



Research paper

Hemicellulose extraction from slash pine sawdust by steam explosion with sulfuric acid



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ABSTRACT

One of the main obstacles to the development of advanced forest biorefineries is the optimal fractionation of the plant biomass. The aim of this work was to design a two-stage process for the fractionation of slash pine sawdust: alkaline deresination for extractives removal and sulfuric acid-catalyzed steam explosion (SE) for hemicellulose extraction. SE was carried out in a high-pressure steam reactor under different conditions of temperature (180 °C–200 °C), time (5 min–10 min) and acid concentration (1:100 H₂SO₄ - 3:100 H₂SO₄ (on mass of dry wood)) according to a factorial design. Extractives, lignin, carbohydrates, and degradation by-products were quantified. The effect of pretreatment on enzymatic hydrolysis (EH) was also evaluated. SE was very efficient for hemicelluloses extraction (90% at 200 °C with 3:100 H₂SO₄ (on mass of dry wood) for 5 min) with a high glucan recovery. EH yield increased 6-fold after SE. There was a trade-off between maximizing carbohydrate recovery, hemicellulose extraction, and cellulose digestibility.

1. Introduction

Elliottii and *taeda* pines are the most planted species in the northeast region of Argentina (NEA Region). In the primary industrialization of wood, almost 50% of industrially processed wood becomes sawdust (1.5 Mt y⁻¹ on a dry basis) [1], which can be an excellent raw material for a biorefinery. Forest biorefineries can be defined as a group of biomass conversion processes that provides the efficient use of woody materials at their full potential. In order to achieve an efficient utilization of lignocellulosic materials and to develop economical, robust, and reliable processes for a biorefinery, an effective fractionation of the biomass into its main constituents (cellulose, hemicellulose, and lignin) is essential [2].

The high content of resins in pine sawdust (2 %–4%) usually complicates its direct use in acid pretreatments because acid hydrolysis causes pitch formation. However, the resin acids can be recovered in a mild alkaline medium as sodium salts [3]. For instance, 91% of the slash pine sawdust extractives was removed by treating it with 5:100 NaOH (on mass of dry wood) at 90 °C for 1 h [4].

Softwoods are recognized as highly recalcitrant for both chemical

and biological processes that are based on the deconstruction of the plant cell wall for the production of fuels and chemicals [5]. Various types of pretreatments have been studied for hemicelluloses extraction from softwoods in general [6–10] and from pine in particular [11–14] with the aim of enhancing enzymatic hydrolysis. Pine sawdust is an interesting raw material to produce high-value compounds but its fractionation is complex due to the composition, properties, and distribution of resin acids, hemicelluloses and guaiacyl lignin. Pine fractionation usually requires the combination of chemical and mechanical pretreatment methods and the use of an acid catalyst such as sulfuric acid (H₂SO₄) or sulfur dioxide [15]. Steam explosion (SE) is one of the most successful pretreatment methods for fractionating lignocellulosic materials into their three main macromolecular components while enhancing cellulose accessibility to enzymatic hydrolysis [16].

In general, acid catalysts such as SO₂ or H₂SO₄ are mandatory for the pretreatment of softwood materials, typically in the range of 0.3:100 H₂SO₄ to 3:100 H₂SO₄ (both on mass of dry wood), because autohydrolysis is rather ineffective when compared to agricultural residues and hardwoods [15]. Previous studies have shown that SO₂ is the most effective catalyst for the steam treatment of softwoods [17], but it

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Nomenclature

AIL	Acid-insoluble lignin	pine fraction	
AnAra	Anhydroarabinose from pine heteroxylans	ASL	Acid-soluble lignin
AnGal	Anhydrogalactose from pine galactoglucomanans	C	Concentration
AnGlc	Anhydroglucose from β -(1-4)-d-glucans (mostly cellulose)	CS	Combined severity factor
AnMan	Anhydromanose from pine galactoglucomanans	Ext	Extractives
AnXyl	Anhydroxylose from pine heteroxylans	OAc	Acetyl groups
APP	Alkaline pre-extracted sawdust	n.d.	Not detected
APP	Alkaline pre-extracted pine	SEP-WI	Water insoluble steam-exploded pine fraction
AP-SEP-WI	Water insoluble alkaline pre-extracted steam-exploded	T	Temperature
		t	Time

is highly toxic to human health and may cause a negative environmental impact [18]. Several authors have obtained high hemicellulose extraction yields (60 %–90%) from softwoods that were pretreated by high-pressure steam after impregnation with dilute sulfuric acid [17,19–21]. Moreover, likewise other acid pretreatment methods, most of the lignin component remain in the water-insoluble fraction after SE.

The aim of this work was to apply a two-step fractionation process to slash pine sawdust: alkaline deresination for extractives removal and sulfuric acid-catalyzed steam explosion for hemicellulose extraction. The influence of temperature, time and acid load in both steam explosion and enzymatic hydrolysis was evaluated.

2. Materials and methods

2.1. Raw material

Samples of slash pine sawdust (*Pinus elliotti*) were taken personally from a local sawmill (Forestal AM, Misiones). The sawdust was air-dried, and then it was sieved with screens with square openings of three different sizes: 12.5 mm, 5 mm and 3 mm. The one retained in a 3 mm square screen was used for pretreatment.

2.2. Fractionation sequence

Fig. 1 shows the experimental procedure employed in this study. Pine sawdust was treated with dilute alkali to remove extractives. The resulting solids were exhaustively washed with water and air dried

before the next step. These solids were then impregnated with dilute sulfuric acid and steam-exploded under different conditions. The resulting materials were washed with water and their susceptibility to enzymatic hydrolysis was evaluated.

2.2.1. Alkaline deresination

The alkaline stage was carried out in a 7 L reactor (M/K Systems, Inc., Maryland) with liquor circulation using 500 g oven dry sawdust (od) with 5 L of alkaline solution. The extraction was performed at 90 °C for 1 h using 5:100 NaOH (on mass of dry wood) in relation to the dry biomass. Upon reaction completion, the spent liquor was separated by centrifugation and the extracted biomass was recovered at approximately 40% total solids (TS). Subsequently, the solid fraction was exhaustively washed with water, filtered and air dried to reveal the resulting mass loss. A sample of the deresinated material was reserved for chemical analysis.

