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SECOND GENERATION BIOETHANOL FROM *Eucalyptus globulus labill* AND *Nothofagus pumilio* USING IONIC LIQUIDS.

María Cristina Ravanal E.

Centro de Biotecnología y Bioingeniería
Universidad de Chile

mravanal@ing.uchile.cl

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BIO-ETHANOL PRODUCTION
with various feedstocks

1st generation

sucrose-containing feedstocks

sugar beet

sugar cane

sweet sorghum

starchy materials

wheat

corn

barley

2nd generation

lignocellulosic biomass

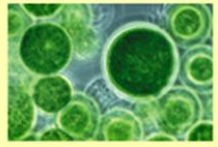
wood

straw

grass

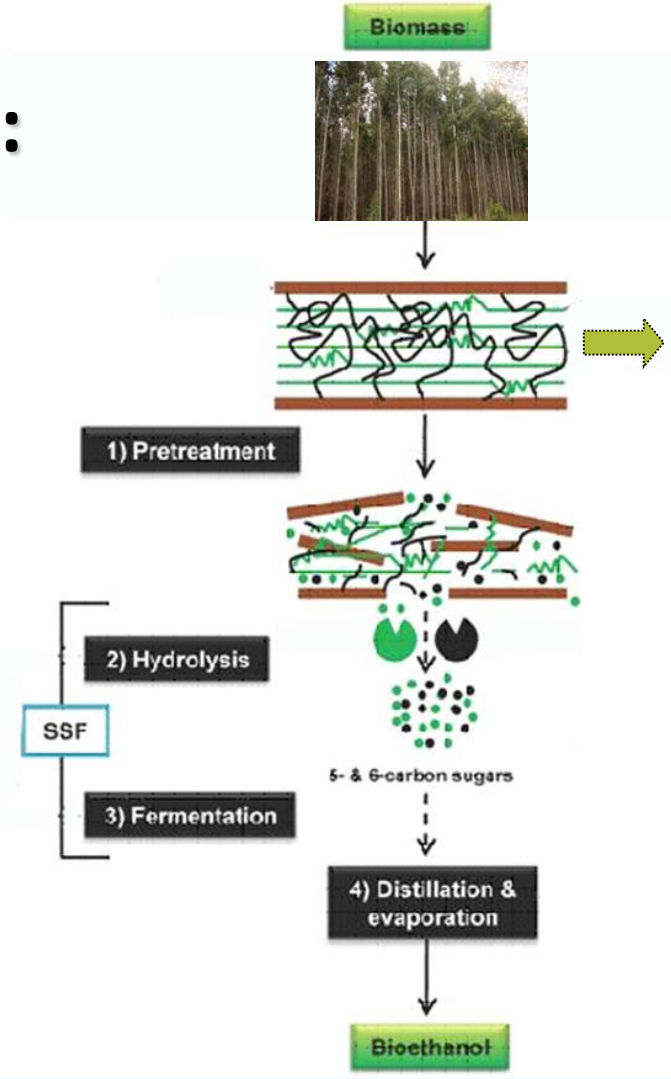
3rd generation

algal biomass



Bioethanol production:

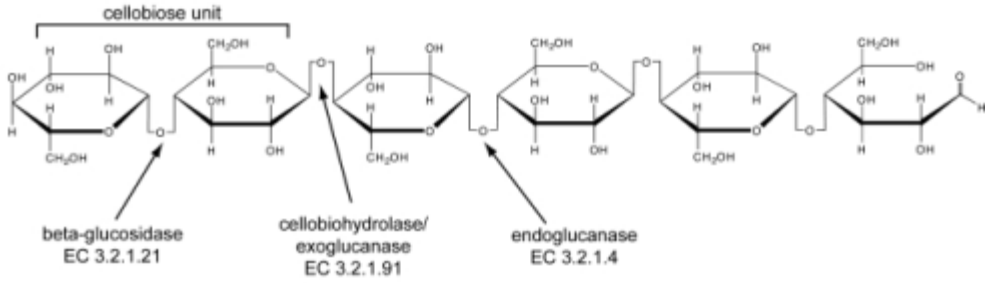
2nd generation



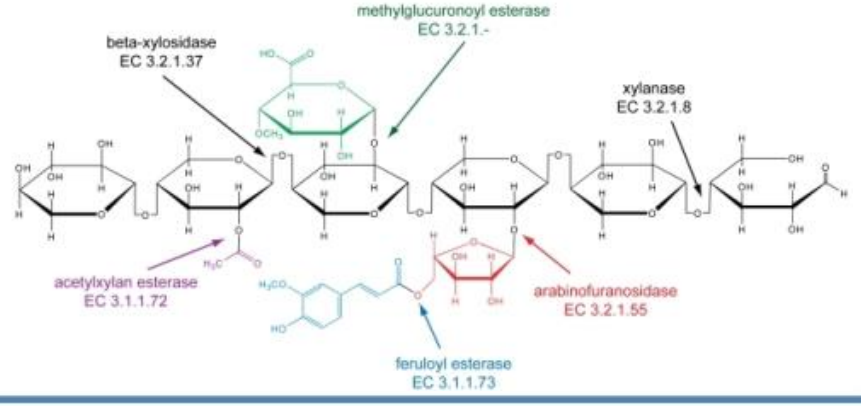
The lignocellulose is composed of carbohydrate polymers: **cellulose**, **hemicelluloses and pectin,** and a polyphenol polymer: **lignin**.

Polysaccharides presents in lignocellulose

- **Cellulose** is the main component of the primary cell wall of plants. It is a polysaccharide consisting of a linear chain of β-1,4-linked D-glucose units.

