca(OH)₂, commonly referred as overliming, followed by acid addition till pH 6.5 [2, 7]. Overliming has been one of the most efficient and less expensive methods to detoxify lignocellulosic hydrolysates [12]. Moreover, the physiological adaptation of *P. stipitis* cells to the hydrolysate was used with success to decrease some inhibition effects because it enabled decreasing the lag phase both in yeast growth and in ethanol production, thus improving ethanol productivity [7]. The same advantage has been observed in other works [10]. Our former assays showed that the fermentation of overlimed acid-hydrolysates led to higher ethanol concentrations [2, 7]. Up to 12.3 g L⁻¹ of ethanol was obtained, at a production rate of 0.221 g h⁻¹ L⁻¹ and with a yield of 0.49 g g⁻¹ based on the reducing sugars consumed [7], which compares well with the literature data [9-11]. On the other hand, only ~2.0 g L⁻¹ of ethanol was achieved in the fermentation of overlimed secondary-auto-hydrolysates, with a low productivity (0.079 g h⁻¹ L⁻¹), but high yield (0.47 g g⁻¹) [2].

The purpose of this work was to improve the fermentation performance of eucalypt wood hemicelluloses, extracted prior to kraft cooking, by using an auto-hydrolysis pre-treatment, and by applying a secondary-hydrolysis and a vacuum evaporation processes to raise the monosaccharides content. The goal was to achieve or even to overcome the ethanol amount produced in the fermentation of acid-hydrolysates. Impacts of the auto- and acid-hydrolysis conditions on kraft pulping and ethanolic fermentation results are compared, in order to analyse the viability of this biorefinery concept.

EXPERIMENTAL

Pre-hydrolysis of Eucalyptus globulus wood

As described in previous studies [2, 6-7], several hydrolysis conditions were performed in rotary

reactors, where 200 g of *Eucalyptus globulus* wood chips (dry basis) were mixed with the extraction liquor (liquor-to-wood ratio of 4:1), to check their effects on hydrolysis yield, hydrolysates composition, cooking conditions and pulp quality. The present work emphasizes four sets of operating conditions: AuH120 and AuH180 - an auto-hydrolysis carried out in water, at 150 °C during 120 min or 180 min, respectively; AH120 and AH180 - acid hydrolysis catalysed by 0.4% (w/w) sulphuric acid, at 140 °C for 120 min or 180 min, respectively. The extracted wood was washed with water and used to produce pulp, whilst the collected hydrolysates were used as raw-material for ethanolic fermentation.

Secondary-hydrolysis of oligosaccharides

A secondary hydrolysis was applied on wood hydrolysates obtained from the primary-hydrolysis, to convert the oligosaccharides into monosaccharides. A preliminary study following a factorial design [6] showed that the secondary hydrolysis is worthless upon the acid-hydrolysates, as it induced monosaccharides degradation. Contrarily, when secondary hydrolysis was performed on the auto-hydrolysates, the monosaccharides content increased up to twice its initial value. This was observed when secondary hydrolysis conditions were carried out with 4% (w/w) H₂SO₄, at 100 °C for 180 min. From now on these hydrolysates will be referred to as secondary-auto-hydrolysates (labelled SAuH).

Kraft pulping and EFC bleaching

Original wood and extracted wood samples (by auto- and acid-hydrolysis) were submitted to kraft cooking in rotary reactors as described elsewhere [2]. The corresponding unbleached pulps were submitted to the bleaching sequence, DEDED (D – chlorine dioxide stage; E – NaOH extraction stage) using a consistency of 10%, also detailed elsewhere [2].

Detoxification and concentration of hydrolysates

Acid- and secondary-auto-hydrolysates were detoxified by overliming, since this method has led to the best fermentation results so far [2, 7]. Ca(OH)₂ was added until pH 10 was reached and the precipitates formed were removed by centrifugation (2500 rpm for 15 min). Sulphuric acid was then added to the hydrolysates until pH ~6.5 was achieved, followed by filtration. These detoxified hydrolysates were used as fermentation culture media. In a second set of experiments the secondary-auto-hydrolysates were submitted to concentration by vacuum evaporation, follow by detoxification by overliming. The vacuum evaporation process was carried out in a 1 L rotary evaporator, at 70°C and 200 mbar, to obtain a reducing sugar content of 30 g L⁻¹ (concentration factor of 3). In a third set of experiments, secondary-auto-hydrolysates were first detoxified and then concentrated by vacuum evaporation under the same conditions, to study the effect of the sequence of these two steps on the inhibitors and sugars concentration. A higher concentration factor was also tested within this set of trials, in which a sugar content of 60 g L⁻¹ was obtained (concentration factor of 8).