2.2.2. Steam explosion

Steam explosion (SE) was carried out in a 10 L high-pressure stainless steel reactor that is located at the Department of Chemistry, Federal University of Paraná (Curitiba, Brazil). The reactor has automated temperature and pressure controls and is coupled to a high-pressure boiler (Weco, GVO 10/30) for the steam supply. Impregnated sawdust was loaded directly into the reactor vessel and steam-treated sawdust was subsequently released by rapid decompression, causing the material to expand (explode) into a stainless steel container.

Acid impregnation was carried out by placing 280 g (dry basis) of

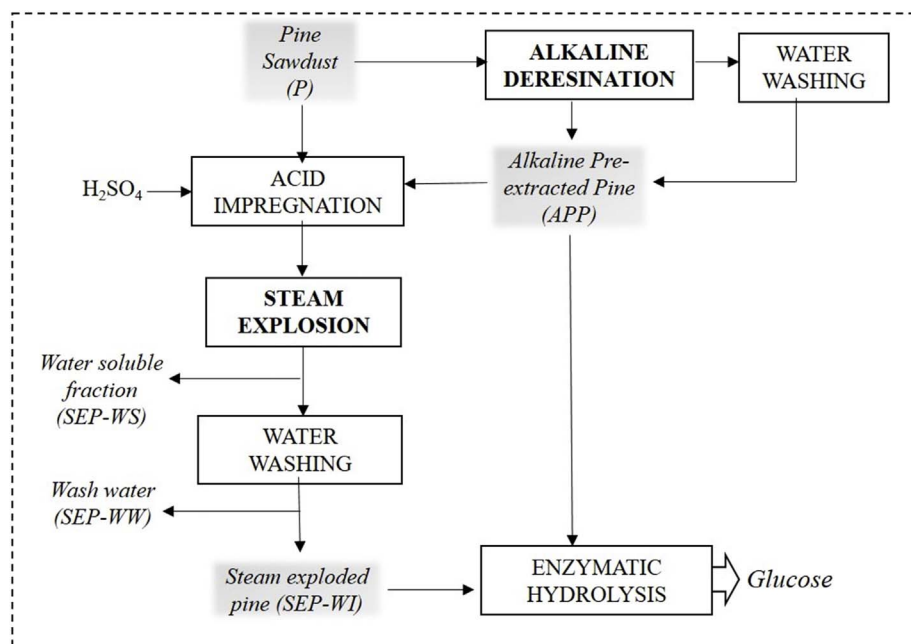


Fig. 1. Experimental set-up used for the two-step pretreatment evaluation.

biomass containing 14% initial moisture content. The material was placed in plastic bags and sprayed with dilute H₂SO₄ until a moisture content of 50% was reached. The bags were vacuum-sealed and kept at room temperature overnight. Steam explosion experiments were carried out under different conditions of temperature (180 °C–200 °C), time (5 min–10 min) and acid concentration 1:100 H₂SO₄ (on mass of dry wood) to 3:100 H₂SO₄ (on mass of dry wood) according to a 2³ factorial design with two replicates at the center point. Pretreatment was applied to alkali pre-extracted pine sawdust (APP) and a batch of the untreated material (P) was used as a reaction control. Furthermore, one additional test at 200 °C, 5 min, and 3:100 H₂SO₄ (on mass of dry wood) with wet APP (i.e., containing a 56% initial moisture content) was performed to evaluate the effect of moisture on pretreatment performance.

The resulting materials were filtrated at reduced pressure to separate the water-soluble fraction (SEP-WS) from the steam-exploded sawdust, which was then washed with water under mechanical stirring at a 5% TS. The water-insoluble fraction was named steam-exploded pine (SEP-WI), whereas the water-soluble fraction was designated as wash water (SEP-WW) (see Fig. 1 for details).

For a better comparison of the process conditions used for pretreatment, the corresponding severity factors were calculated. The severity concept was developed from previous similar concepts, such as the H-Factor and P-Factor, which are used to control the extent of pulping and/or prehydrolysis in the pulp and paper industry. This factor combines severity temperature and reaction time in a single parameter. Although the severity concept is a phenomenological description, it allows for a fair comparison of different pretreatment processes [22]. The severity factor is a variant of H-Factor that is defined by the following equation,

$$H - Factor = \int_0^t \exp((T - T_{ref})/(R * T_{ref} * T / E_a)) * dt \quad (1)$$

where t is the pretreatment residence time (min), T is the pretreatment temperature (°C), T_{ref} is the reference temperature (100 °C), and E_a is the reaction average activation energy. If one approximates T_e as a constant,

$$T_e \approx R * T_{ref} * (T / E_a) \quad (2)$$

Eq. (1) can be written as:

$$H - Factor \approx \int_0^t \exp((T - T_{ref})/T_e) * dt = t * \exp(T - T_{ref})/T_e \quad (3)$$

For the hot-water extraction of woody biomass, the frequently used T_e value is 14.75 °C, assuming it as a hydrolytic first-order reaction [23]. Then, the severity factor (SF) is defined as the log of the H-Factor, as expressed in Eq. (4):

$$SF = \log(t * \exp(T - 100)/14.75) \quad (4)$$

When the pretreatment is performed under acidic conditions, the effect of pH can be taken into account as shown in Eq. (5), which defines the pretreatment combined severity factor [22]:

$$Combined\ severity\ factor = SF - pH \quad (5)$$

The pH was calculated from the amount of sulfuric acid that was added to the material in relation to its total water content. Then, the obtained pH value had to be corrected because the initial acid concentration was partially neutralized by sodium carboxylates (mainly from resin and fatty acids) and residual alkali (remaining from the washing stages) that were present in the slash pine sawdust. To calculate the pH of the liquor, the amount of sulfuric acid that is consumed in the neutralization of the residual alkali was determined. To do this, 24 g of the alkali pre-extracted pine sawdust (APP) was suspended in water and stirred for 1 h. The solution was then titrated with 0.1 mol/L H₂SO₄ until pH 4. The spent sulfuric acid was calculated according to Eq. (6).

$$Spent\ H_2SO_4(g) = Volume\ H_2SO_4\ added\ (L) * 0.1 \left(\frac{mol}{L} \right) * 98.08 \left(\frac{g}{mol} \right) \quad (6)$$

