



## Optimization of sequential alkaline–acid fractionation of pine sawdust for a biorefinery



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### ABSTRACT

The aim of this work was to apply an alkaline–acid sequence for the fractionation of slash pine sawdust, to obtain a solid lignocellulosic material (which can be subsequently delignified to obtain pure cellulose), and separate liquid fractions containing extractives and hemicelluloses. Processes were optimized using a central composite design of two variables for the alkaline extraction stage (NaOH concentration and temperature), and another of three variables for the acid treatment ( $H_2SO_4$  concentration, time and temperature). Yield and contents of extractives, lignin, carbohydrates and degradation products, all by NREL Standards were determined. Maximum removal of extractives in the alkaline stage (90.7%) was reached using 5% NaOH (oven dry basis, od) at 90 °C for 1 h, and maximum hemicelluloses extraction in the acid stage (57%) was obtained using 7.5 g/L  $H_2SO_4$  at 150 °C for 30 min. The alkaline–acid sequence has shown to be effective for the sequential extraction of resin and hemicelluloses from slash pine sawdust, making possible its use for the production of high value products.

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## 1. Introduction

The environmental care has become in a factor that adds value to industrial production, making it more competitive by the use of renewable resources and advanced technologies. Following the concept of oil refineries, the forest biorefinery consist of lignocellulosic biomass processing (wood debris, sawdust, shavings) to produce energy, chemicals and biomaterials. Pine sawdust is one of the main residues of the primary industrialization of wood in the Northeast of Argentina (NEA Region). About 50% of industrially processed wood is become in waste, generating 1.5 million of dry ton wood wastes per year, which are not properly exploited (Uasuf and Hilbert, 2012).

The chemical composition of *Pinus elliottii* grown in Misiones, Argentina, depending on soil type and climate, involves about

41–44% of cellulose, 28–31% of lignin, 27–33% of hemicelluloses, and 2–4% of extractives in dichloromethane (Area et al., 1992). This high resin content makes difficult the direct application of acid processes because of pitch formation. Considering that resin content in pine sawdust is usually higher than in solid wood, we seek to find a technological alternative to make possible the extraction of hemicelluloses, allowing the recovery of extractives, as well as the integral utilization of these wastes.

Chemical fractionation involves the separation of biomass components, so that each component can be industrialized. One important obstacle to overcome is to find efficient and cost effective methods of fractionation (FitzPatrick et al., 2010). Sawdust has the additional advantage of not requiring milling pretreatment.

Pretreatments of resinous softwoods have been not extensively researched mainly because this material presents high contents of crystalline cellulose, lignin and extractives. However, it is a very promising raw material due to its high content of hexoses and its wide availability (Bengoechea et al., 2012).

The extractives in organic solvents of pines are composed of resin acids (abietic and hydroabietic acids), fatty acids (oleic and linoleic acids) and a neutral fraction, often called unsaponifiables (phytosterols, fatty and wax alcohols, terpenes and hydrocarbons).

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Fatty and resin acids can be extracted in mild alkaline conditions, since they are dissolved forming sodium soaps. The concentration of the pulping solution (black liquor) prior of the inorganic pulping chemicals recovery allows the skimming of the insoluble soaps from the surface. Acidification of the skimmed soaps yields tall oil resin and fatty acids. Fatty acids can be recovered from tall oil by vacuum distillation. These tall oils by-products are a source of valuable chemicals which could improve the economic balance of the pulping industry (Demirbas, 2011). When liquors are very dilute and cannot be concentrated, the extractives can be removed by other techniques such as flotation, with removal efficiency of 80–90% of flocculated extractives (Tanase et al., 2010).

Fatty and resin acids are source of valuable chemicals as surfactants, detergents, adhesives, plastics, glues, inks, soaps, medicines, health promoting agents, and biodiesel (Hillis, 1962; Arshadi et al., 2013). It could also make more attractive the production of bioethanol from loblolly pine (Frederick et al., 2008). On the other hand, despite their small amount, they generate deposits and foam in production processes, and provide toxicity to the mill effluents (Foran, 1992).

Extractives removal prior to Kraft pulping (Baptista et al., 2006) sulfite pulping (Arrabajal and Cortijo, 1995) and bleaching (Sitholé et al., 2010) has also been studied to improve pulp properties.

Once the resinous material has been removed, fractionation must focus on the macromolecular components present in plant tissues. Dilute acid treatment with sulfuric acid has the advantage to be inexpensive, simple and effective to remove hemicelluloses, producing fewer amounts of degradation products than other processes (Wyman, 1994; Larsson et al., 1999; Frederick et al., 2008). The oligomers and monosaccharides extracted from hemicelluloses have multiples uses, as chemicals products, bioethanol, biopolymers and other applications (Hayes et al., 2006; Vallejos, 2012). The residual porous structure consisting mainly of cellulose and lignin can be subsequently delignified by an alkaline process to obtain cellulosic pulps, which can be used to produce bioethanol or dissolving pulp. Lignin in spent liquor can be precipitated and transformed into high-value products such as vanillin and other phenolic components (Stoffel et al., 2012).

The most studied pretreatments for softwood are steam explosion, which combines physical and chemical methods (Kim, 2005; Nguyen et al., 1998; Söderström et al., 2003) and treatment with dilute acid (Shuai et al., 2010; Marzalletti et al., 2008; Lim and Lee, 2013). However, no work applying alkaline deresination prior to the above mentioned treatments were found.

Despite the relevance of extractives components and their possible exploitation, no studies have been found about alkaline deresination of pine sawdust as part of neither wood fractionation nor antecedents concerning the dilute acid fractionation of slash pine.

The aim of this work was to study the fractionation of slash pine sawdust by a sequential alkaline–acid process. The influence of the main parameters of both processes (concentration, time and temperature) and their optimization were established by Central Composite Experimental Designs (CCD).

## 2. Materials and methods

### 2.1. Raw material

Slash pine sawdust was supplied by a local sawmill (Forestal Eldorado, Misiones). The sawdust was air-dried, screened and maintained in closed plastic bag. The fraction passing a 3 square mm<sup>2</sup> screen was used for the treatments.

### 2.2. Experimental design

Sawdust was subjected to alkaline and acid fractionation to sequentially extract the extractives and hemicelluloses. The experiments were arranged according to a Central Composite Design (Barker, 1985) in each case. The variables for the alkaline extraction were NaOH charge and temperature, and for the acid treatment, H<sub>2</sub>SO<sub>4</sub> concentration, time and temperature.