Microorganisms, culture media and fermentation conditions

Pichia stipitis DSM 3651 (provided by DSMZ, Germany) was the yeast selected to ferment the eucalypt wood hydrolysates. The yeast was grown in liquid synthetic media containing xylose (10 g L⁻¹), malt extract (3 g L⁻¹), yeast extract (3 g L⁻¹), peptone (5 g L⁻¹) and transferred to solid synthetic media on agar slants, being sealed at 4 °C. To enhance the yeast performance in the hydrolysates fermentation process, *Pichia stipitis* cells were progressively transferred from the synthetic solid media into agar slants containing increasing percentages of detoxified acid-hydrolysates (30, 50 and 75% (v/v), diluted in water, and non-diluted, *i.e.* 100% v/v), supplemented with the amount of peptone, yeast extract and peptone referred above, added as powder [7]. Natural liquid media, *i.e.* the supplemented and detoxified acid- and secondary-auto-hydrolysates (concentrated and non concentrated) were then inoculated with the physiological adapted yeast. The inocula were prepared with the same natural culture media that were intended to be used. Batch fermentations were performed in 250 mL Erlenmeyer flasks with a cotton plug, in an orbital shaker (Stuart S150) at 150 rpm and 30°C. 150 mL of natural medium (hydrolysates) and 10 mL of fresh inoculum were used.

Analytical methods

The extraction yield after the two wood hydrolysis processes was measured by the dissolved solids

quantification (evaporation at 105 °C of a sample of the filtrated hydrolysates). For the kraft cooking tests, pulps were characterised in terms of kappa number, hexenuronic acid content, intrinsic viscosity, reflectance and metal content described elsewhere [2]. During the fermentation stage, the sugar content in the hydrolysates samples was measured as reducing sugars using the colorimetric method with dinitrosalicylic acid reagent (DNS). Calibration curves based on xylose were used and the reducing sugars were estimated as xylose equivalents. After the DNS reaction, the absorbance of standards and samples was read at 540 nm in a Beckman D.U. 650 UV-Vis spectrophotometer. Yeast growth was evaluated by reading the optical density of cells suspension at 540 nm in the spectrophotometer. Ethanol and xylose concentrations were analysed by HPLC (Knauer model K 301, RI detector), with PL Hi-Plex Ca 8 μm columns, at 85°C, and ultra-pure water as eluente at a flow rate of 0.6 mL min⁻¹. For DNS method and HPLC analysis samples were previously centrifuged. The samples were also filtered through 0.2 μm pore size Whatman membrane filter for HPLC analysis. Productivity in ethanol, P (g L⁻¹ h⁻¹), was determined dividing the maximum ethanol concentration produced by the fermentation time needed to achieve it. Ethanol yield, y (g g⁻¹), was calculated dividing the maximum ethanol concentration produced by the corresponding concentration of reducing sugars consumed. Fermentation efficiency, η (%), was measured by the ratio between the observed yield (y) and the theoretical yield (0.51 g g⁻¹).

RESULTS AND DISCUSSION

Pre-extraction of wood material

The chemical composition of the *Eucalyptus globulus* wood used in this work was determined elsewhere [15]: 21.5% lignin, 52.5% glucose, 14.8% xylose, 1.4% galactose, 1.2% mannose, 0.4% arabinose, 0.3% rhamnose, 3.5% acetyl groups, 3% methylglucuronic acid, 0.2% ash and 1.3% extractives. Having in mind that about 50% of the wood hemicelluloses are lost in the traditional cooking stage, *i.e.* ~13% of the original wood, the hemicelluloses extracted should not be higher than this value, in order to achieve the same overall pulp yield and quality when using non pre-treated wood. Actually, acid-hydrolysis conditions (AH120 and AH180) had lead to extraction yields of 131 and 160 g kg⁻¹ of wood, respectively, based on the dissolved solids, whereas auto-hydrolysis (AuH120 and AuH180) promoted an extraction of 75 g kg⁻¹ and 125 g kg⁻¹ of wood material, respectively. Considering only the wood extraction yield point of view, an acid-hydrolysis pre-treatment is more efficient, due to a greater cleavage extension of hydrogen and covalent bonds of the lignocellulosic structure. However, stronger negative impacts upon kraft pulping yield and pulp properties are expected especially in the case of the extraction yield had overcome the maximum acceptable value (13% w/w).