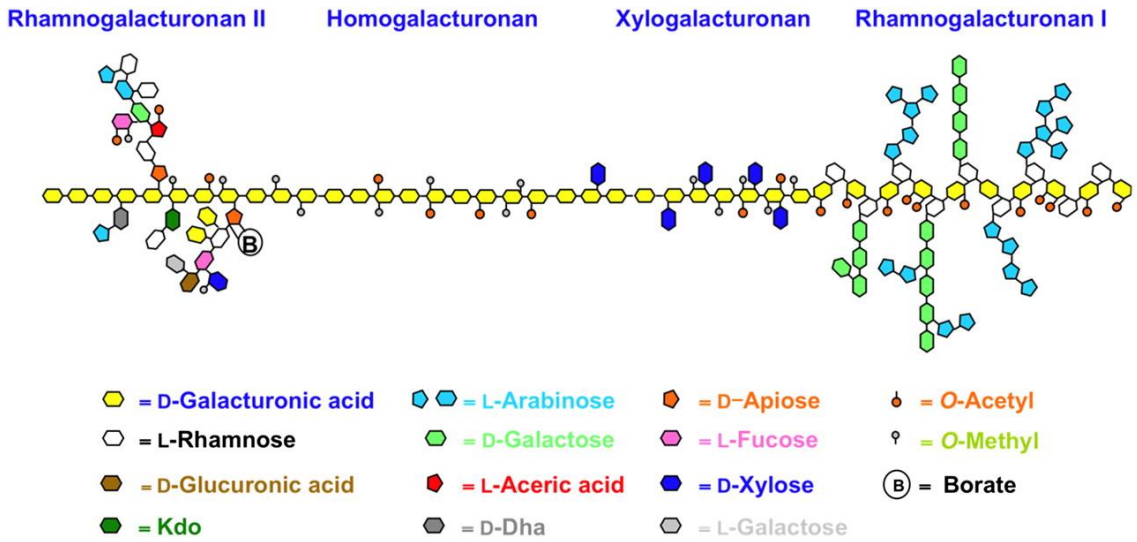


- **Hemicelluloses:** Xylan, the major component of hemicelluloses, is composed of a linear chain of D-xylopyranoses linked by ß(1→4) and is substituted by several types of residues: methyl glucuronate, L-arabinofuranose and acetate.



Yang et al., 2009.

Pectin is composed of two basic structures: a “smooth” region and a “hairy” region. The “smooth” region (homogalacturonan) is a linear polymer of galacturonic acid residues. The “hairy” region is more complex, containing xylogalacturonan and rhamnogalacturonans.



Scheller et al., 2007

Eucalyptus and Lenga residues in Chile

- Eucalyptus is the second most abundant lignocellulosic material in Chile; equals to a 23 % of the total forest plantations in this country.

- Lenga is a Chilean native tree that grows preferably in the extreme south of the country and represents 26.5% of native forests.

- The forest plantations are concentrated in certain regions of Chile:

- 38.1% of them are located in the Maule Region and 62.8% (mainly native forest) in the Los Lagos and Aysén (General Carlos Ibáñez del Campo) Regions.

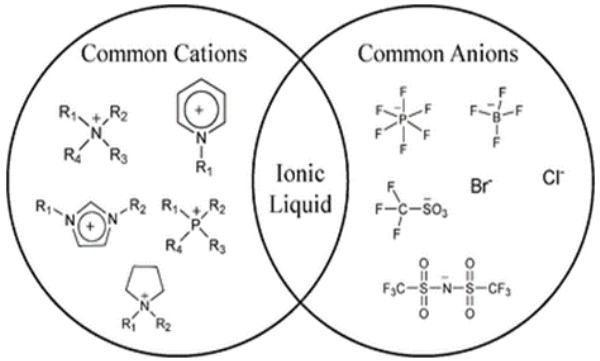
Pretreatment

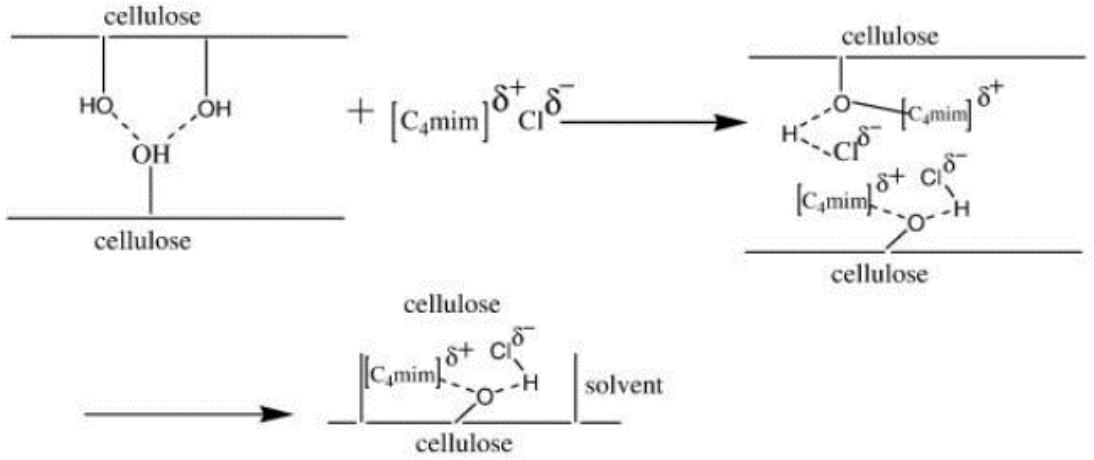
- Different pretreatment strategies have been developed throughout the years for lignocelluloses, including physical, biological, chemical and physicochemical processes.

- Each of them is associated with some disadvantages:
- Physical treatments, such as steam explosion is energy-demanding.
- Biological processing methods, such as lignin degradation by fungi necessitate long times to be effective.
- Chemical methods, such as dilute acid hydrolysis, produces toxic products.

Ionic Liquids “Green Solvents”

- Ionic liquids (ILs) are organic salts able to melt under 100ºC.
- Excellent physical characteristics such as the ability to dissolve polar and non-polar organic and inorganic materials, as well as polymers.
- 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.