Where 98.08 is de mass of one mol of sulfuric acid.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis was used to assay the effect of pretreatment on substrate accessibility. This was performed following the Laboratory Analytical Procedure (LAP) that are found in the Technical Reports of the National Renewable Energy Laboratory (NREL/TP-510-42629) [24]. Commercial enzymes (Sigma Aldrich) were used for this purpose: *Trichoderma reesei* cellulases (51 FPU/mL of cellulase) and *Aspergillus niger* β-glucosidase (339 IU/mL against cellobiose). To determine the total cellulase activity, the NREL/TP-510-42628 standard method was used, in which Whatman #1 filter paper is used as substrate and the results are given as filter paper units (FPU) per milliliter of original (undiluted) enzyme solution [25]. The β-glucosidase activity was determined by the ability of the enzyme preparation to hydrolyze *p*-nitrophenyl-β-D-glucopyranoside (PNPG) to *p*-nitrophenol [26].

Enzymatic hydrolysis of the water-washed solids was performed at 50 °C with 2% TS in acetate buffer 50 mmol/L pH 4.8, using 20 FPU/g glucans (cellulose activity) and 40 IU/g glucans (β-glucosidase activity). Hydrolyses were carried out in 50 mL Erlenmeyer flasks that were kept in a thermostatic bath with orbital agitation. The reaction was monitored every 24 h up to 72 h. All hydrolysis experiments were performed in duplicate. Enzymatic hydrolysis (EH) yields were calculated according to Eq. (7):

$$EH\ Yield\ (\%) = (Glucose\ released * 0.9) / (glucans\ initially\ present) * 100 \quad (7)$$

2.4. Analysis

The raw material was characterized using the Laboratory Analytical Procedures (LAP) of the National Renewable Energy Laboratory (NREL). These measurements included its total solids, moisture content and total extractable materials in water and ethanol (NREL/TP 510-42619) [27], as well as its structural carbohydrate components (glucose, mannose, galactose, xylose, arabinose and acetic acid) and both acid-insoluble and acid-soluble lignins (NREL/TP 510-42618) [28]. The same analyses were carried out with the resulting solids from all pretreatment stages.

The concentrations of sugars (glucose, xylose, mannose, galactose, and arabinose), acetic acid and dehydration by-products (furfural and 5-hydroxymethylfurfural) were determined by HPLC (Waters HPLC System). Liquor samples from the acid stage were neutralized with Ba (OH)₂ following the method used by Kaar et al. [29]. Carbohydrates were analyzed in an SHODEX SP810 column operating at 85 °C with ultrapure water as the mobile phase at a flow rate of 0.6 mL/min and detection was carried out by differential refractometry. The system was equipped with Deashing Refill Cartridges (Bio-Rad) and a Carbo-P Refill Cartridge (Bio-Rad) prior to the column. Furfural, HMF, and acetic acid were analyzed in an Aminex-HPX87H column (Bio-Rad) with the following chromatographic conditions: 35 °C, 4 mmol/L H₂SO₄ as eluent, 0.6 mL/min and quantification by UV spectrophotometry using a diode array detector.

The pretreatment liquors were characterized by the determination of sugars (glucose, xylose, mannose, galactose, and arabinose), water-soluble oligomeric sugars and degradation products (acetic acid, furfural and 5-hydroxymethylfurfural) contents using the NREL/TP-510-42623 standard method [30].

Sugar losses by dehydration (hexoses and pentoses) were calculated from HMF and furfural using 1.43 and 1.56 as the corresponding stoichiometric factors. The concentration of the polymeric sugars were

calculated from the concentration of the corresponding monomeric sugars using the anhydrous correction factors of 0.99 (or 132/150) for C-5 sugars (xylose and arabinose) and of 0.90 (or 162/180) for C-6 sugars (glucose, galactose, and mannose), hereinafter referred to as anhydroglucose (AnGlc), anhydroxylose (AnXyl), anhydroarabinose (AnAra), anhydromannose (AnMan) and anhydrogalactose (AnGal).

Water-soluble oligomeric sugars were determined by difference between the monomeric sugars determined by HPLC before and after hydrolysis with 3:100 H₂SO₄ (on mass of dry wood) at 121 °C for 1 h.

The recovery yield of cellulose and hemicellulose in both liquid and solid fractions was calculated using the following equation:

$$\text{Recovery yield (\%)} = (\text{Amount after pretreatment in liquid or solid fraction} / \text{Initial amount present in raw material}) * 100 \quad (8)$$

2.5. Statistical analysis

The statistical analysis of the experimental results, including analysis of variance (ANOVA), regression equations and optimization by the Desirability function, was performed using Statgraphics Centurion software at the significance of 95% ($p < 0.05$).

3. Results and discussion

3.1. Raw material

An alkaline step was performed at 90 °C for 1 h with 5:100 NaOH (on mass of dry wood) to remove fatty and resin acids from the pine sawdust. The chemical composition of the original pine sawdust (control) and the alkaline pre-extracted pine sawdust (APP) is shown in Table 1 as percentage in relation to the dried wood. Slash pine sawdust presented high contents of both hexosans and acid-insoluble lignin. Similar values of anhydroglucose, hemicellulosic sugars and lignin were obtained by Clark and Mackie [11], Huang and Ragauskas [12] and Marzialetti et al. [13] for pine species.

The mass loss was 8% in the alkaline step and the treated sawdust was completely deacetylated in the applied experimental conditions. By contrast, no significant extraction of carbohydrates and lignin was observed.

3.2. Hemicelluloses extraction

The alkaline pre-extracted sawdust was steam-exploded in order to extract the hemicelluloses using sulfuric acid as catalyst. The process was applied to the alkaline pre-extracted pine sawdust (APP) and also to the original material as a reaction control. The design conditions, combined severity (CS) factors, yields and chemical composition of the steam-exploded pine sawdust are presented in Table 2.

Since an amount of the initially added sulfuric acid (0.73:100 H₂SO₄ (on mass of dry wood), according to Eq. (6)) was consumed to neutralize both sodium carboxylates and the alkalinity remaining from alkaline deresination, the actual CS applied to APP was lower than the value expected from the amount of acid applied to the original sawdust.