The experimental designs for the alkaline and acid stages are shown in Fig. 1a and b, respectively. Each axis corresponds to a factor and each point on the cube represents an experimental combination of conditions. CCD consists of  $2^k + 2k + m$  runs, where  $k$  is the number of factors,  $2^k$  is the number of the factorial points at the corners of the square or cube,  $2k$  is the number of the axial points on the axis of each design factor at a distance of  $\pm\alpha$  ( $\alpha = 2^{k/4}$ ) from the center of the square or cube and  $m$  is the number of the center points at the center of the square or cube. In this study, three replicas of the center point were performed to estimate the experimental error. Accordingly, the total number of experiments was  $4 + 4 + 3 = 11$  for the alkaline stage and  $8 + 6 + 3 = 17$  for the acid one. The experiments were performed randomly. Factors and levels of each treatment with coded and uncoded variables are shown in Fig. 1c.

The Desirability function is the most popular method for the solution of multiresponse optimization problems. This approach to simultaneously optimize multiple equations, translates the functions to a common scale ([0,1]), and combines them using the geometric mean and optimizing the overall metric.

Statistical analysis of results (ANOVA and optimization by the desirability function) was performed using Statgraphics Centurion XV software at 95% significance ( $p < 0.05$ ).

### 2.3. Alkaline deresination

All reactions were performed in 250 mL glass vessels heated in a hot water bath for one hour. Ten grams (dry basis) of pine sawdust was used, with a liquid to dry wood ratio (v/w) of 10 in all tests. The vessels were closed with a plastic film and placed in the bath at the desired temperature. Sawdust and liquor were mixed manually before closing the vessels. Stirring was not necessary since the liquid to dry wood ratio was high, and heating promoted the movement of the solid in the liquid. After the reaction, the vessels were cooled down with ice, and the liquor was separated from the solid by filtration at reduced pressure.

In order to verify the representativeness of data obtained at laboratory scale, a scaling-up trial was performed, for which a point near the optimum was chosen. The selected experience was reproduced in a 7 L reactor (M/K Systems, Inc., Maryland) with liquor circulation, using 500 g of dry sawdust with 5 L of alkaline solution (liquor to wood ratio of 10, v/w). Once the reaction finished, the spent liquor was separated from the solid by centrifugation to approximately 40% of solids. Subsequently, the wood residue was exhaustively washed with water, it was filtered and air dried to measure the weight loss. A sample for chemical analysis was taken.

### 2.4. Dilute acid treatment

Acid stage with diluted sulfuric acid was conducted in 200 mL stainless steel reactors heated in a glycerin bath. The treatment was applied on the alkaline-pre-extracted sawdust and on the original sawdust, as control. The reactors were loaded with 15 g of dry sawdust and 150 mL of the acidic aqueous solution, at the concentration established by the experimental design (liquor to wood ratio of 10, v/w). As in the case of the alkaline treatment, sawdust and liquor were mixed manually before closing the vessels. Stirring was not necessary since the liquid to dry wood ratio was high, and

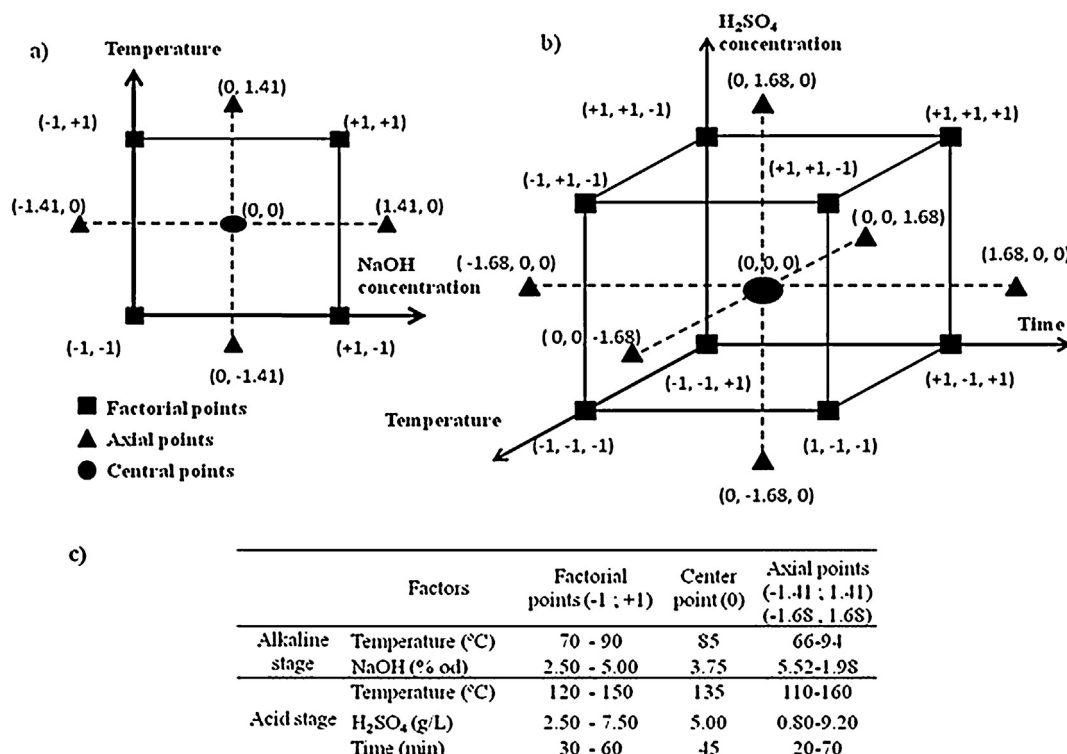


Fig. 1. Central composite designs. (a) CCD of two factors with two levels for the alkaline stage. (b) CCD of three factors with two levels for the acid treatment. (c) Factors and levels of each treatment with code and uncoded variables.

heating promoted the movement of the solid in the liquid. Upon the reaction was finished, the reactor was instantly ice-cooled. This reaction system prevents ramps of heating and cooling since they are almost instantaneous. The solid was filtered with reduced pressure to separate the spent liquor. Then the residual solid was washed with water. All liquid samples were sealed in a vial and stored in a refrigerator until further characterization. Samples of residual solids were air dried to measure the weight loss.

The severity factor ( $\log RO$ ) of the treatment can be estimated as a function of treatment time and temperature to describe the impact of the treatment severity on lignocellulosic components:

$$\text{Log}(RO) = \text{Log } t \times \exp \left[ \frac{T - T_{ref}}{R \times T_{ef} \times (T/Ea)} \right] \quad (1)$$

where  $t$  is the residence time (min),  $T$  is the treatment temperature (°C),  $T_{ref}$  is the base temperature (100°C), and  $Ea$  is the activation energy. If one approximates,

$$Te \approx R \times T_{ef} \times \left( \frac{T}{Ea} \right) \quad (2)$$

For hot water extraction of woody biomass,  $Te$  values of 14.75°C is frequently used, based on the assumption that the reaction is hydrolytic and the overall conversion is of first order (Liu, 2010). When the pretreatment is performed under acidic conditions, the effect of pH can be taken into consideration by the combined severity (CS), defined as:

$$\text{Combined severity (CS)} = \text{Log}(RO) - \text{pH} \quad (3)$$

The pH can be calculated from the amount of sulfuric acid solution added to the sample, corrected by its moisture content (Söderström et al., 2003).

