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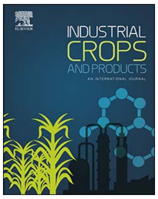

Selective arabinose extraction from *Pinus* sp. sawdust by two-step soft acid hydrolysis

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ABSTRACT

Acid hydrolysis of *Pinus* sp. sawdust is optimized in this work in order to selectively recover arabinose from the hemicellulose fraction by an environmental-friendly process (reactions at atmospheric pressure and mild conditions and using dilute HCl as catalyst). The influence of temperature, HCl concentration and reaction time was studied in the first experiments considering one step hydrolysis. One step hydrolysis was studied at different conditions, being the best results obtained at 65 °C for 18 h with 3% of HCl, with more than 3.8 g/L of arabinose in the liquid phase. This value corresponds to more than 47.5% of recovery of the arabinose present in the original sawdust and a selectivity of a 52% in the liquid phase (hydrolysis of other hemicellulosic sugars). In a second approach, a two steps process was considered in order to maximize the arabinose recovery. The optimum results were obtained combining a first cycle at 65 °C with a second one at 80 °C. More than 54% of the initial arabinose was recovered in the liquid phase as monomers. This percentage increases to 100% if the oligomers are also considering (no arabinose detected in the solid phase after the treatment). At these conditions, more than 56% of the global hemicellulose sugars were removed from the solid phase, without having a relevant degradation of glucose (pentose selectivity in liquid phase is higher than 88%, corresponding the resto mainly to hemicellulosic glucose).

1. Introduction

Lignocellulosic biomass is considered nowadays as the most promising alternative to fossil resources, not only to produce fuels and energy but also to obtain many different chemical products (Chin et al., 2013; Ghaffar et al., 2015; Faba et al., 2015). For the last point, a correct isolation of the different lignocellulosic fractions – lignin (~30%), cellulose (~40%) and hemicellulose (~30%) – is required (Menon and Rao, 2012). Lignin is a very complex aromatic fraction with high industrial value for the food and pharmaceutical industries. Cellulose is a crystalline homopolymer of glucose, typical raw material for the paper industry. Besides, new uses have been reported for these two fractions during the last few years, being considered the highest industrial valuable fractions of biomass (Gallezot 2012; Ragauskas et al., 2014; Deuss et al., 2014). Hemicellulose is a non-crystalline heteropolymer of different sugars, being the glucose, xylose and the arabinose the most important ones (Saha, 2003). Arabinose is a five carbon sugar, one of the main monomers in the hemicellulose of softwoods. It can be used as a non-caloric sweetener, dietary fiber, in flavors and as an intermediate for the synthesis of different drugs, mainly related to human diabetes, bacteriological diagnostics, antivirus, production of vitamin B2 (Aguedo et al., 2013; Jiang et al., 2015). Despite of these important uses, arabinose commercial production is still nowadays a complex and expensive process consisting of acid hydrolysis of Arabic gum or other raw materials followed by multiple procedures of purification (Cheng et al., 2011), so the development of an alternative and easier process (starting, for example, from a low-value raw material such as the hemicellulose) would be very interesting.

Nowadays, there are several alternatives proposed to separate cellulosic and hemicellulosic polymers from the lignin and obtain the corresponding monomers by a partial degradation of the polymeric structure. The acid hydrolysis using sulfuric acid is the most common one (Alonso et al., 2010; Mäki-Arvela et al., 2011). Other methods developed for this purpose are hot water and alkaline treatments, AFEX, steam explosion, pyrolysis, COSLIF and ionic liquids (Capolupo and Faraco, 2016). However, the reaction conditions are so harsh that these processes cannot be considered as green-processes and sugars are easily dehydrated and transformed to small molecular weight degradation products (Klinke et al., 2004). Concerning to the acid hydrolysis, most of the previous literature suggests the H_2SO_4 as catalyst for this reaction, concluding that the complete sugar hydrolysis (complete but

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not selective) is directly related to a good combination of temperature and acidity, in such a way that optimum results can be obtained after few minutes working at low temperature (< 50 °C) using concentrated acid (30–70%) or using dilute acid (< 1%) at high temperatures (> 200 °C) (Rinaldi and Schüth, 2009; Kim and Mazza, 2008; Dong et al., 2016). Acid hydrolysis conditions could be modified, working at mild temperature with dilute acid and controlling the reaction time, in order to selectively hydrolyze the hemicellulose fraction without breaking the strong bonds between glucose monomers in the cellulose. This procedure balances the autohydrolysis and hydrolysis of biomass.