Kraft cooking and pulp properties

The repercussions of the above pre-treatments on the kraft cooking process and some pulp properties to reach comparable kappa numbers were studied in a previous work [2] and the main results are shown in Table 1. In general, the wood auto-hydrolysis is shown to be a better option as a pre-treatment prior to cooking. The yield in the cooking stage decreased independently of the pre-hydrolysis nature as a result of carbohydrates removal. Even so, a smaller decrease was observed for the auto-hydrolysis pre-treatment. The overall yield, including both pre-hydrolysis and cooking stages, was obviously lower due to the extracted material in the first one: 45.0 and 39.7%, respectively for auto- and acid-hydrolysis. An extra consumption of alkali (lower residual effective alkali), more noticed in the wood acid-hydrolysis was also observed. Yoon and van Heiningen (2008) also obtained lower total pulp yields for the kraft cooking of hot water pre-extracted pine wood, compared to kraft control cooks as well as an increase in the effective alkali consumed [1]. A decrease in pulp cooking yield was verified by Helmerius et al (2010) as well for birch wood [3]. Table 1 shows that pulp viscosity was severely influenced by acid-hydrolysis conditions. Hence this fact results in a strength loss, which can limit pulps application. Auto-hydrolysis conditions promoted a slight viscosity increase. This fact can be a result of lower cellulose degradation due to the decrease in cooking time (cooking H-factor of 239 h) or to the removal of polysaccharides with low molecular weight in a higher extension than in the traditional cooking process. Minimal differences were registered by Yoon

and van Heiningen (2008) between intrinsic viscosities of the pulps obtained from conventional kraft cooks and kraft cooks of hot-water pre-extracted wood chips [1]. These results suggest that the fibre strength properties will not be significantly altered by an auto-hydrolysis pre-treatment. However, Helmerius et al (2010) detected negative impacts of water extractions in some pulp properties [3]. Table 1 shows that pre-hydrolysis step decreased the reflectance of unbleached pulps. Nevertheless wood pre-hydrolysis promoted a decrease in the ClO₂ consumption in the conventional method used to achieve ISO brightness of 90%, regardless the initial lower reflectance. In addition the pre-treatment prevented the formation of leuco-chromophores thus improving the stability of bleached pulps as shown by the lower brightness reversion values (Table 1) [2].

Table 1. Pulp properties obtained with the untreated *Eucalyptus globulus* wood chips (reference) and wood submitted to auto- and acid-hydrolysis (experiments design to reach a kappa number of ~13.5) [2].

| Wood sample | Overall H-factor ^(a) (h) | Cooking yield (%) | Unbleached pulp viscosity (mL g ⁻¹) | Unbleached pulp reflectance (%) | ClO ₂ consumption (%) ^(b) | Pulp brightness reversion (post-color number) |
|--------------------------------|--|-------------------|---|---------------------------------|---|---|
| Reference | 738 | 54.7 | 1268 | 45.5 | 4.4 | 0.43 |
| Auto-hydrolysis ^(c) | 735 | 51.5 | 1390 | 43.2 | 3.8 | 0.35 |
| Acid-hydrolysis ^(d) | 404 | 45.7 | 908 | 40.9 | 4.3 | 0.16 |

(a) – Including both pre-hydrolysis and cooking stages; (b) – as active chlorine, to reach 90% ISO brightness; (c) – AuH180: 150°C, 180min, H-factor ~500h; (d) – AH120: 140°C, 120min, H-factor ~130h.