## 2.5. Characterization trials

### 2.5.1. Characterization of solids

The characterization of the raw material was accomplished using the Laboratory Analytical Procedure (LAP), Technical Reports of the National Renewable Energy Laboratory (NREL). The measures included total solids, humidity, and extractable substances in water and ethanol (NREL/TP 510-42619), structural carbohydrates (glucans, mannans, galactans, xylans, arabinans and acetyl groups), and acid insoluble and soluble lignin (NREL/TP 510-42618). The same analyses were carried out on the resulting solids from all stages.

Cellulose content in the raw material was determined by measuring total glucose content in sawdust and discounting the glucose from glucomannans. It was assumed that the mannose to glucose ratio in the hemicelluloses of pine is 4.15 (Yoon et al., 2008). The glucose from cellulose (named Mono-C) and hemicellulosic sugars (named Mono-H) in the original sawdust were calculated using the following equations:

$$\text{Mono-C} = \text{glucose} - \text{mannose}/4.15 \quad (4)$$

$$\text{Mono-H} = \text{arabinose} + \text{xylose} + \text{galactose} + \text{mannose} + \text{glucose} - \text{mannose}/4.15 \quad (5)$$

Crystallinity of the original and treated residual solids was determined by X-ray diffraction. The sawdust was milled and the fraction passing 80 mesh was used for analysis. Spectra were obtained using a Philips X Pert diffractometer. The diffraction patterns were recorded using a copper X-ray source and data were collected at 0.02° ( $2\theta$ ) resolution, from 5° to 40° ( $2\theta$ ). After recording the diffraction patterns, the intensity as a function of the scattering angle  $2\theta$  is processed using Microsoft Excel spreadsheet tool. Crystallinity index ( $CrI$ ) was calculated from the height ratio between the intensity of the crystalline peak ( $I_{002} - I_{am}$ ) and total

**Table 1**  
Experimental conditions of the alkaline stage and chemical composition of the residual solids (original and alkaline extracted sawdust).

Trial	Temperature (°C)	NaOH (% od)	Composition of the solid material (% based on treated wood)								
			Gl <sup>a</sup>	Xy <sup>a</sup>	Ga <sup>a</sup>	Ar <sup>a</sup>	Ma <sup>a</sup>	Ac <sup>a</sup>	Ex <sup>a</sup>	Lig <sup>a</sup>	Weight loss
		Original sawdust	46.62	7.30	2.24	1.13	10.18	1.13	4.60	27.20	
1	70	2.50	42.27	6.91	1.99	0.92	10.28	N.d.	0.72	28.73	5.34
2	90	2.50	43.18	6.52	1.92	0.96	10.26	N.d.	0.62	28.71	6.50
3	70	5.00	42.32	6.38	1.82	0.94	10.32	N.d.	0.57	28.58	6.81
4	90	5.00	43.77	6.70	1.74	0.86	10.09	N.d.	0.44	28.67	8.59
5	66	3.75	43.12	6.33	1.89	0.92	10.46	N.d.	0.95	28.72	6.21
6	94	3.75	43.04	6.47	1.60	0.68	10.61	N.d.	0.55	28.58	7.78
7	80	1.98	44.48	6.92	2.25	1.09	10.60	N.d.	0.87	29.10	7.06
8	80	5.52	43.92	6.76	1.76	0.93	10.32	N.d.	0.35	29.55	4.07
9	80	3.75	43.96	6.70	2.11	1.07	10.40	N.d.	0.45	29.80	7.14
10	80	3.75	44.35	6.81	2.10	1.03	10.41	N.d.	0.38	29.22	6.64
11	80	3.75	43.31	6.82	2.11	1.02	10.28	N.d.	0.44	29.13	6.25

N.d., Not detected.

<sup>a</sup> Ex, extractives; Lig, lignin.

intensity ( $I_{002}$ ). The peaks  $I_{002}$  and  $I_{am}$  correspond to  $2\theta = 22.6^\circ$  and  $18.0^\circ$ , respectively.

$$CrI = \left( \frac{I_{002} - I_{am}}{I_{002}} \right) \times 100 \quad (6)$$

### 2.5.2. Characterization of liquids

The liquid samples were characterized by the determination of sugars (glucose, xylose, mannose, galactose and arabinose) and degradation products (acetic acid, furfural and 5-hydroxymethylfurfural) contents.

The quantification of organic acids and degradation products was carried out by HPLC liquid chromatography (Waters HPLC System), using an Aminex-HPX87H column (BIO-RAD) with the following chromatographic conditions: 4 mM of  $H_2SO_4$  as eluent, 0.6 mL/min, 35 °C, and diode array detector.

Liquor samples from the acid stage were neutralized with  $Ba(OH)_2$  following the methodology used by Kaar et al. (1991). Carbohydrates were determined by HPLC, using a SHODEX SP810 column, under following conditions: water as eluent, 0.6 mL/min, 85 °C, and refractive index detector.

The HPLC analysis was performed both before and after hydrolysis of the extracts with 3%  $H_2SO_4$  at 121 °C for one hour in an autoclave.

The extraction yields ( $Ye$ ) of wood components were defined by Eq. (7).

$$Ye = \frac{m_{dissolved}}{m_{initial}} \times 100 \quad (7)$$

where  $m_{dissolved}$  represents the amount of the component dissolved in each reaction condition and  $m_{initial}$  is the original amount of the same component in pine sawdust, both expressed as percentage of initial dry wood.

## 3. Results and discussion

### 3.1. Raw material

Slash pine sawdust presented the chemical composition of a typical softwood (Table 1), with high content of insoluble lignin and hexoses. Mono-C and Mono-H, according to Eqs. (4) and (5), were 49.1% and 26.1% based on dry wood, respectively. The glucose from the galactoglucomannan was approximately 2.7% on dry wood.

### 3.2. Alkaline fractionation

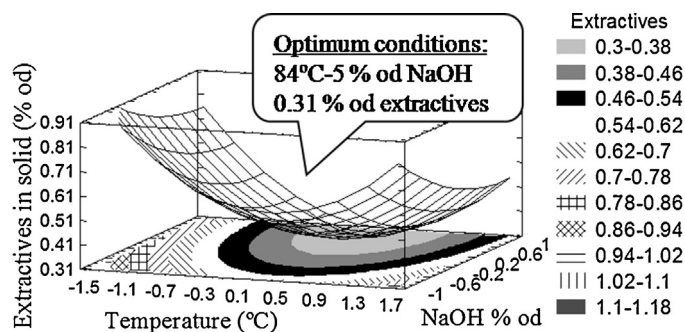
Sawdust was subjected to an alkaline stage in order to remove the resin and fatty acids. The extraction conditions and the composition of the extracted sawdust (as percentage of initial dry wood) according to the points set by the experimental design are presented in Table 1.