The process yields (obtained solid material) ranged from 60% to 90%. The yields decreased as pretreatment severity increased due to higher levels of solubilization of pine sawdust macromolecular components. After the steam explosion, the solid fraction became a fine powdered suspension and its complete recovery from the collector inner walls was rather difficult, particularly at higher pretreatment severities. Nevertheless, Schwald et al. [18] obtained similar yields after the steam explosion of 2.5% SO₂-impregnated spruce at 190 °C - 200 °C for 2.5 min, reaching 75% and 65% in relation to the dry mass of the original material, respectively.

Extraction of hemicelluloses and glucans increased at higher CS as described in Table 2. Also, hemicellulose extraction was nearly

complete in trials carried out at the highest pretreatment temperature (trials 6 and 8 at 200 °C). For example, most of the hemicelluloses were solubilized in test 6 200 °C, 5 min, and 3:100 H₂SO₄ (on mass of dry wood) where the steam-exploded sawdust contained 55% hexosans, 0.8% pentosans and 42% total lignin in relation to the dry biomass.

At equal CS (2.7), hemicellulose removal was significantly higher for the untreated sawdust (or samples that were not alkali extracted) compared to APP as can be seen in Table 2. Therefore, the alkaline stage had a negative influence on hemicellulose extraction and this may have been caused by changes in chemical composition that occurred during extraction such as in the case of deacetylation and partial loss of acid-labile branching points. Moreover, the acid-insoluble lignin (AIL) ranged from 34% to 48% (Table 2) in both steam-exploded materials and this was mostly a result of hemicellulose removal.

Cellulose degradation was almost negligible at less severe pretreatment conditions but it became an important variable at higher temperatures and acid concentrations. For instance, cellulose losses reached 34% at the highest pretreatment severity (test 8, CS = 3.5). This result is consistent with those of Shahbazi et al. [21] with the steam explosion of pine sawdust, who obtained 14% cellulose losses at 190 °C and nearly 70% when the temperature was increased to 215 °C.

3.3. Composition of the pretreatment liquor

Hemicelluloses and cellulose were dissolved in the reaction medium as oligomers and monosaccharides. However, due to the use of high temperatures and acid concentrations, some of the released pentoses and hexoses were dehydrated to furfural and 5-hydroxymethylfurfural (HMF), respectively.

Mass balances approached 100% at low severities but mass losses were about ± 10% at higher severities due to the formation of by-products and the release of volatiles that were not analyzed by liquid chromatography, therefore requiring further studies for their identification and quantification. Longer residence times and higher temperatures promoted steam condensation and dilution of the pretreatment liquor. The amount of sugars extracted in the wash water was very small (< 2% as mass fraction on the initial dry wood), such that the use of an additional washing step was not justified.

Given the standard error of the means, there was no difference in the anhydroglucose content of steam-exploded materials when pretreatment was carried out at low severities. However, the glucose content became more important at higher severities because of the onset of cellulose degradation (Fig. 2a). In general, glucose yields are difficult to interpret because glucose is released from both hemicellulose and cellulose. The maximum glucose content (7.5%) in the pretreatment liquor was reached at the highest CS (200 °C, 10 min, 3:100 H₂SO₄ (on mass of dry wood)). By contrast, water-soluble hemicellulose sugars in the liquor behaved quite differently, reaching a

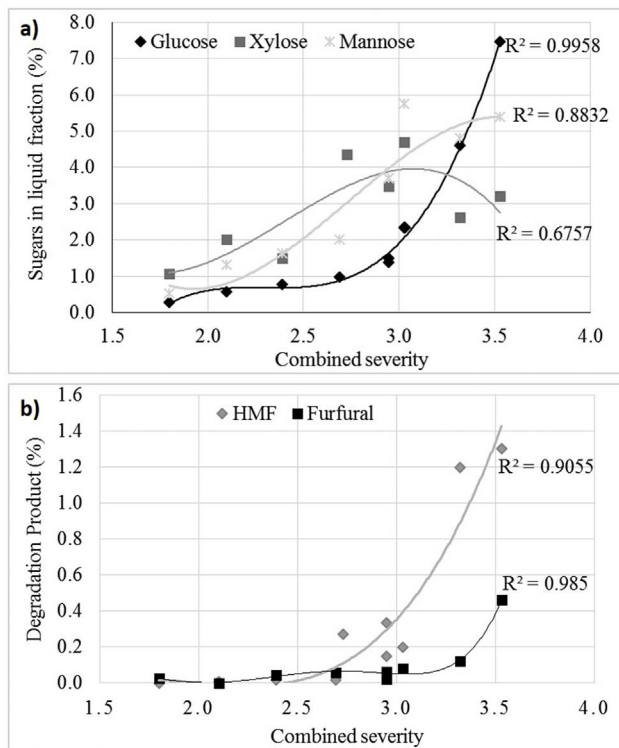
Table 1
Chemical composition (mass fraction) of the original pine sawdust (control) and the alkaline pre-extracted pine sawdust (APP).

Components	Composition (%)	
	Sawdust	APP
AnGlc	39.4 ± 0.25	44.0 ± 1.00
AnXyl	6.42 ± 0.18	6.77 ± 0.10
AnGal	1.97 ± 0.49	2.37 ± 0.10
AnAra	1.32 ± 0.11	1.04 ± 0.10
AnMan	10.6 ± 0.44	11.8 ± 0.30
OAc	2.24 ± 0.65	n.d.
Ext	2.28 ± 0.03	1.32 ± 0.03
AIL	30.5 ± 0.18	29.8 ± 0.01
ASL	0.93 ± 0.09	0.99 ± 0.09
Total	95.7	99.3

Table 2

Experimental conditions, combined severity (CS) factors and chemical composition of the steam exploded pine sawdust (as mass fraction on dry wood).