Ethanol fermentation of wood hydrolysates

Monosaccharides were determined as reducing sugars and followed during the fermentation processes. A concentration of 36 and 40 g L⁻¹ was observed in the wood hydrolysates obtained from the primary acid-hydrolysis at 140 °C, for 120 and 180 min (AH120 and AH180, respectively). The extraction of hemicelluloses by auto-hydrolysis at 150°C for 120 min (AuH120) generated 6 g L⁻¹ of reducing sugars. Since auto-hydrolysis is a milder pre-treatment than acid-hydrolysis, the corresponding hydrolysates contained a mixture of mono- and oligosaccharides. Therefore, after the secondary hydrolysis of auto-hydrolysates (SAuH120), the reducing sugars concentration raised to 12 g L⁻¹ (a 100% increase). All hydrolysates were then submitted to detoxification by overliming. However, up to 3.6 g L⁻¹ of reducing sugar was lost during this treatment, corresponding to 9 and 29 % decrease in the acid-hydrolysates and secondary-auto-hydrolysates, respectively. Hórvath et al (2005) registered nearly 11% of sugar loss during the overliming treatment of acid-hydrolysates [12]. Other authors observed a sugar concentration decrease up to 17% during the detoxification of acid-hydrolysates with lime [13]. The sugar losses could be explained by the co-precipitation of reducing sugar moieties and/or alkaline degradation catalysed by the presence of calcium ions. The monosaccharides can form the corresponding enolate species, leading to an alkali-induced sugar degradation [12].

Detoxified AH180 acid-hydrolysates were fermented by adapted *P. stipitis* [7], producing up to 12 g L⁻¹ of ethanol, with a productivity of 0.221 g L⁻¹ h⁻¹ and a yield of 0.49 g g⁻¹, based on the reducing sugars consumed (Table 2). An efficiency of 96% on reducing sugars-to-ethanol conversion was determined. Lag phase on yeast growth and ethanol production have been reduced and productivity has been notably increased with the utilization of adapted cells combined with overliming treatment of hydrolysates [7]. Ferrari et al (1992) obtained a maximum ethanol concentration of 12.6 g L⁻¹ with a volumetric productivity of 0.167 g L⁻¹ h⁻¹ and a yield of 0.35 g g⁻¹ based on sugars consumed in the fermentation of *Eucalyptus globulus* wood hydrolysates (~40 g L⁻¹ of monosaccharides) by *Pichia stipitis* [9]. A slightly higher ethanol concentration was produced by Amartej and Jeffries (1996). These authors used corn cob acid-hydrolysates in ethanolic fermentation by previous adapted *P. stipitis* cells. A concentration of 13.3 g L⁻¹ of ethanol was achieved and a yield of 0.41 g g⁻¹ was determined, emphasizing the combined advantages of overliming and strain adaptation [10]. Telli-

Okur and Eken-Saraçoglu (2008) have obtained a lower ethanol concentration in the fermentation of sunflower seed hull hydrolysates containing a bigger amount of total reducing sugars (48 g L⁻¹). An ethanol concentration of 11 g L⁻¹ was produced. The volumetric productivity and yield were 0.065 g L⁻¹ h⁻¹ and 0.32 g g⁻¹, respectively [11]. A lower ethanol concentration (9.6 g L⁻¹) was obtained in the fermentation of AH120 acid-hydrolysates, which slightly milder conditions had released a smaller monosaccharides amount from the hemicellulosic fraction [2]. A productivity of 0.199 g L⁻¹ h⁻¹ and a yield of 0.32 g g⁻¹ (a reducing sugars-to-ethanol conversion of 63%) were calculated [2], as shown in Table 2.

Table 2. Fermentation of *Eucalyptus globulus* hydrolysates by *Pichia stipitis*.

| Hydrolysates treatment | Red. Sugars Conc. ^(a) (g L ⁻¹) | Red. Sugars Consumed (%) | Ethanol conc. (g L ⁻¹) | P (g L ⁻¹ h ⁻¹) | y (g g ⁻¹) | η (%) |
|---------------------------------|---|--------------------------|------------------------------------|--|------------------------|-------|
| AH180^(b) | | | | | | |
| Overliming | 36.4 | 85 | 12.3 | 0.221 | 0.49 | 96 |
| AH120^(c) | | | | | | |
| Overliming | 34.5 | 87 | 9.6 | 0.199 | 0.32 | 62.7 |
| SAuH120 | | | | | | |
| Overliming | 8.5 | 84 | 2.5 | 0.101 | 0.42 | 82.4 |
| Concentrated (3x) Overliming | 19.5 | 88 | 6.0 | 0.218 | 0.42 | 82.4 |
| Overliming Concentrated (3x) | 29.0 | 86 | 9.9 | 0.225 | 0.50 | 98 |
| Overliming Concentrated (8x) | 61.0 | 80 | 7.5 | 0.170 | 0.49 | 96 |