The treated sawdust was completely deacetylated in these conditions. Chen et al. (2012) have reported that the deacetylation improves glucose and xylose yields during dilute acid pretreatment and enzymatic hydrolysis. It also reduces the toxicity of the hydrolyzate, improving ethanol yields in the fermentation.

Weight loss of the solid increased both with increasing alkali charge and temperature. Variations in lignin and carbohydrates were minimal in all cases, but the applied treatments produced significant differences in the extractives content. The empirical model derived from the application of the experimental design that explains the residual extractives content in the material is:

$$\begin{aligned} \text{Extractives} = & 0.39 - 0.09 \times T - 0.13 \times C + 0.13 \times T^2 \\ & + 0.07 \times C^2 \quad R^2 = 85\% \end{aligned}$$

Both the temperature and the NaOH charge ( $p$ -value < 0.05) influenced deresination. According to the coefficients of this equation, alkali concentration is the most influential factor in the removal of extractives. The amount of residual extractives in the sample decreased with the increase of temperature and concentration, but the quadratic terms in the equation indicate the presence of a minimum (Fig. 2). This means that there is a limit in the alkaline extraction, i.e. there is a remaining amount of extractives in wood



**Fig. 2.** Response surface for extractives removal (temperature and NaOH concentration are as codified variable).

**Table 2**  
Experimental conditions and yields of dilute acid treatment.

Trial	Temp. (°C)	Time (min)	H <sub>2</sub> SO <sub>4</sub> (g/L)	CS <sup>a</sup>	Yield (% od based on initial wood)	
					Original sawdust	Alkaline pre-extracted sawdust
1	120	30	2.50	0.78	94.90	87.80
2	150	30	2.50	1.25	91.10	84.00
3	120	60	2.50	1.08	92.30	84.00
4	150	60	2.50	1.55	85.20	81.80
5	120	30	7.50	1.66	79.90	80.20
6	150	30	7.50	2.14	74.90	73.30
7	120	60	7.50	1.96	76.20	76.20
8	150	60	7.50	2.44	70.70	69.90
9	110	45	5.00	0.96	94.20	86.10
10	160	45	5.00	2.43	70.20	70.00
11	135	20	5.00	1.34	90.30	83.40
12	135	70	5.00	1.89	81.90	71.40
13	135	45	0.80	0.90	90.50	87.60
14	135	45	9.20	1.96	78.00	76.90
15	135	45	5.00	1.70	80.00	79.00
16	135	45	5.00	1.70	80.50	79.90
17	135	45	5.00	1.70	80.90	79.20

<sup>a</sup> CS, combined severity.

that cannot be removed with alkali, being probably the fraction of unsaponifiables.

The conditions corresponding to trial 4 (90 °C and 5% NaOH, od) were selected to perform the scaling-up in a 7 L reactor. At this scale, the yield was 92%, and the removal of extractives and lignin were 90.7% and 3.3% respectively. Glucans and mannans were easily removed, but their extraction was low with respect to the original values of sawdust (12% in both cases). Galactoglucomannans were easily dissolved in mild alkaline conditions unlike what happened with arabinoglucuroxilans that are more stable (Schild et al., 2010; Sjoström, 1977). The amount of hemicellulosic sugars extracted in this stage was 22.6% on basis of original sawdust, probably in the form of hydroxy acids in spent liquors (Sjoström, 1977). The obtained results at this scale were similar to those obtained in lab-scale experiments, showing the prediction capacity of those experiences. Despite a high yield of extractives removal has been obtained, this stage is susceptible to optimization. Further experiences should include low liquor to dry wood ratios to facilitate the recovery of extractives, with simultaneous reduction of water and energy.

**Table 3**  
Chemical composition of spent liquors from the acid stage applied to alkaline extracted sawdust (g/L).

Trial	Total sugars (g/L)						Degradation products (g/L)		Total (g/L)	
	CS <sup>a</sup>	Gl <sup>b</sup>	Xy <sup>b</sup>	Ga <sup>b</sup>	Ar <sup>b</sup>	Ma <sup>b</sup>	HMF	Furfural	Hexoses <sup>c</sup>	Pentoses <sup>d</sup>
1	0.78	0.03	0.91	0.22	0.98	0.14	0.01	N.d.	0.39	1.90
2	1.25	1.09	3.31	1.42	1.46	2.09	0.02	N.d.	4.60	4.77
3	1.08	0.23	1.52	0.53	1.17	0.24	0.02	N.d.	0.99	2.69
4	1.55	1.55	4.69	1.90	1.42	4.16	0.02	0.07	7.61	6.11
5	1.66	0.34	2.42	0.89	1.39	0.66	0.02	N.d.	1.88	3.81
6	2.14	2.12	4.88	2.32	1.34	5.52	0.06	0.13	9.96	6.23
7	1.96	0.71	2.95	1.44	1.43	1.56	0.02	N.d.	3.71	4.38
8	2.44	3.22	4.21	1.61	1.39	4.94	0.32	0.47	9.78	5.60
9	0.96	0.14	1.67	0.49	1.29	0.10	0.01	N.d.	0.73	2.96
10	2.43	2.84	4.46	1.63	1.20	5.84	0.28	0.39	10.32	5.66
11	1.34	0.52	2.58	1.38	1.38	0.81	0.02	N.d.	2.71	3.96
12	1.89	1.15	3.90	1.97	1.38	3.42	0.01	0.04	6.55	5.28
13	0.90	0.05	0.95	0.18	0.68	0.21	0.02	N.d.	0.44	1.63
14	1.96	1.37	3.94	1.97	1.41	3.56	0.01	0.04	6.90	5.36
15	1.70	1.05	3.54	1.94	1.50	2.71	0.00	0.02	5.71	5.03
16	1.70	0.92	3.29	1.72	1.52	2.29	0.02	0.02	4.93	4.81
17	1.70	0.99	3.49	1.69	1.50	2.60	0.02	0.01	5.28	4.99

<sup>a</sup> CS, combined severity.

<sup>b</sup> Gl, glucose; Xy, xylose; Ga, galactose; Ar, arabinose; Ma, mannose.

<sup>c</sup> Hexoses = Gl + Ga + Ma.

<sup>d</sup> Pentoses = Xy + Ar.