Saccharification and fermentation

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 (SHF), a process in which hydrolysis of polysaccharides and the fermentation of monosaccharides is performed separately.

b) Simultaneous Saccharification and Fermentation (SSF), a process in which polysaccharides hydrolysis and fermentation are carried out in one container.

c) Consolidated BioProcessing (CBP), a process in which the enzymes are produced by the fermenting organisms.

d) Simultaneous Saccharification and Co-Fermentation of hexoses and pentoses (SSCF).

Objective

The aim of the present work is to study the effect of the use of different process configurations for the saccharification and fermentation steps (SSF and SHF).

Nothofagus pumilio (LENGA)

- Residual Lenga (about 40-60 years of age) from a sawmill located in the Santa Alicia, Tierra del Fuego (XII región).

- Chip size: 1- 2 mm high, 1-3 mm wide and 5-7 mm long.

- Lenga was dried at 80° C overnight prior to pretreatments.

Sawmill

Hammermill

Tamiz

Composition of *Nothofagus pumilio* lignocellulose.

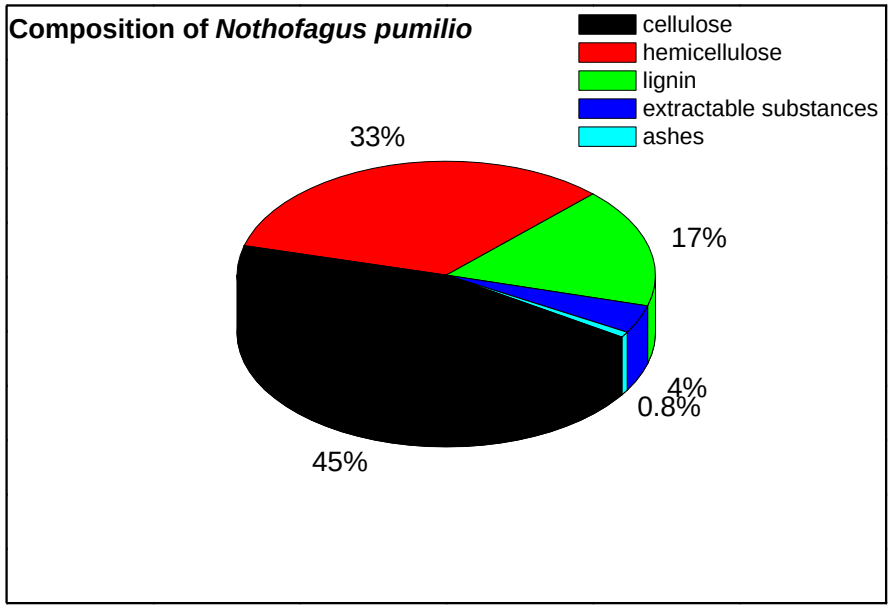
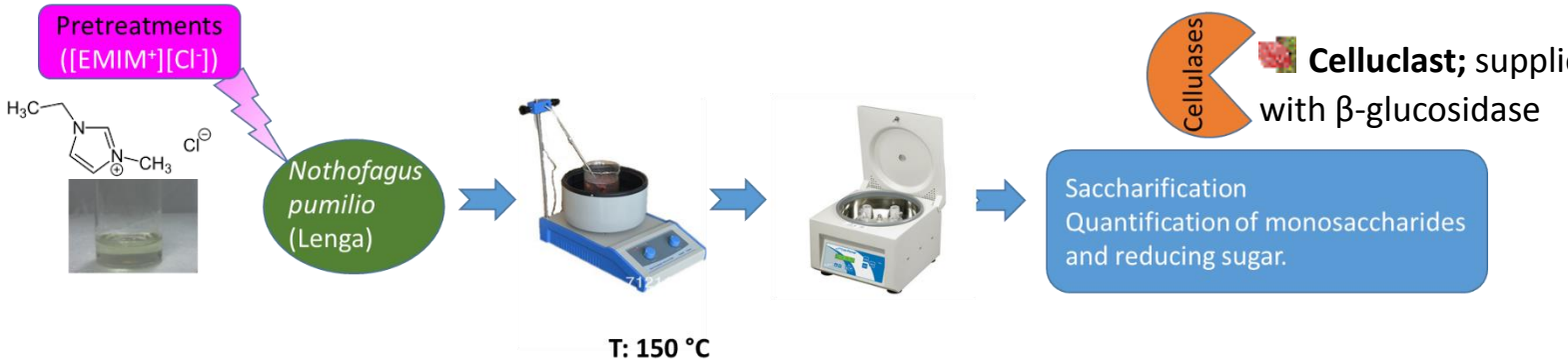


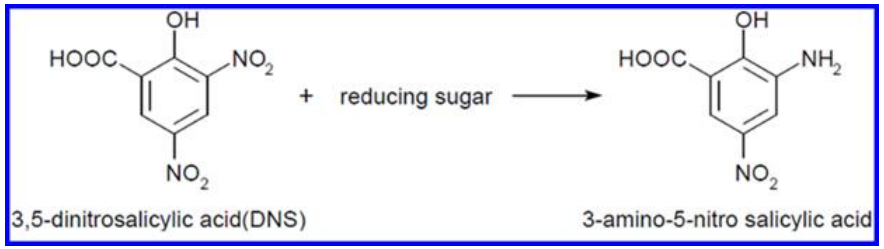
Table 1: Carbohydrate content (mg/g of dry mass) in Lenga. Monosaccharides' content analyzed by the acid methanolysis method.

Monosaccharides	Fresh Lenga [mg/g dry mass]
Arabinose	6.58
Fructose	0.45
Galactose	9.37
Galacturonic acid	16.89
Glucose	**434.02**
Glucuronic acid	3.64
Mannose	4.06
Rhamnose	4.43
Xylose	146.78
Total	**643.76**

Experimental Procedure



Reducing sugar quantitation by the dinitrosalicylic colorimetric method



This method involves the oxidation of the aldehyde functional group present; simultaneously DNS is reduced to 3-amino,5-nitrosalicylic acid (Bailey et al., 1992).