(a) – Reducing sugars concentration after hydrolysates treatment; (b) – data from reference [7]; (c) – data from reference [2]

The fermentation of secondary-auto-hydrolysates produced a lower ethanol concentration due to its lower reducing sugars content, and consequently a low productivity was achieved. Nevertheless a high efficiency on the conversion of the reducing sugars to ethanol was calculated, as it can be seen in Table 2. These results show that *P. stipitis* makes a good use of reducing sugars for ethanol production in the fermentation of secondary-auto-hydrolysates. Therefore, further studies with this hydrolysate were performed to enhance productivity by increasing the reducing sugars available for ethanol production.

Untreated secondary-auto-hydrolysate was thereafter submitted to vacuum evaporation, raising the sugar concentration to nearly 33 g L⁻¹ (concentration factor = 3), similar to the reducing sugars content of AH120 acid-hydrolysates (36 g L⁻¹ before overliming). The concentrated secondary-auto-hydrolysates were then overlimed, after what a 13.5 g L⁻¹ reducing sugars concentration was lost (a 41% decrease). Therefore, a concentration of 19.5 g L⁻¹ of reducing sugars was available for the fermentation process. An ethanol concentration of 6.0 g L⁻¹ was obtained, with high values of productivity (0.218 g L⁻¹ h⁻¹) and yield based on reducing sugars consumed (0.42 g g⁻¹). An equal sugars-to-ethanol conversion, compared to non concentrated secondary-auto-hydrolysates, was determined (82.4%), as shown in Table 2.

A second approach was tested, in which untreated secondary-auto-hydrolysates were first overlimed followed by vacuum evaporation. After overliming, a loss of 4.7 g L⁻¹ of reducing sugars was observed (a 39% decrease of the initial concentration, 12 g L⁻¹). The detoxified secondary-auto-hydrolysate was concentrated by vacuum evaporation raising the sugar content to 29 g L⁻¹ (higher value in contrast to the one observed in the process described above, 19.5 g L⁻¹). Table 2 shows that an ethanol concentration of 9.9 g L⁻¹ was achieved, overcoming the ethanol concentration obtained with AH120 acid-hydrolysates fermentation, as well as the volumetric productivity (0.225 g L⁻¹ h⁻¹) and yield (0.50

g g⁻¹). A reducing sugar-to-ethanol conversion of 98% was registered (Table 2). Hydrolysates obtained from auto-hydrolysis of olive prunings were also used in ethanolic fermentation after vacuum evaporation and pH adjustment (~5) with NaOH [5], where *Candida tropicalis* produced an ethanol concentration of 7.2 g per 100 g of olive prunings (nearly 12 g L⁻¹) with a yield of 0.44 g g⁻¹ [5].

As Fig.1 illustrates, fermentation profiles of AH120 hydrolysates and concentrated secondary-auto-hydrolysates were very similar. No lag phase was registered on yeast growth and a small delay on ethanol production was observed (Fig.1). A remaining amount of reducing sugars was not utilized by *P. stipitis*. Probably it corresponds to the sum of uronic acids, furfural (which were identified as reducing compounds measured by the DNS method) and some reducing disaccharides not fermentable by the yeast. In fact, when reducing sugars were no longer metabolised, ethanol concentration reached a stationary phase or started to decline. Since yeast cells continued to grow it is believable that the ethanol was used as carbon and energy source for the yeast growth.