### 3.3. Dilute acid fractionation

The resulting solid material from the alkaline deresination (90 °C, 5% NaOH, od) was subjected to an acid stage in order to find the conditions that optimize the removal of hemicelluloses. Combined severity parameters and yields of this stage, corresponding to each condition of the experimental design for the original sawdust alkaline and the treated sawdust, are presented in Table 2.

Alkali pre-extracted sawdust was more easily impregnated by the dilute acid solution than the original material. It has been shown that deacetylation and glucomannan loss in pine are associated with an increased capillarity of the wood (Montagna et al., 2012).

Arabinoglucuroxilans and galactoglucomannans were dissolved in the reaction medium as oligomers and monosaccharides. Due to the high temperatures and the acid conditions of the treatment, some released sugars were degraded, generating furfural (degradation of pentoses) and 5-hydroxymethylfurfural or HMF (degradation of hexoses). Total sugars and degradation products contents in spent liquor are shown in Table 3.

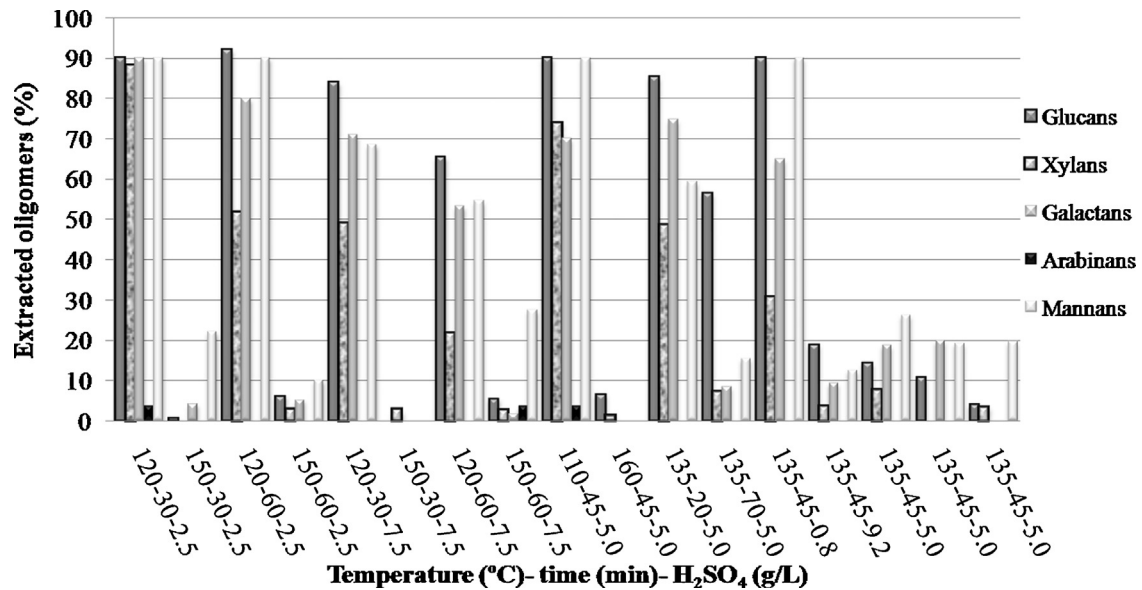


Fig. 3. Oligomers removed (as % of total sugars removed) in the acid stage applied to alkali pre-extracted sawdust.

The amount of hemicellulosic sugars in the spent liquor was high, whereas that of glucose was low. These results can be attributed to the high reactivity of hemicelluloses (amorphous and more accessible) as compared with cellulose (crystalline and packed in a microfibrillar array). Sugars degradation was low and increased at higher CS.

The presence of soluble oligosaccharides after hydrolysis was confirmed by applying a secondary acid hydrolysis to the spent liquor. The percentages of hemicellulosic sugars extracted from the alkali pre-extracted sawdust as oligomers (pentosans and hexosans as % of total sugar extracted) are exposed in Fig. 3.

Arabinans are extracted principally as sugars in all trials. In general, with milder conditions of temperature, time and sulfuric acid concentration, glucose, xylose, mannose and galactose are extracted mainly as oligomers. This differs from what happened

in autohydrolysis, where glucomannans are obtained primarily as oligomers, even in the most severe conditions (Yoon et al., 2008).

Total carbohydrates (hexoses and pentoses) content in spent liquors for each sample, as percentages, are shown in Fig. 4. The obtained yields in all conditions were higher for original sawdust (control) than for alkaline pre-extracted sawdust.

The highest yields of Mono-H (hexoses and pentoses) were 74% for the original sawdust and 57% for the alkaline pre-extracted sawdust, both treated in the same conditions (150 °C, 30 min, 7.5 g/L H<sub>2</sub>SO<sub>4</sub>). This difference can be attributed to the fact that the more labile components have already been extracted in alkaline stage.

The obtained yields of glucose and hemicellulosic sugars were similar to those obtained by others authors using different CS (Table 4). Marzioletti et al. (2008) have obtained the same yield with a lower CS. Increasing CS produce an increase in sugar degradation.

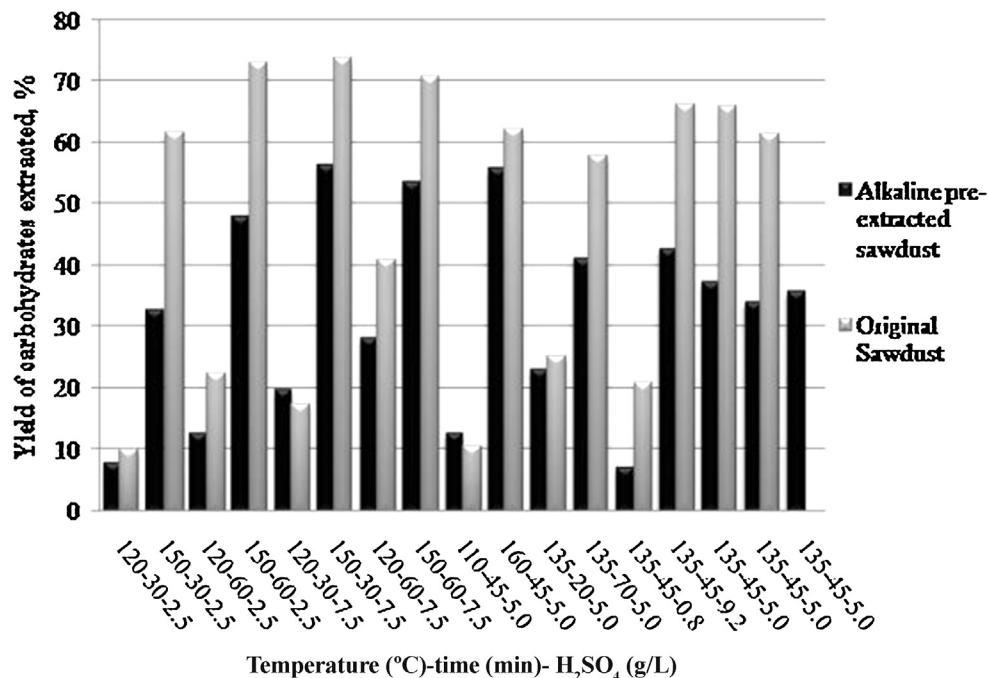


Fig. 4. Total carbohydrates extracted during the acid treatment of the original sawdust and the alkali pre-extracted sawdust.