The monosaccharides profiles were determined by Gas Chromatography (GC)

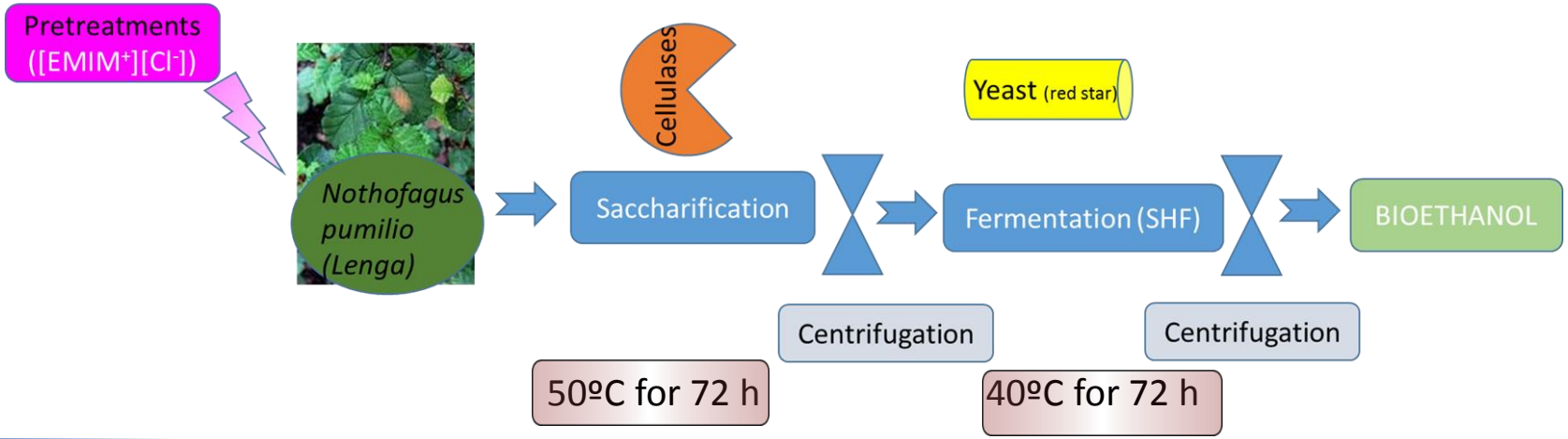
Table 2. Effect of temperature and time of pretreatment on the release of sugars from Lenga after enzymatic hydrolysis, using a 1:20 biomass/IL ratio.			
Ionic Liquid	Temperature [°C]	Time [min]	Sugar yield [wt-%]
Without pretreatment			**8.5**
[EMIM$^+$][Cl$^-$]	150	60	**36.5**
[EMIM$^+$][Cl$^-$]	150	30	**51.9**

Table 3. Influence of different biomass-IL ratios and pretreatment times on the release of sugars from Lenga.

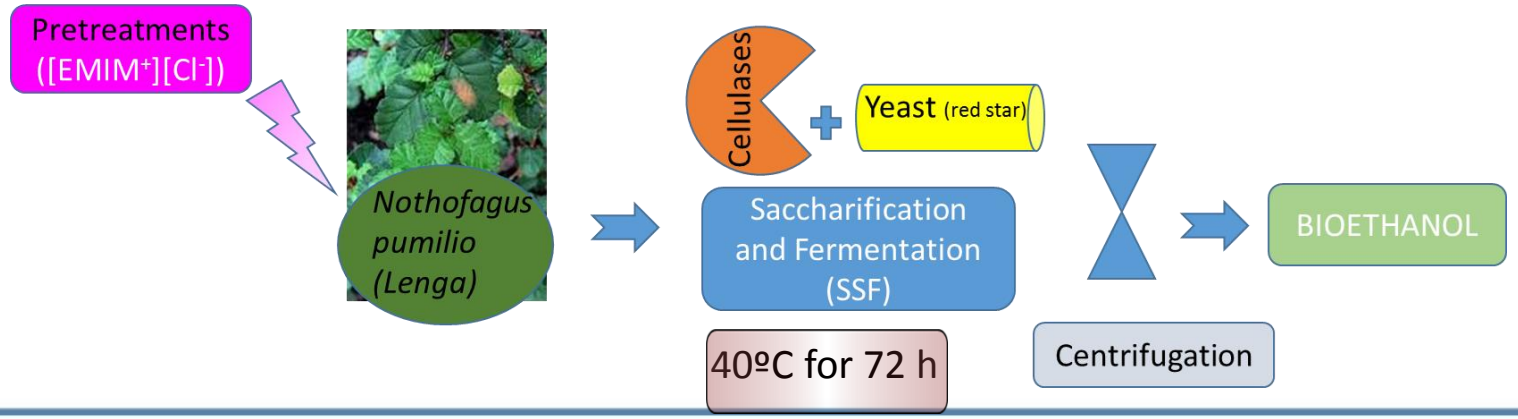
Biomass/IL ratio [wt:wt]	Time [min]	Sugar	Sugar productivity [g/g IL/h]	
1:3	15	Glucose		0.393
		Total reducing sugars		0.595
1:3	30	Glucose		0.198
		Total reducing sugars		0.305
1:5	15	Glucose		0.259
		Total reducing sugars		0.375
1:10	30	Glucose		0.065
		Total reducing sugars		0.087

The best condition was pretreatments with [EMIM$^+$][Cl$^-$] at 150° C, a 1:3 biomass-IL loading ratio and an exposure time of 15 min.

Experimental strategy for Separate Hydrolysis and Fermentation (SHF).



Experimental strategy for Simultaneous Saccharification and Fermentation (SSF).



Glucose and ethanol were quantified by HPLC (Aminex HPX-87H -0,005 M H_2SO_4: 0,6 mL/min:45° C).