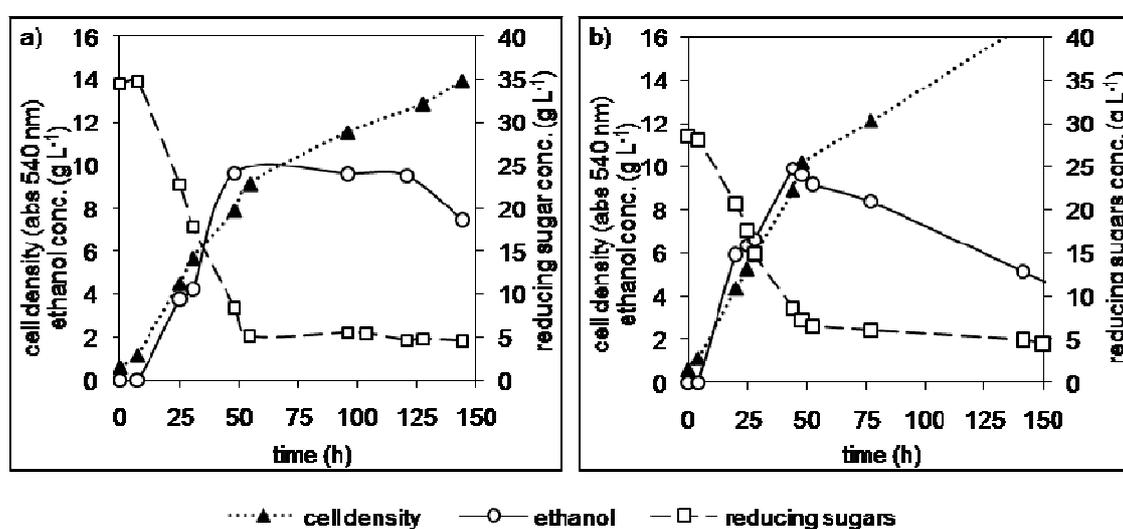


Figure 1 – Yeast growth, reducing sugars consumption and ethanol production profiles in the fermentation of: a) acid-hydrolysates; b) secondary-auto-hydrolysates overlimed and concentrated (3x).

A different concentration factor (8) was tested, aiming to reach double content of reducing sugars (~60 g L⁻¹). A concentration of 61 g L⁻¹ was in fact obtained and a higher ethanol concentration was expected after fermentation. Contrarily, only 7.5 g L⁻¹ of ethanol was achieved, with a smaller rate production (0.17 g L⁻¹ h⁻¹), as presented in Table 2. A slightly lower yield and consequently, a smaller conversion efficiency, were determined, 0.49 g g⁻¹ and 96%, respectively (Table 2). A lag phase on yeast growth (data not shown) was also observed. The decrease of the fermentation performance was probably due to a raise in inhibitors content above the tolerance level of toxicity of the yeast (particularly lignin and its derivatives), in spite of the detoxification step before the vacuum evaporation.

The results obtained above, using the sequence overliming followed by vacuum concentration, are promising for the utilization of auto-hydrolysates in ethanolic fermentation processes, and combine well with the most suitable findings obtained with the extracted wood kraft pulping process. However further studies must be performed to clarify the reasons for the observed decrease in the fermentation performance using higher concentration factors. The utilization of activated charcoal as selective adsorber is under study. The income with ethanol production by auto-hydrolysates fermentation must overcome the pulp yield losses and the energy costs required in the operating stages of the overall fermentation process.

CONCLUSIONS

The aim of this work was to study the viability of the forest biorefinery concept, incorporating lignocellulosic biomass conversion process into an existing chemical pulp mill to produce bioethanol by fermentation. A pre-hydrolysis was applied to *Eucalyptus globulus* wood chips, before kraft cooking, to extract hemicelluloses. The goal was to get profit of hemicelluloses energetic value, but seeking not to jeopardise the quality of the pulp. Acid- and auto-hydrolysis pre-treatment were tested. From the pulp production perspective, wood auto-hydrolysis is, generally, a better option as a pre-treatment prior to cooking, because it does not compromise so harshly the quality of the final pulp. Acid-hydrolysis promotes a higher monosaccharides extraction to be used in the ethanolic process and leads to better ethanol production parameters. Nonetheless auto-hydrolysates can be turned into a more sustainable raw material for bioethanol production, by applying a secondary hydrolysis, detoxification and vacuum evaporation. Overliming followed by evaporation (with a concentration factor of 3) gave better results than the inverse method. This procedure led to $\sim 10 \text{ g}_{\text{eth}} \text{ L}^{-1}$ of ethanol with a productivity of $0.23 \text{ g}_{\text{eth}} \text{ L}^{-1} \text{ h}^{-1}$ and a yield of $0.50 \text{ g}_{\text{eth}} \text{ g}_{\text{xy}}^{-1} \text{ eq}^{-1}$, which compares well with the results obtained with the fermentation of acid-hydrolysates. Optimum conditions for secondary-hydrolysates treatment are currently under study.