**Table 4**  
Conditions and results of acid pretreatments on softwoods.

Raw material	Conditions	CS	Yield	Degradation products	References
Mixed softwood (Pinus)	pH: 1.34; 30 min; 170 °C	2.49	100% of mannose and xylose; 5% of cellulose	1.49 g/L furfural; 5.57 g/L HMF	Lim and Lee (2013)
Spruce ground chips	5 g/L H <sub>2</sub> SO <sub>4</sub> ; 60 min, 180 °C	3.15	57% of hemicelluloses; 3% of cellulose	2.7 g/L furfural; 4.7 g/L HMF	Shuai et al. (2010)
Loblolly pine sawdust	1 g/L H <sub>2</sub> SO <sub>4</sub> ; 60 min, 150 °C	1.57	60% of hemicelluloses; 2.1% of cellulose	2.9% HMF + furfural	Marzalletti et al. (2008)
Loblolly pine chips	H-factor: 1500 (170 °C; 90 min time at T <sub>max</sub> ) (autohydrolysis)	–	11.9% of hemicelluloses; 0.54% of cellulose	Not reported	Yoon et al. (2008)
Picea abies sawdust	0.5% H <sub>2</sub> SO <sub>4</sub> ; 10 min, 180 °C (steam explosion)	2.36	88% of mannose; 12% of glucose	0.6% HMF; 0.3% furfural	Söderström et al. (2003)
Softwood sawdust	1.5 wt% H <sub>2</sub> SO <sub>4</sub> ; 190 °C; 100 seg (steam explosion)	2.37	60–70% of hemicelluloses; 16.6% glucose	0.2% HMF; furfural not detected	Kim (2005)
Softwoods chips	0.35 wt% H <sub>2</sub> SO <sub>4</sub> ; 212 °C; 105 seg (steam explosion)	2.41	90% of hemicelluloses; 20% of cellulose	3.1% HMF; 13.5% furfural	Nguyen et al. (1998)
Slash pine sawdust	7.5 g/L H <sub>2</sub> SO <sub>4</sub> ; 30 min, 150 °C	2.14	57% of hemicelluloses; 4% of glucose	0.11% HMF; 0.22% furfural	This work

Lim and Lee (2013) reported the complete extraction of mannans and xylans with a CS of 2.5.

Yields obtained during the acid-catalyzed treatment were considerably higher than those reported by Yoon et al. (2008) using autohydrolysis (11.9% of total hemicelluloses extracted with water at 180 °C for 15 min).

Temperature (*T*), acid concentration (*C*) and time (*t*) produced significant effects on the extraction of sugars in the acid process (*p*-value < 0.05). Regression equations (obtained with coded variables) representing the influence of these factors on sugars extraction in the acid treatment of the alkali pre-extracted sawdust were:

$$\text{Glucose} = 0.82 + 0.75 \times T + 0.21 \times t + 0.38 \times C + 0.21 \times T^2 + 0.11 \times T \times t + 0.22 \times T \times C + 0.09 \times t \times C \quad R^2 = 99$$

$$\text{Xylose} = 3.04 + 0.94 \times T + 0.27 \times t + 0.61 \times C - 0.21 \times T \times C - 0.24 \times t \times C - 0.25 \times C^2 \quad R^2 = 93$$

$$\text{Galactose} = 1.60 + 0.41 \times T + 0.35 \times C - 0.22 \times T^2 - 0.21 \times C^2 \quad R^2 = 74$$

$$\text{Mannose} = 2.01 + 1.60 \times T + 0.46 \times t + 0.78 \times C + 0.23 \times T^2 \quad R^2 = 95$$

All the equations showed significant adjustments at 95% confidence (*p*-value < 0.05). Lack of fit of the models for arabinose, HMF and furfural was significant because their amounts were too small in relation to the experimental error.

According to the coefficients of the model, temperature was the most influential factor, followed by acid concentration, and time. Janga et al. (2012) have found similar results working with concentrated acid. In all cases, high levels of these factors resulted in greatest extraction. Mannose and glucose were affected by a slight positive quadratic effect of temperature, indicating that the extraction is accelerated at high values of this factor. The positive effects on glucose extraction of the interactions: temperature–time, and temperature–concentration, indicate that the influence of the other factors become more important at high temperature. This can be attributed to the dissolution of the less crystalline cellulose under severe conditions. In the case of xylose concentration, the negative quadratic effect represents a maximum followed by a subsequent decrease due to degradation thereof. This behavior of glucose and xylose at high severity agrees with the findings of others authors (Marzalletti et al., 2008; Jensen et al., 2010).

The desirability function was used to maximize the extraction of mannose, xylose, and galactose, while minimizing glucose removal. The resulting optimum conditions were 7.5 g/L H<sub>2</sub>SO<sub>4</sub> at 150 °C for 30 min, with a maximum desirability of 0.73 and 57% of hemicelluloses extraction, mainly as sugars (Fig. 5). There is a maximum in the extraction of sugars from hemicelluloses that reflects that degradation of carbohydrates is very high at the most severe conditions. This agrees with the work of Marzalletti et al. (2008), who found that hemicelluloses yield dropped dramatically by increasing the hydrolysis temperature (150 °C vs. 200 °C).

### 3.4. Fibrous material

Chemical composition and crystallinity of sawdust before and after the treatment in the optimum operation conditions are shown in Table 5.

**Table 5**

Extraction yields and composition of the original sawdust, the alkaline pre-extracted sawdust (90 °C, 5% NaOH, 60 min), the alkaline pre-extracted/acid treated sawdust (90 °C, 5% od NaOH, 60 min/150 °C, 7.5 g/L H<sub>2</sub>SO<sub>4</sub>, 30 min) and the acid treated sawdust (150 °C, 7.5 g/L H<sub>2</sub>SO<sub>4</sub>, 30 min).