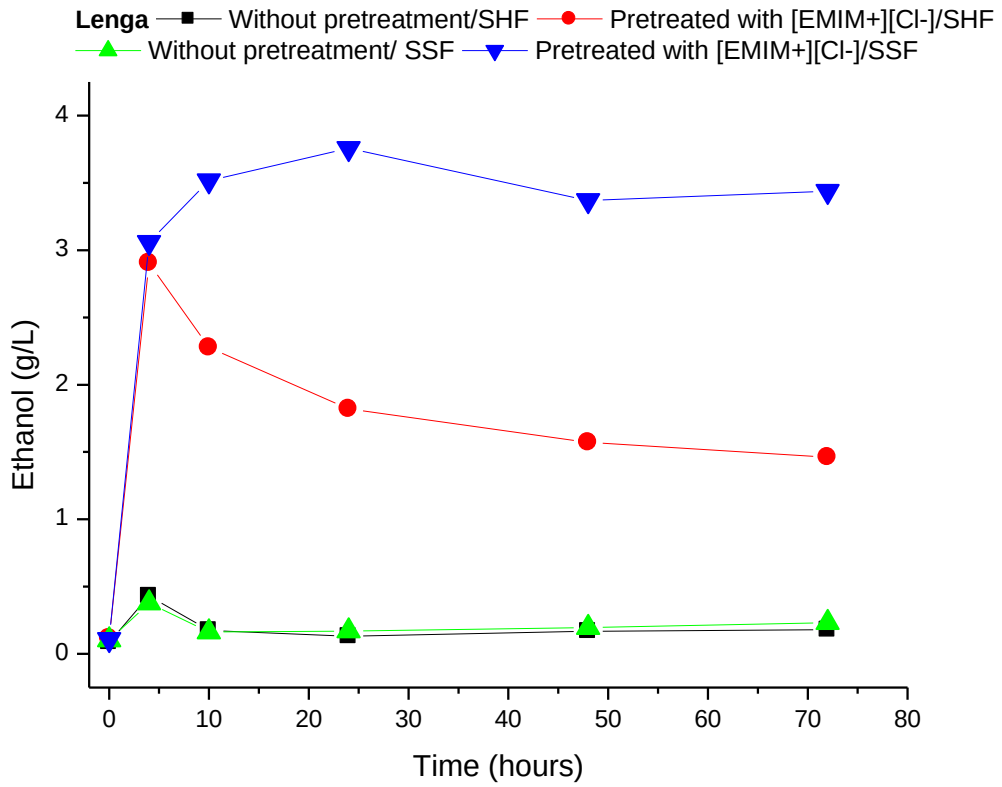


Figure 1. **The effect of the different fermentation and saccharification processes (SHF and SSF) on ethanol production from Lenga.** Ethanol production in SHF from Lenga is shown in squares and pretreated Lenga is shown in circle; Ethanol production in SSF from Lenga is shown in up triangles and pretreated Lenga is shown in down triangles.

Table 4: The yield of ethanol production from Lenga for different fermentation and saccharification processes (SHF and SSF).

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

(*) Fermentation for 4 hours; (**) fermentation for 24 hours.

Eucalyptus globulus

- Residual Eucalyptus (15 years of age) from a sawmill located in the V Region.
- Chip size: 0.5-1 mm wide, 0.5-1 mm high and 10-20 mm long.
- Eucalyptus was dried at 65° C for 18 hours.

Sawmill

Hammermill

Tamiz

Composition of *Eucalyptus globulus* lignocellulose.

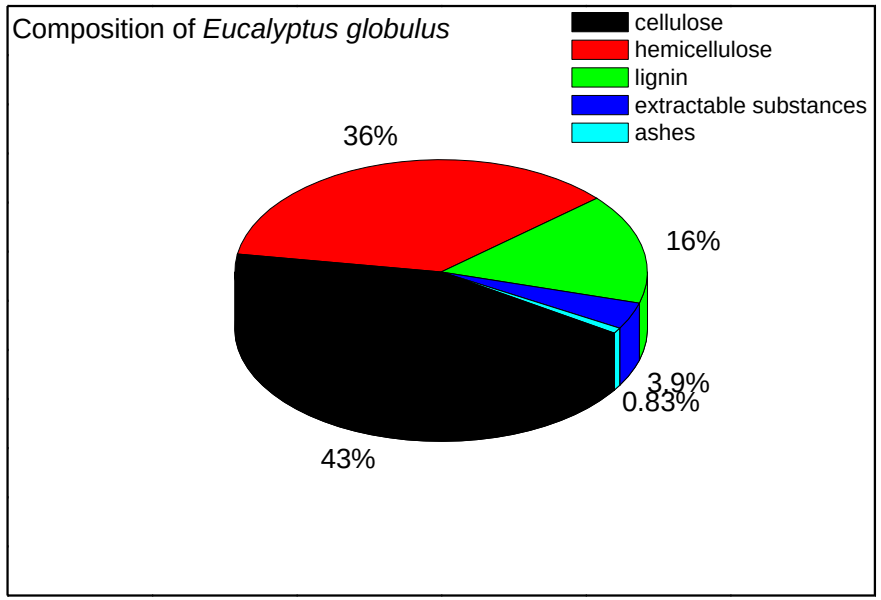
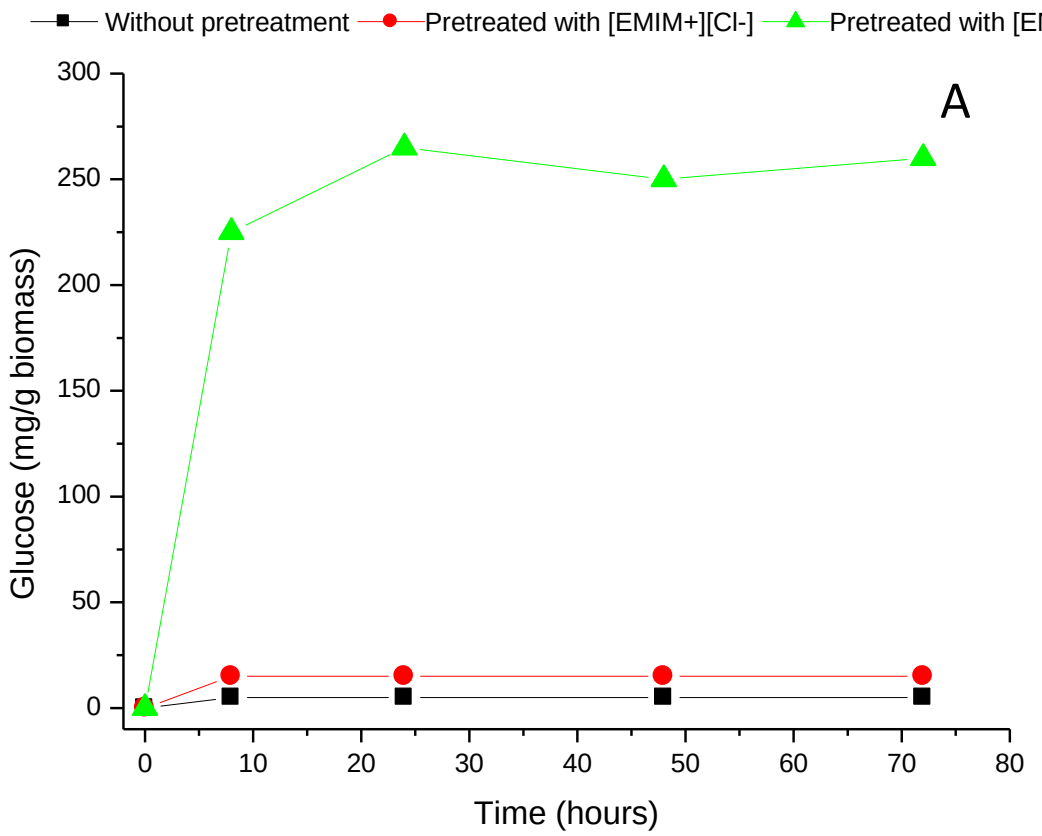