REFERENCES

1. S.H. Yoon, K. Macewan, A. van Heiningen, Kraft pulping and papermaking properties of hot-water pre-extracted loblolly pine in an integrated forest products biorefinery, *TAPPI J*, **7**(6): 22–27 (2008).
2. C.V.T. Mendes, M.G.V.S. Carvalho, C.M.S.G. Baptista, J.M.S. Rocha, B.I.G. Soares, G.D.A. Sousa, Valorisation of hardwood hemicelluloses in the kraft pulping process by using an integrated biorefinery concept, *Food Bioprod Process*, **87**(3): 197–207 (2009).
3. J. Helmerius, J.V. von Walter, U. Rova, K.A. Berglund, D.B. Hodge, Impact of hemicellulose pre-extraction for bioconversion on birch Kraft pulp properties, *Bioresource Technol*, **101**(15): 5996–6005 (2010).
4. Y. Sun, J. Cheng, Hydrolysis of lignocellulosic materials for ethanol production: a review, *Bioresource Technol*, **83**(1): 1–11 (2002).
5. J.F.G. Martín, M. Cuevas, V. Bravo, S. Sánchez, Ethanol production from olive prunings by autohydrolysis and fermentation with *Candida tropicalis*, *Renew Energ*, **35**(7): 1602–1608 (2010).
6. M.G.V.S. Carvalho, C.F.A. Baptista, C.V.T. Mendes, J.M.S. Rocha, Hemicelluloses: from wood to the fermenter, Proc. II Int Conf Environmental, Industrial and Applied Microbiology, BioMicroWorld2007, Seville, Spain, A. Mendez-Vilas (ed.), 46–50 (2009).
7. C.V.T. Mendes, C.M.S.G. Baptista, J.M.S. Rocha, M.G.V.S. Carvalho, Pre-hydrolysis of *Eucalyptus globulus* Labill. hemicelluloses prior to pulping and fermentation of the hydrolysates with the yeast *Pichia stipitis*, *Holzforchung*, **63** (6), 737–743 (2009).
8. E. Palmqvist, B. Hahn-Hägerdal, Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition, *Bioresource Technol*, **74**(1): 25–33.
9. M.D. Ferrari, E. Neirotti, C. Albornoz, E. Saucedo, Ethanol Production from Eucalyptus Wood Hemicellulose Hydrolysate by *Pichia stipitis*, *Biotechnol Bioeng*, **40**(7): 753–759 (1992).
10. S. Amartey, T. Jeffries, An improvement in *Pichia stipitis* fermentation of acid-hydrolysates hemicellulose achieved by overliming (calcium hydroxide treatment) and strain adaptation, *World J Microb Biotech*, **12**(3): 281 – 283 (1996).
11. M. Telli-Okur, N. Eken-Saraçoglu, Fermentation of sunflower seed hull hydrolysate to ethanol by *Pichia stipitis*, *Bioresource Technol*, **99**(7): 2162 – 2169 (2008).
12. I.S. Horváth, A. Sjöde, B. Alriksson, L.J. Jönsson, N. Nilvebrant, Critical Conditions for Improved Fermentability During Overliming of Acid Hydrolysates from Spruce, *Appl Biochem Biotech*, **121-124**: 1031–1044 (2005).
13. A. Martinez, M.E. Rodriguez, M. L. Wells, S.W. York, J.F. Preston, L.O. Ingram, Detoxification of Dilute Acid Hydrolysates of Lignocellulose with Lime, *Biotechnol Prog*, **17**(2): 287–293.
14. L. Canilha, J.B. Almeida e Silva, A.I.N. Solenzal, Eucalyptus hydrolysate detoxification with activated charcoal adsorption or ion-exchange resins for xylitol production, *Process Biochem*, **39**(12): 1909–1912 (2004).
15. A. Santiago, C.P. Neto, C. Vilela, Impact of effective alkali and sulfide profiling on *Eucalyptus globulus* kraft pulping. Selectivity of the impregnation phase and its effect on final pulping results, *J Chem Technol Biot*, **83**(3): 242-251 (2008).