Trial	T (°C)	t (min)	C (mass of H ₂ SO ₄ :mass of dry wood)	CS	Yield (%)	Chemical Composition (%)					
						AnGlc	AnXyl	AnGal	AnAra	AnMan	AIL
APP	–	–	–	–	92	44 ± 1.0	6.8 ± 0.1	2.4 ± 0.1	1 ± 0.2	12 ± 0.3	31 ± 0.2
APP-SEP-WI											
1	180	5	1:100	1.8	90	46 ± 1.5	3.8 ± 0.1	1.2 ± 0.1	n.d.	10 ± 0.3	34 ± 0.4
2	200	5	1:100	2.4	78	48 ± 1.5	1.9 ± 0.1	0.6 ± 0.0	n.d.	7.3 ± 0.3	38 ± 0.0
3	180	10	1:100	2.1	91	46 ± 2.5	3.3 ± 0.3	1.1 ± 0.2	n.d.	11 ± 0.7	34 ± 0.0
4	200	10	1:100	2.7	74	53 ± 1.0	2.0 ± 0.1	1.0 ± 0.1	n.d.	5.9 ± 0.3	39 ± 0.3
5	180	5	3:100	2.6	72	51 ± 0.7	1.7 ± 0.0	n.d.	n.d.	3.5 ± 0.0	40 ± 0.2
6	200	5	3:100	3.3	60	53 ± 1.8	0.8 ± 0.2	n.d.	n.d.	2.1 ± 1.0	43 ± 0.1
7	180	10	3:100	3.0	77	45 ± 1.0	1.9 ± 0.1	0.7 ± 0.1	n.d.	5.5 ± 0.0	38 ± 0.0
8	200	10	3:100	3.5	59	52 ± 0.2	n.d.	n.d.	n.d.	1.6 ± 0.3	48 ± 0.2
9	190	7.5	2:100	3.0	75	51 ± 0.6	2.0 ± 0.1	0.7 ± 0.2	n.d.	6.8 ± 0.3	38 ± 0.1
10	190	7.5	2:100	3.0	68	51 ± 0.5	2.2 ± 0.1	0.6 ± 0.2	n.d.	6.4 ± 0.4	37 ± 0.2
SEP-WI											
1	190	7.5	0.75:100	2.7	69	49 ± 0.1	0.9 ± 0.0	0.2 ± 0.1	n.d.	1.0 ± 0.1	44 ± 0.2
2	190	7.5	0.75:100	2.7	69	49 ± 0.4	1.3 ± 0.0	n.d.	n.d.	1.2 ± 0.1	43 ± 0.3

**Fig. 2.** Relationship between the combined severity and the concentration of both (a) monosaccharides (glucose, mannose, and xylose) and (b) dehydration by-products (furfural and HMF) in pretreatment liquors.

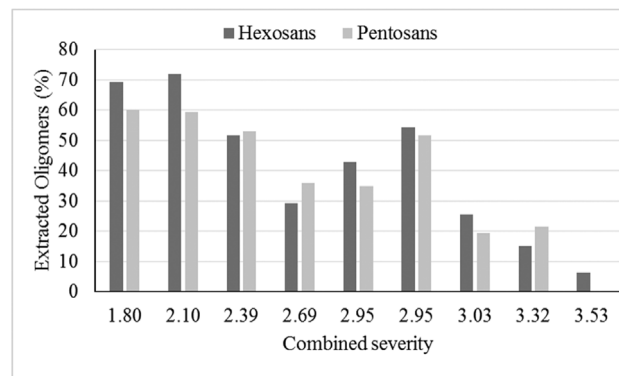
maximum value at CS ~3 and then dropping considerably due to sugar degradation at the most severe pretreatment conditions. These results agree with those obtained by other authors [6,17,19,20,31]. Both HMF and furfural contents increased along with an increase in CS but their concentrations were lower than 0.1% at CS lower than 3 (Fig. 2b). Maximum concentrations of HMF (1.30%) and furfural (0.46%) were detected in experiment 8, which corresponds to the highest CS in the experimental design (CS of 3.5). Nguyen et al. [17] reported the generation of higher HMF and furfural levels with the use of slightly less severe pretreatment conditions. However, low furfural and HMF contents were found at high CS because these compounds were converted

to secondary degradation compounds such as levulinic and formic acids and to acid-insoluble macromolecular components (often referred to as pseudo-lignin) that were not quantified in this work. This decrease in HMF and furfural was also reported by other authors, who demonstrated their conversion to such organic acids [6,20,31]. Acetic acid was always absent in the pretreatment liquor because it was completely removed during alkaline deresination.

The presence of soluble oligosaccharides after SE was confirmed by further acid hydrolysis of the pretreatment liquor. The percentage of hemicellulose sugars extracted as oligomers is shown in Fig. 3. More than 50% of the slash pine sawdust hemicelluloses were found as oligomers at low CS. By contrast, at high CS, monosaccharides were the predominant form of hemicellulose sugars in the corresponding pretreatment liquors.

3.4. Statistical analysis and optimization

Statistical analysis of the biomass chemical composition indicated that both the acid concentration and the temperature ($p < 0.05$) influenced the extraction of polysaccharides (hemicelluloses and cellulose). However, the effect of time was not statistically significant ($p > 0.05$) under the conditions applied in this study. This means that, in this case, time and temperature are not interchangeable in the severity actor. The reason is the high influence of temperature and acid concentration respect of the short considered periods. The regression

**Fig. 3.** Concentration of oligosaccharides in the pretreatment liquors as a function of combined severity (values expressed in relation to the amount of total sugars).

equations (in coded variables) representing the influences of these factors on the chemical composition of AP-SEP are:

$$\text{Anhydroglucose} = 36.5 - 3.36 * T - 3.12 * C \quad R^2 = 0.8278 \quad (9)$$

$$\text{Anhydroxylose} = 1.59 - 0.74 * T - 0.81 * C \quad R^2 = 0.9615 \quad (10)$$

$$\text{Anhydromannose} = 4.84 - 1.53 * T - 2.64 * C \quad R^2 = 0.9629 \quad (11)$$

$$\text{Anhydrogalactose} = 0.51 - 0.28 * T - 0.25 * C \quad R^2 = 0.9864 \quad (12)$$

In general, hemicelluloses and glucans (mostly cellulose) decreased in the pretreated solids with an increase in both temperature and acid concentration. The equation coefficients indicated that acid concentration was the factor that most influenced the extraction of hemicelluloses, followed by temperature. However, the temperature was the most influential factor for cellulose hydrolysis. Other authors observed the same trends regarding the effect of time in the steam explosion of softwoods, even when SO_2 was used instead of H_2SO_4 [17,32].