Table 5: Carbohydrate content (mg/g of dry mass) in *Eucalyptus globulus*. Monosaccharides' content analyzed by the acid methanolysis method.

Monosaccharide	Fresh sample [mg/g of fresh sample]
Arabinose	4.14
Fructose	0.80
Galactose	13.30
Galacturonic acid	13.67
Glucuronic acid	2.54
4-O-Me-Glucuronic acid	13.15
Mannose	7.29
Rhamnose	5.34
Xylose	146.56
Glucose	427.55



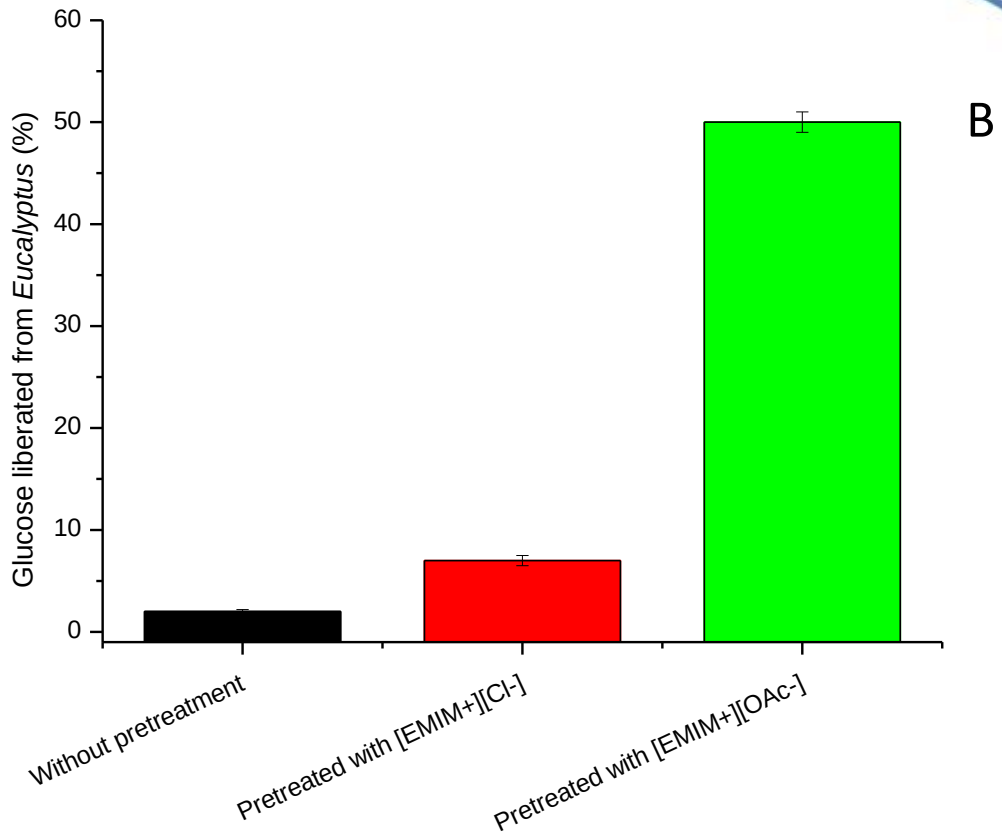
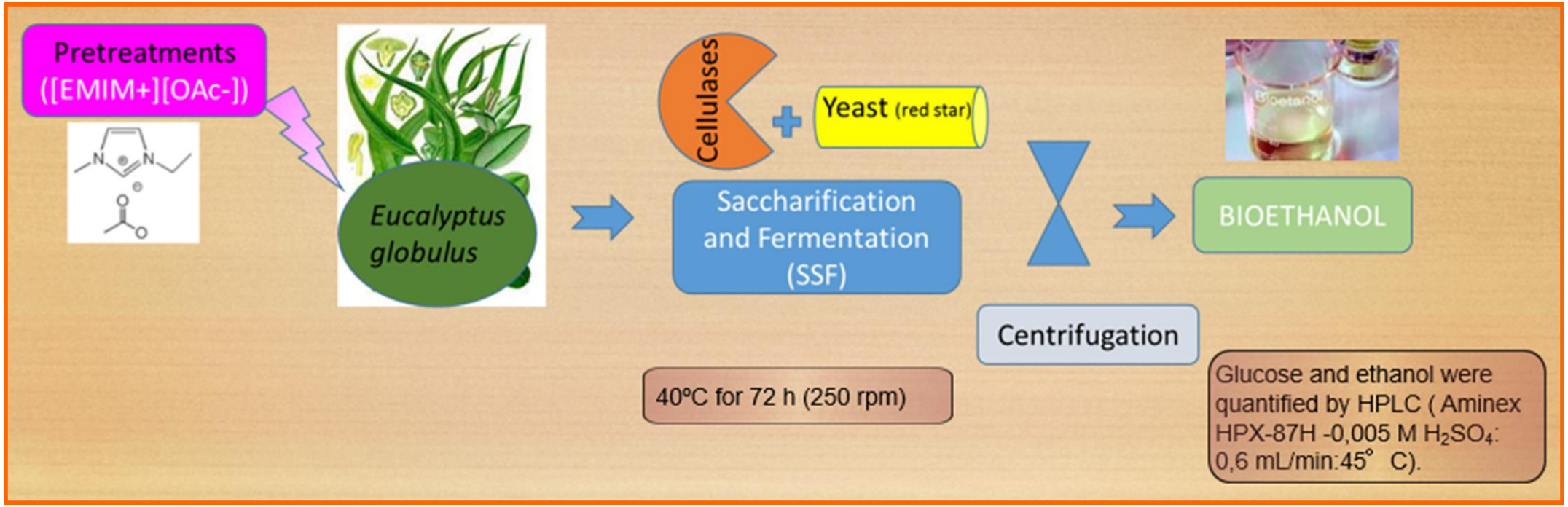