% od based on initial wood	Original sawdust	Alkaline pre-extracted sawdust	Ye <sup>a</sup> (%)	Alkaline pre-extracted/acid treated sawdust	Ye (%)	Acid treated sawdust	Ye (%)
Glucans	46.6	41.2	11.7	38.3	6.9	39.1	16.2
Xylans	7.3	6.4	12.9	1.9	69.5	2.1	71.8
Galactans	2.2	1.7	23.2	N.d.	100	N.d.	100
Arabinans	1.1	1.0	15.9	N.d.	100	N.d.	100
Mannans	10.2	9.0	11.7	4.1	54.8	2.0	80.6
Acetyl groups	1.1	N.d.	100.0	N.d.	–	N.d.	100
Lignin	27.2	26.3	3.3	26.8	–1.7	29.4	–8.0
Extractives	4.6	0.4	90.7	0.4	0.0	2.8	38.5
Total	100.4	85.9		71.5		75.3	
CrI <sup>b</sup>	61.0	66.9		70.0		69.7	

N.d., not detected.

<sup>a</sup> Ye, extraction yield.

<sup>b</sup> CrI, crystallinity index.

At the end of the alkaline stage, the material was free of acetyl groups and showed low resin content. As part of the carbohydrates was degraded, remaining as acids in the spent liquors, they cannot be exploited as sugars.

Mannose removal was greater in the sawdust treated with acid than in that treated with the alkaline–acid sequence. The lignocellulosic material obtained after both stages had high hexoses content (glucose and mannose), with a great potential for bioethanol production. Nevertheless, its high lignin content makes necessary the application of a subsequent delignification stage to improve the yield of both the enzymatic hydrolysis and fermentation, allowing also the lignin exploitation.

No significant delignification took place during the acid stage. On the contrary, an increase in lignin content was observed. This phenomenon has previously been reported by several authors, and is believed to be consequence of the formation of a material called pseudolignin. This aromatic material is produced by the condensation of degradation products from polysaccharides and increases as the conditions become more severe (Sannigrahi et al., 2008a, 2011b). The presence of pseudolignin is negative for the enzymatic processes (Hu et al., 2012). This study revealed high formation of pseudolignin, as determined by the technique used by (Sannigrahi et al., 2011). It was greater for the acid treated sawdust than for the alkaline–acid treated sawdust, which is an advantage for this last treatment. Acid treatments can also increase the degree of lignin condensation (Pu et al., 2013; Sannigrahi and Ragauskas, 2008).

Crystallinity increased with all treatments, but the main change was produced by the acid stage. An increase in the crystallinity index of cellulose, due to the preferential degradation of the less crystalline regions during acid treatments, has been reported by

other authors (Pu et al., 2013; Sannigrahi and Ragauskas, 2008). This could mean that the extracted glucans not only come from hemicelluloses but also from part of the cellulose.

#### 4. Conclusions

The alkaline–acid sequence has shown to be effective for extractives and hemicelluloses removal from slash pine sawdust. Using this sequence, a lignocellulosic material with low hemicelluloses content and two liquid fractions, one containing the saponified extractives and the other rich in sugars were obtained. The highest removal of extractives (90.7%) was reached with the alkaline treatment at 90 °C for 60 min and 5% NaOH (oven dry basis, od). The acid stage produced a maximum of carbohydrates extraction because of sugars degradation under severe conditions. Utmost removal of hemicelluloses (57%), mainly as sugars, was attained with 7.5 g/L H<sub>2</sub>SO<sub>4</sub> at 150 °C for 30 min

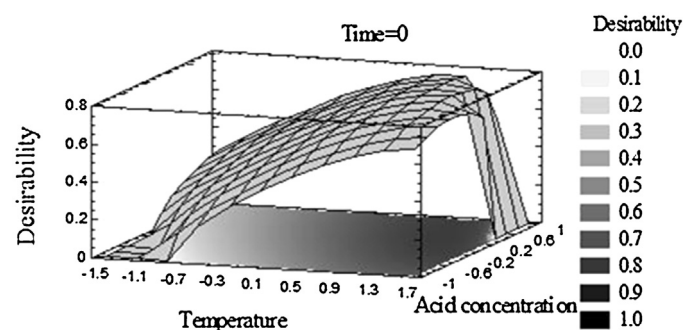
These results provide useful information that can be used to develop technologies that allow integrated use of pine sawdust, by the recovery of valuable products for several uses.

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#### References

- Area, M., Gavazzo, G., Barbosa, O., Villalba, L., 1992. Pastas de alto rendimiento de pinus elliotti y taeda: caracterización de la madera y respuesta a los pulpados RMP y CMP. *El papel*, Junio/Julio., pp. 89–92.
- Arrababal, C., Cortijo, M., 1995. Fracción ácida de extractos de madera de Pinus pinaster ait. producción a partir de madera triturada. *Sist. Recur. For.* 4, 127–142.
- Arshadi, M., Backlund, I., Geladi, P., Bergsten, U., 2013. Comparison of fatty and resin acid composition in boreal lodgepole pine and Scots pine for biorefinery applications. *Ind. Crops Prod.* 49, 535–541.
- Baptista, C., Belgacem, N., Duarte, A., 2006. The effect of wood extractives on pulp properties of maritime pine kraft pulps. *Appita J.* 59 (4), 311–316.
- Barker, T.B., 1985. *Quality by Experimental Design*, 1st ed. Marcel Dekker Inc., New York.
- Bengoechea, D.I., Stoffel, R.B., Area, M.C., 2012. Preatratamientos o Fraccionamientos. In: Area, M.C., Vallejos, M.E. (Eds.), *Biorrefinería a partir de residuos lignocelulósicos. Conversión de residuos a productos de alto valor*. Editorial Académica Española, Saarbrücken, pp. 109–150.
- Chen, X., Shekiri, S., Franden, M.A., Wang, W., Zhang, M., Kuhn, E., Johnson, D., Tucker, M., 2012. The impacts of deacetylation prior to dilute acid pretreatment on the bioethanol process. *Biotechnol. Biofuels* 5 (8), 1–14.
- Demirbas, A., 2011. Methylation of wood fatty and resin acids for production of biodiesel. *Fuel* 90, 2273–2279.



**Fig. 5.** Surface response and contour lines plot of the desirability function for maximization of sugars extracted from hemicelluloses (temperature, time and H<sub>2</sub>SO<sub>4</sub> concentration are as codified variable).