The desirability function identifies the combination of factors that simultaneously optimize multiple answers. This function, expressed in a scale from 0 to 1, was used to maximize the extraction of anhydromannose and anhydrogalactose from galactoglucomannans and anhydroxylose from heteroxylans while minimizing anhydroglucose removal from the cellulose of the steam-exploded pine sawdust. The response surface of the desirability function for the optimization of Equations (9)–(12) using the above mentioned criteria is shown in Fig. 4.

The desirability function increased to a maximum value and dropped abruptly at high levels of temperature and acid concentration mostly due to glucan hydrolysis and dehydration. The maximum desirability value (0.70) in the experimental region was achieved at 200 °C (+1), 3:100 H_2SO_4 (on mass of dry wood) (+1), and 5 min (−1), which corresponded to trial 6 of the experimental design. In such conditions, 89% anhydromannoses, 93% anhydroxyloses, and 100% anhydroarabinose and anhydrogalactose were extracted from pine sawdust while 21% glucans were lost to the pretreatment liquor.

Acid penetration into fibers is a critical issue to obtain a uniform catalytic effect and an optimal hemicellulose extraction from lignocellulosic biomass such as slash pine sawdust. Therefore, further improvements in pretreatment performance may be achieved by modifying the method used for acid impregnation, such as in the case of soaking the biomass with the acid solution and pressing it to remove excess of liquid prior to pretreatment.

3.5. Effect of the initial moisture content of slash pine sawdust

To evaluate the effect of air drying on SE, the optimal condition (200 °C, 5 min, 3:100 H_2SO_4 (on mass of dry wood)) was reproduced

using never dried APP sawdust with an initial moisture of 56%. The use of such high moisture contents led to acid dilution during impregnation, resulting in a CS value lower than that of trial 6 of the experimental design (3.0 vs. 3.3). The mass recovery yield at this pretreatment condition was similar to that obtained for the dry sawdust (58.8%) but the hemicellulose extraction was higher, probably due to a better acid impregnation of the sawdust. Anhydroxylose, anhydroarabinose, and anhydrogalactose residues were completely extracted from both materials (never dried APP and dry APP). By contrast, 37% and 93% of glucans and mannans were extracted from the wet material, whereas such values for the dry sawdust were 21% and 89%, respectively. In addition, both pretreatment liquors had very similar concentrations of furfural and HMF.

3.6. Enzymatic digestibility

Enzymatic hydrolysis of steam-exploded pine sawdust under different experimental conditions was carried out for 72 h using an enzyme loading of 20 FPU/g glucan (Fig. 5). Glucose yield was higher after steam explosion indicating an increase in cellulose accessibility due to the disruption of the fiber structure, which increased both substrate surface area and pore volume by the selective hydrolysis and removal of hemicelluloses [7,14]. Nevertheless, glucose yields were still low after pretreatment probably due to the distribution and chemical/physical composition of steam-exploded lignin. For example, lignin droplets are formed as a result of polymerization/repolymerization reactions and these may redeposit on the cellulose surface, blocking the pores formed by SE and decreasing the rate and extent of enzymatic hydrolysis [33].

Glucose yield was not increased after alkaline stage indicating that extractives removal caused not apparent effect on the susceptibility of cellulose to enzymatic hydrolysis. The best hydrolysis performance was obtained when SE was carried out at the highest CS of 3.23 (200 °C, 5 min and 3:100 H_2SO_4 (on mass of dry wood)), resulting in glucose yields (24.3%) 6 times higher than those obtained from the untreated sawdust. These results agree with those of Söderström et al. [20], who obtained a 35% glucose yield by enzymatic hydrolysis (28 FPU/g glucan) of substrates that were derived from the two-step dilute H_2SO_4 pretreatment of Douglas fir.

Similar hemicellulose extraction yields were obtained from softwoods by SE when the material was pre-impregnated with SO_2 or H_2SO_4 but the enzymatic hydrolysis performance was better when SO_2 was used for impregnation [8]. For instance, glucose yields from SO_2 -impregnated steam-exploded softwoods were about 50–60% after 72 h of hydrolysis using 20 FPU/g glucan [9–12]. This was so because SO_2 impregnation led to a higher delignification degree probably due to lignin sulfonation, which reduces the glass transition temperature of

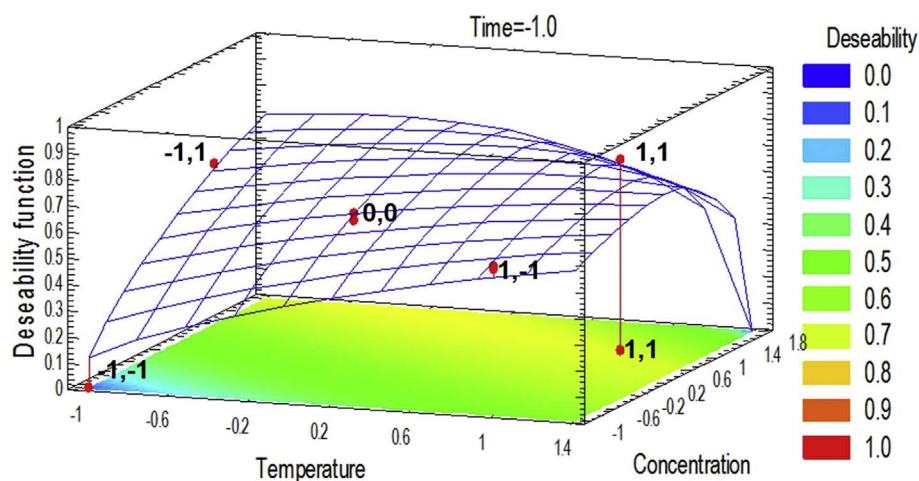


Fig. 4. Response surface and contour lines of the desirability function for the maximization of hemicellulose removal (galactoglucomannans and heteroxylans) and the minimization of cellulose loss (temperature, time and H_2SO_4 concentration as coded variables). The experimental points of temperature, H_2SO_4 concentration and time are indicated in black.