Figure 2. **Effect of different Ionic liquids on glucose liberation from Eucalyptus. A)** Eucalyptus without pretreatment is shown in squares; pretreated with [EMIM+][Cl-] is shown in circles and pretreated with [EMIM+][Oac-] is shown in up triangles. **B)** Percentage of glucose liberated from Eucalyptus.

Experimental strategy for Simultaneous Saccharification and Fermentation (SSF).



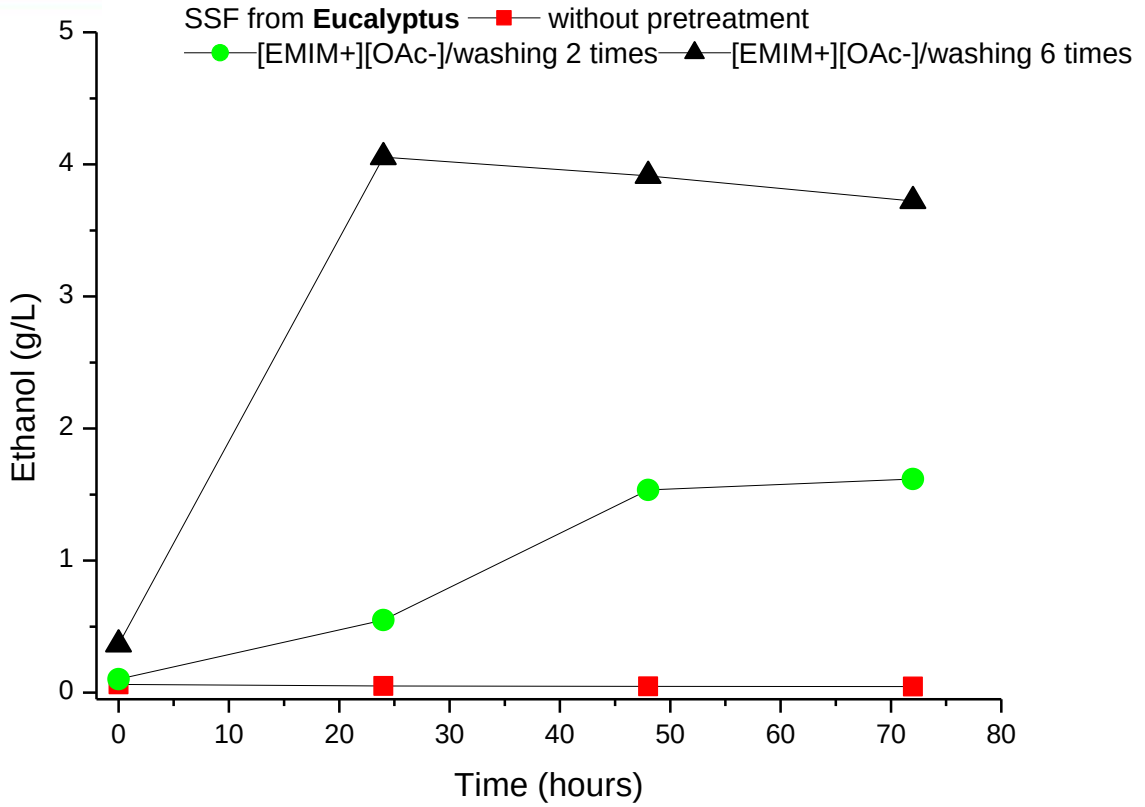


Figure 3. Effect of the Simultaneous Fermentation and Saccharification processes (SSF) for 3 days on ethanol production. Eucalyptus residues without pretreatment is shown in squares. Eucalyptus pretreated with [EMIM+][OAc-]/ fibers washed 2 times is shown in circles, and Eucalyptus pretreated with [EMIM+][OAc-]/ fibers washed 6 times is shown in triangles. Eucalyptus residues were pretreated with [EMIM+][OAc-] at 150°C, for 30 min, using a 1:3 ratio (wt RL/wt IL).