- FitzPatrick, M., Champagne, P., Cunningham, M.F., Whitney, R.A., 2010. A biorefinery processing perspective: treatment of lignocellulosic materials for the production of value-added products. *Bioresour. Technol.* 101, 8915–8922.
- Foran, C.D., 1992. Tall oil soap recovery. In: Green, G., Hough, R.P. (Eds.), *Chemical Recovery in the Alkaline Pulping Processes*. TAPPI Press, New York, pp. 45–56.
- Frederick, W.J., Lien Jr., S.J., Courchene, C.E., DeMartini, N.A., Ragauskas, A.J., Lisa, K., 2008. Co-production of ethanol and cellulose fiber from southern pine: a technical and economic assessment. *Biomass Bioenergy* 32, 1293–1302.
- Hayes, D.J., Fitzpatrick, S., Hayes, M.H.B., Ross, J.R.H., 2006. The biofine process – production of levulinic acid, furfural and formic acid from lignocellulosic feedstocks. In: Gruber, P.R., Kamm, M. (Eds.), *Biorefineries – Industrial Processes and Products*. Status Quo and Future Directions, vol. 1. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, pp. 139–164.
- Hillis, W.E., 1962. *Wood Extractives and Their Significance to the Pulp and Paper Industries*. Academic Press Inc., New York.
- Hu, F., Jung, S., Ragauskas, A., 2012. Pseudo-lignin formation and its impact on enzymatic hydrolysis. *Bioresour. Technol.* 117, 7–12.
- Janga, K.K., Hägg, M., Moe, S.T., 2012. Influence of acid concentration, temperature, and time decrystallization in two-stage concentrated sulfuric acid hydrolysis of pinewood and aspenwood: a statistical approach. *BioResources* 7 (1), 391–411.
- Jensen, J.R., Morinelly, J.E., Gossen, K.R., Campbell-Brodeur, M.J., Shonard, D.R., 2010. Effects of dilute acid pretreatment conditions on enzymatic hydrolysis monomer and oligomer sugar yields for aspen, balsam, and switchgrass. *Bioresour. Technol.* 101, 2317–2325.
- Kaar, W., Cool, L., Merriman, M., Brink, D., 1991. The complete analysis of wood polysaccharides using HPLC. *J. Wood Chem. Technol.* 11 (4), 447–463.
- Kim, K.H., 2005. Two-stage dilute acid-catalyzed hydrolytic conversion of softwood sawdust into sugars fermentable by ethanologenic microorganisms. *J. Sci. Food Agric.* 85, 2461–2467.
- Larsson, S., Palmqvist, E., Tengborg, C., Stenberg, K., Zacchi, G., Nilvebrant, N., 1999. The generation of fermentation inhibitors during dilute acid hydrolysis of softwood. *Enzyme Microb. Technol.* 24, 151–159.
- Lim, W.S., Lee, J.W., 2013. Influence of pretreatment condition on the fermentable sugar production and enzymatic hydrolysis of dilute acid-pretreated mixed softwood. *Bioresour. Technol.* 140, 306–311.
- Liu, S., 2010. Woody biomass: niche position as a source of sustainable renewable chemicals and energy and kinetics of hot-water extraction/hydrolysis. *Biotechnol. Adv.* 28, 563–582.
- Marzalletti, T., Valenzuela Olarte, M.B., Sievers, C., Hoskins, T.J.C., 2008. Dilute acid hydrolysis of loblolly pine: a comprehensive approach. *Ind. Eng. Chem. Res.* 47, 7121–7140.
- Montagna, P., Inalbon, M., Paananen, M., Sixta, H., Zanuttini, M., 2012. Chemical reactions and dynamic capillarity changes in Kraft impregnation of *pinus sylvestris*. In: *Proceedings of the ABTCP 2012 + VII CIADICYP*.
- Nguyen, Q.A., Tucker, N.P., Boynton, B.L., Keller, F.A., Schell, D.J., 1998. Dilute acid pretreatment of softwoods. *Appl. Biochem. Biotechnol.* 70–72, 77–87.
- Pu, Y., Hu, F., Huang, F., Davison, B.H., Ragauskas, A., 2013. Assessing the molecular structure basis for biomass recalcitrance during dilute acid and hydrothermal pretreatments. *Biotechnol. Biofuels* 6 (15), 1–13.
- Sannigrahi, P., Ragauskas, A., 2008. Effects of two-stage dilute acid pretreatment on the structure and composition of lignin and cellulose in loblolly pine. *Bioenergy Res.* 1, 205–214.
- Sannigrahi, P., Kim, D.H., Jung, S., Ragauskas, P., 2011. Pseudo-lignin and pretreatment chemistry. *Energy Environ. Sci.*, 1306–1310.
- Schild, G., Sixta, H., Testova, L., 2010. Multifunctional alkaline pulping, delignification and hemicellulose extraction. *Cellul. Chem. Technol.* 44 (1–3), 35–45.
- Shuai, L., Yang, Q., Zhu, J.Y., Lu, F.C., Weimer, P.J., Ralph, J., Pam, X.J., 2010. Comparative study of SPORL and dilute-acid pretreatments of spruce for cellulosic ethanol production. *Bioresour. Technol.* 101, 3106–3114.
- Sitholé, B., Shirin, S., Zhang, X., Lapierre, L., Pimentel, J., Paice, M., 2010. Deresination options in sulphite pulping. *BioResources* 5 (1), 187–205.
- Sjostrom, E., 1977. The behavior of wood polysaccharides during alkaline pulping process. *TAPPI* 60 (9), 151–155.
- Söderström, J., Pilcher, L., Galbe, M., Zacchi, G., 2003. Two-step steam pretreatment of softwood by dilute H<sub>2</sub>SO<sub>4</sub> impregnation for ethanol production. *Biomass Bioenergy* 24, 475–486.
- Stoffel, R.B., Felissia, F.E., Vallejos, M.E., Area, M.C., 2012. In: Area, M.C., Vallejos, M.E. (Eds.), *Aplicación de la biorrefinería*. En *Biorrefinería a partir de residuos lignocelulósicos: Conversión de residuos a productos de alto valor*. Editorial Académica Española, Saarbrücken, pp. 171–182.
- Tanase, M., Stenius, P., Gregersen, Ø., Johansson, L., Hill, J., 2010. Removal of extractives from TMP process water using dissolved air flotation. In: *7th International Seminar on Fundamental Mechanical Pulp Research*, Nanjing, China.
- Uasuf, A., Hilbert, J., 2012. El uso de la biomasa de Origen Forestal con destino a bioenergía en la Argentina. Castelar, Ediciones INTA, Buenos Aires.
- Vallejos, M.E., 2012. Productos de la Biorrefinería. In: Area, M.C., Vallejos, M.E. (Eds.), *Biorrefinería a partir de residuos lignocelulósicos. Conversión de residuos a productos de alto valor*. Editorial Académica Española, Saarbrücken, pp. 21–107.
- Wyman, C.E., 1994. Ethanol from lignocellulosic biomass: technology, economics, and opportunities. *Bioresour. Technol.* 50, 3–15.
- Yoon, H.S., Macewam, K., Van Heiningen, A., 2008. Hot-water pre-extraction from loblolly pine (*Pinus taeda*) in an integrated forest products biorefinery. *TAPPI*, 27–31.