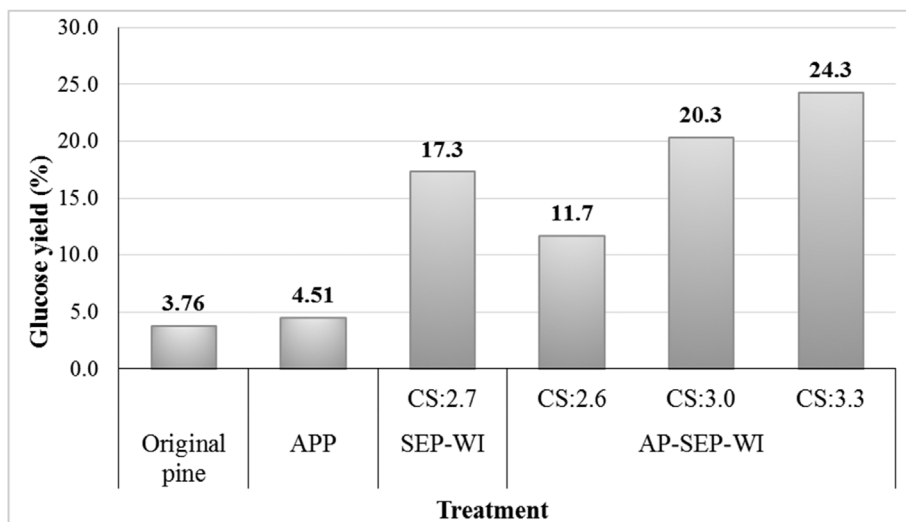


Fig. 5. Hydrolysis profiles of untreated pine sawdust and water-insoluble fractions that were produced by alkaline pre-extraction at 90 °C, 1 h, and 5:100 NaOH (on mass of dry wood) (APP), steam explosion with a combined severity factor (CS) of 2.7 (SEP-WI) and alkaline pre-extraction followed by steam explosion (AP-SEP-WI) at different severities.

lignin and increases its hydrophilicity, promoting the softening of the middle lamella and improving the separation of the fibers [34].

Steam-exploded pine sawdust was more susceptible to enzymatic hydrolysis than the alkaline pre-extracted steam-exploded material that was pretreated at the same CS value (17.0 and 11.7%, respectively). Since the hemicellulose content of the former was lower than that of the latter (anhydroxyloses plus anhydromannoses equal to 2.6% and 5.2%, respectively), the extent of hemicellulose removal appeared to improve the susceptibility of steam-treated substrates to enzymatic hydrolysis [35].

There were no significant differences in EH yield between steam-treated materials that were produced from air-dried and never dried samples of slash pine sawdust (Fig. 5). Santi Junior et al. [36] found that mild drying (such as in the case of air drying) collapsed only small pores that were already inaccessible to the enzymes. Therefore, mild drying seems to have very little influence on the EH efficiencies of steam-treated softwood materials.

3.7. Overall mass balance

The detailed mass balance of the fractionation sequence including the enzymatic hydrolysis stage is shown in Fig. 6. Calculations were based on 100 g of dry slash pine sawdust and consider the results of the experience 6. The “unidentified compounds” fraction corresponds to components that were not quantified in this study, such as glucuronic, levulinic, and formic acids, among other unidentified degradation compounds.

The alkaline stage produced deresinated sawdust and a liquid fraction mainly composed of extractives and organic acids. Softwood extractives (mainly resin and fatty acids) have potential for the production of chemicals such as surfactants, adhesives, pharmaceutical supplies, paints, cosmetics, and biodiesel by esterification of fatty acids [37,38]. Carbohydrates released as hydroxy acids can be separated by techniques such as ultrafiltration, nanofiltration, electrodialysis, and distillation to be used as platform chemicals for the production of

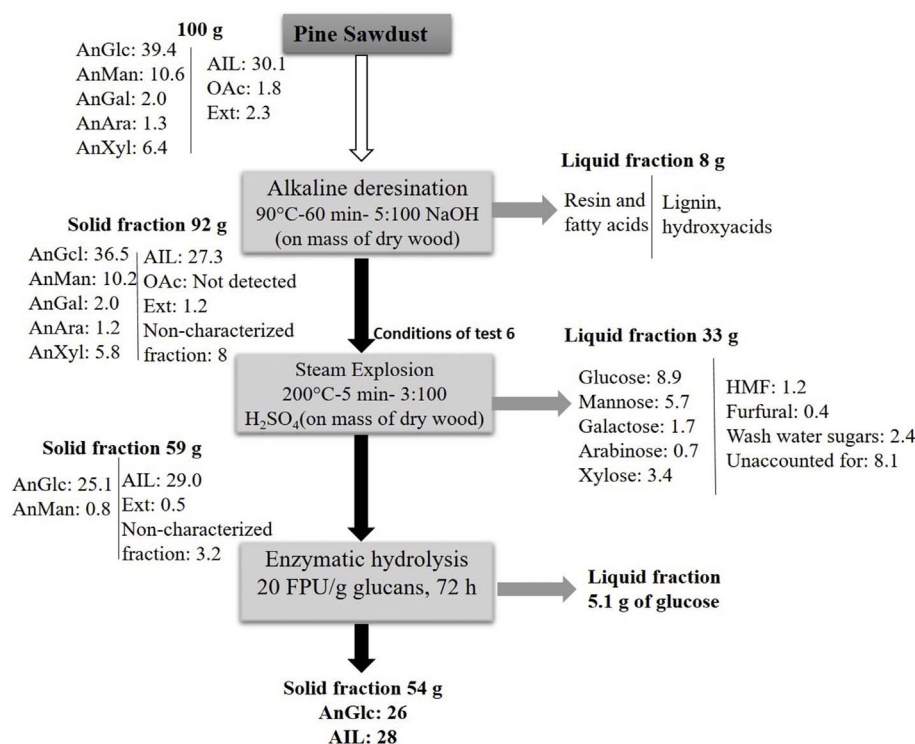


Fig. 6. Overall mass balance for the complete fractionation sequence of slash pine sawdust considering the results of the experience 6.

detergents, cosmetics, polymers and/or resins, plasticizers and surfactants, among others [39].