Table 6: Effect of the number of washing cycles on the yield of ethanol production using Simultaneous Saccharification and Fermentation (SSF) of Eucalyptus lignocellulose.

Pretreatment	Ethanol yield [g ethanol/g glucose]	Percentage relative to theoretical yield (wt-%)
Theoretical yield	0.510	100.0
Without pretreatment	0.002	0.48
Pretreatment with [$EMIM^+$][OAc^-]/ fibers washed two times	0.083	16.5
Pretreatment with [$EMIM^+$][OAc^-]/ fibers washed six times	0.194	**38.0**

Table 7. Composition of monosaccharides in fresh and processed *Eucalyptus globulus* samples after fermentation.

Monosaccharide	Fresh sample [mg/g of fresh sample]	Processed sample [mg/g of processed sample] ([1])
Arabinose	4.14	4.55
4-O-Me-Glucuronic acid	13.15	12.15
Rhamnose	5.34	2.55
Xylose	146.56	107.24
Glucose	427.55	183.89

- **Celluclast;** endo and exo-β-glucanase supplied with β-glucosidase from Sigma.
- **Celluclast** has residual xylanase activity.

([1]) Calculated by gram of remaining mass after fermentation.

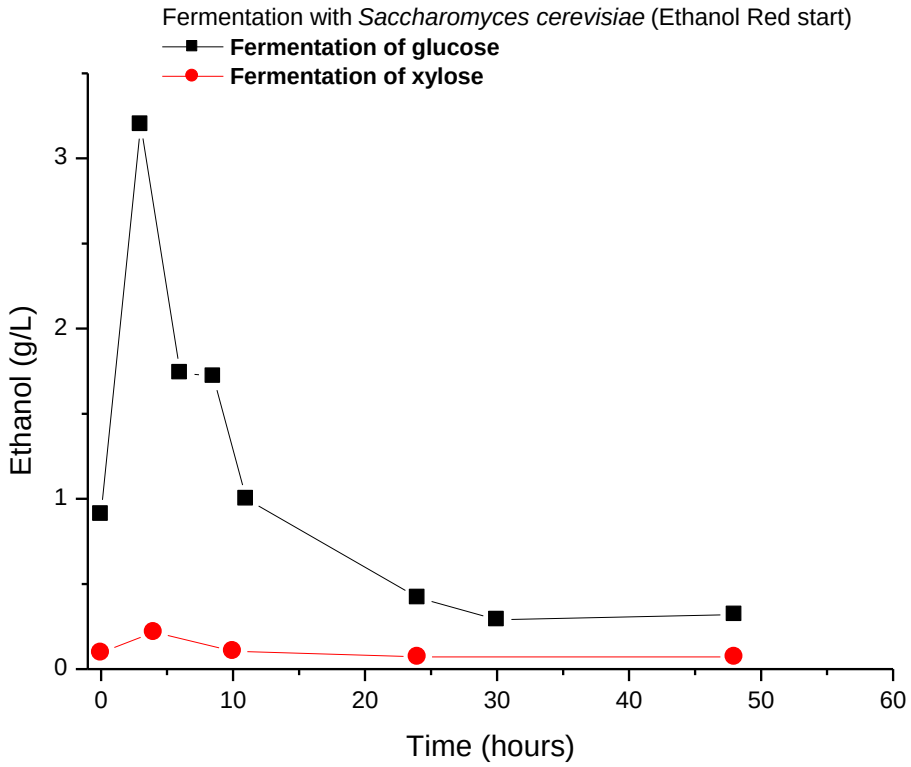


Figure 4: Fermentation of glucose and xylose with S. cerevisiae Ethanol Red®. Ethanol production from glucose is shown in squares and xylose is shown in circles. Fermentation of 9 g/L of carbohydrate for 48 hours at 40°C.

Conclusions and outlook

- The use of [EMIM+][Cl-] and [EMIM+][OAc-] as “structure-disruptive” solvents in the pretreatment of Lenga and Eucalyptus residues was performed.
- The combination of pretreatment with ionic liquid and a SSF process has high potential for bioethanol production from Lenga and Eucalyptus residues.
- Further improvements are still possible by optimization of some operational conditions and the recycling of ILs.
- For saccharification, we will study the effect of the combination of cellulases with xilanases under different incubation periods, temperatures, and pHs.
- For fermentation, we will search for microorganisms that are able to ferment glucose and xylose.

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INDCRO 8519 1–8

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Industrial Crops and Products xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Industrial Crops and Products

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Second generation bioethanol from *Eucalyptus globulus Labill* and *Nothofagus pumilio*: Ionic liquid pretreatment boosts the yields

María Elena Lienqueo[a,*], María Cristina Ravanal[a], Ricardo Pezoa-Conte[b], Victoria Cortínez[a], Loreto Martinez[a], Tomas Niklitschek[a], Oriana Salazar[a], René Carmona[c], Alejandro García[c], Sari Hyvärinen[b], Päivi Mäki-Arvela[b], Jyri-Pekka Mikkola[b,d]

[a] *Centre for Biotechnology and Bioengineering, Universidad de Chile, Beauchef 850, Santiago, Chile*
[b] *Industrial Chemistry and Reaction Engineering, Johan Gadolin Process Chemistry Centre, Åbo Akademi University, Turku, Finland*
[c] *Department of Wood Engineering and Their Biomaterials, Faculty of Forest Sciences and Nature Conservation, Universidad de Chile, Santa Rosa 11.315 La Pintana, Santiago, Chile*
[d] *Technical Chemistry, Department of Chemistry, Chemical-Biological Centre, Umeå University, Sweden*

Co-researchers





Acknowledgments

This research was supported by a grant from **CONICYT, Project AKA-ERNC 0009** and the Centre for Biotechnology and Bioengineering (CeBiB) **FB-0001**.

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