Steam explosion generated a solid fraction that was rich in cellulose and lignin, and a liquid fraction mainly composed of sugars and dehydration by-products. Hexoses (glucose and mannose), the main components of pine hemicelluloses, were the major components of the pretreatment liquors.

From the mass balance of Figure 6, 8.9 g of glucose and 12 g of hemicellulosic sugars (mannose + xylose + galactose + arabinose) can be obtained in the water-soluble fraction after SE, meaning that 53% of the initial hemicelluloses and 23% of the initial glucans are recovered in this fraction. These carbohydrates may be used in a variety of advanced fermentation processes or may undergo conversion to several platform chemicals such as furan compounds, polyols, and levulinic acid, among others.

In modern biorefineries, the greatest challenge of fractionation is to maximize the recovery of all wood components not only in high yields but also at high concentrations and in good conditions for their conversion to value-added co-products. Hence, all process streams must be valorized and if a residual fibrous material is produced, its recalcitrance must be considerably reduced to produce fuels, chemicals, and materials. In this work, the efficiency of the overall fractionation process was estimated by the amount of polysaccharides that were recovered either in monomeric or oligomeric form (see Eq. (7) for details). However, it is noteworthy that the achievement of high sugar yields depends on the efficiency of the subsequent steps of separation and purification.

Carbohydrate recovery yields in both liquid and solid fractions are shown in Table 3. Glucans were mainly recovered in the solid fraction (66%) after pretreatment and enzymatic hydrolysis. Hence, the recovery of glucans in the liquid fraction (32.0%) represents the total amount of glucose equivalents that were released from both pretreatment and hydrolysis stages.

The recovery yields of anhydromannose and anhydroxylose in the pretreatment liquor were 50% and 47%, respectively. Nguyen et al. [17] obtained similar yields when pretreating softwoods chips by SE at 201 °C for 5.1 min with 0.4:100 H₂SO₄ (on mass of dry wood), which corresponded to a CS value of 3.0. The recovery yields reported in this work were 57% mannans, 60% xylans, and 20% glucans.

The overall recovery yield of hemicellulose sugars was low regardless of the high extraction yields achieved after pretreatment (nearly 100%), indicating high levels of sugar dehydration. However, the concentration of HMF and furfural in the liquid fraction was not very high. Hence, most of these hemicellulose sugars were further degraded to others compounds including organic acids and humins (pseudo-lignin). HMF is known to form levulinic and formic acid by rehydration while and furfural is only converted to levulinic acid [31]. These sugar losses are responsible for the large amount of “unidentified compounds” in Table 3.

In general, the use of more severe pretreatment conditions favors hemicellulose removal and cellulose digestibility but the recovery of hemicellulose sugars decreases in the pretreatment liquor. For example, the recovery yields in trial 5 (180 °C for 5 min with 3:100 H₂SO₄ (on mass of dry wood)) were 100% glucans, 85% xylans, and 77% mannans, which are considerably higher than those obtained at the optimum conditions. However, hemicellulose extraction was 10% lower and the yield of enzymatic hydrolysis decreased by two-fold. Therefore, there is a trade-off between maximizing total carbohydrate recovery, hemicellulose extraction, and cellulose digestibility altogether. These results are consistent with those obtained by others using dilute acid pretreatment [6,13] and SE [17]. On the basis of these, many authors have suggested the application of a two-stage pretreatment method, with the first step operating at lower temperatures (170 °C–190 °C) to maximize hemicellulose recovery and the second stage at a higher temperature (200 °C–230 °C) to modify the cellulose structure and increase its accessibility. With the application of steam explosion in two stages, the resulting hemicellulose extraction yields were similar to

those obtained in this work but higher glucan yields were achieved upon EH (34 %–40% glucans in 72 h) [19,20]. However, the use of a two-stage process would increase both capital and operational costs that are associated with the overall biomass conversion process [17].

As a result of the fractionation sequence, a large quantity of non-hydrolyzed materials is obtained (50% of the initial sawdust) as residue and this is mainly composed of lignin and glucans. One possible economical use for this would be in the production of fuel pellets for residential pellet appliances. Öhman et al. [40] have demonstrated that pellets produced from this material have higher heating values due to the higher lignin concentration and lower tendency to excoriation than pellets derived from non-treated wood. On the other hand, the material could be separated and purified for the valorization of lignin and cellulose. Residual glucans can also be used for the production of cellulose nanowhiskers. Oksman et al. [41] showed that the residue from a bioethanol plant is an excellent source for the large-scale production of cellulose nanowhiskers, yielding 48% of cellulose nanocrystals after the purification and isolation of cellulose from this material. Lignin could be depolymerized and thermal deoxygenated for green gasoline and diesel production or could become an ideal precursor for carbon fiber synthesis [42].

Finally, the optimal conditions for fractionation must be defined by the type and amount of the desired products. For example, hemicellulose valorization requires mild conditions of temperature and acid concentration to avoid dehydration. On the other hand, severe pretreatment conditions are needed to produce good substrates for enzymatic hydrolysis. In this case, the liquid fraction could be optimized to produce other platform chemicals such as levulinic acid. Nevertheless, subsequent strategies to increase substrate accessibility may be applied, such as in the case of introducing an alkaline or organosolv delignification stage.

4. Conclusion

Steam explosion was very effective for hemicellulose removal from slash pine sawdust but the recovery of sugars in the pretreatment liquor was compromised by high levels of acid dehydration. Low glucan losses were observed and the digestibility of the steam-exploded substrates was increased considerably after the steam explosion. However, the resulting glucose yields remained relatively low compared to studies carried out with other lignocellulosic materials. Glucose yields were not increased by the alkaline stage, indicating that the removal of extractives caused not apparent effect on sawdust digestibility. Finally, there was a clear trade-off between maximizing carbohydrate recovery, hemicellulose extraction, and cellulose digestibility.

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Table 3
Carbohydrate recovery yields (as mass fraction) in the solid and in the liquid fractions derived from acid-catalyzed steam explosion.

Component (%)	Liquid fraction	Solid fraction	Total	Unidentified compounds
AnGlc	32.0	63.6	95.6	4.4
AnXyl	46.6	0.0	46.6	53.4
AnGal	77.6	0.0	77.6	22.4
AnAra	46.7	0.0	46.7	53.3
AnMan	48.6	7.2	55.9	44.1

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