The particular properties of each wood can affect the results obtained with this pretreatment. In this work, sawdust of different pinewoods, all of them included in the *Pinus* sp. genus (mainly *Pinus pinaster*) is used as raw material. *Pinus* sp. is a softwood species, very common in woods of the north of Spain, with high value in the timber and wood industry. According to the literature, *Pinus* sp. is typically composed of 37.1–40% of cellulose, 28.3–28.5% of hemicellulose and 27.2–27.9% of lignin; with more than 10% of arabinose, being this percentage among the highest in woody species (Evtuguin and Neto, 2007; Mäki-Arvela et al., 2011). The manufacturing of *Pinus* sp. generates a high amount of sawdust, considered nowadays as a waste. The few references about the use of this material are focused in the pretreatment of this wood by the Organosolv process (Ballina-Casarrubias et al., 2015), using soft acid media at high temperatures (Rivas et al., 2016) or even microbiological systems (Barbosa et al., 1992) whereas, to the best of our knowledge, there are not systematic studies about the soft hydrolysis at low temperatures of this sawdust. Hardwood hemicellulose is generally easier to hydrolyze than softwood hemicellulose (Marzialetti et al., 2008), so different parameters must be optimized in order to obtain an effective hydrolysis of this type of sawdust.

The aim of this work is to identify the reaction conditions that allow obtaining the maximum hemicellulose extraction, with main focus on recovering the arabinose in the liquid phase without altering the cellulose fraction (different temperatures, acid concentration, and number of cycles). We propose the use of HCl as homogeneous catalyst because its ecotoxicity is considerably lower than the toxicity of H_2SO_4, with values of 25 and 10 mg/L, respectively (values published in their MSDS files).

2. Materials and methods

2.1. Raw material

Pinus sawdust was supplied by a local sawmill (*Maderas Prieto*, Asturias, Spain). The sawdust was air-dried and maintained in sealed plastic bags. The particle size distribution of this sawdust was analyzed using a Mastersizer S long bench equipment (Malvern Instruments, Ltd.) obtaining a pseudo-Gaussian distribution with an average diameter of 629.7 ± 12 µm.

2.2. Reaction conditions

The hydrolysis was carried out in a 1 L double-walled, thermostatically temperature controlled, glass reactor. 50 g of wood sawdust was added to 500 mL of water (1:10 ratio). The resulting mixture was stirred at 500 rpm until it reaches the reaction temperature (from 60 to 80 °C as function of the reaction). The hydrochloric acid was then introduced in the reactor (ranging HCl concentrations from 1 to 3%), considering this moment as the starting point of the hydrolysis. Liquid samples are collected for 4 or 18 h, neutralized with $CaCO_3$ and filtered (0.45 µm pore size).

The harshness of the dilute acid pretreatment conditions was quantified by the combined severity factor (CSF), a parameter firstly introduced by Chum et al. (1999) that relates the harshness to the reaction time (t, min), the temperature (T, °C) and pH as function of the following equation:

$$CSF = log\left[t\cdot exp\left(\frac{T-100°C}{14.7}\right)\right] - pH$$

2.3. Characterization of liquids

Liquid phase concentration of the different sugars was analyzed by high performance liquid chromatograph (HPLC) in an Agilent 1200, using a Hi-Plex H column and a refractive index detector. Milli-Q water was used as mobile phase, with a flow rate of 0.6 mL/min. Column and detector temperatures were fixed at 50 °C. In order to obtain quantitative data, commercial samples of D(+)-glucose (Panreac, ACS quality), D(+)-galactose (Panreac, ≥98%), L(+)-arabinose (Panreac, ≥98%), D(+)-xylose (Fluka, ≥99%) and D(+)-manose (Sigma Aldrich, ≥99%) were used.

2.4. Characterization of solids

The chemical composition of the solid phase (structural carbohydrates and lignin biomass) was determined following the Standard Biomass Analytical Methods provided by the National renewable Energy Laboratory (NREL) of the American Department of Energy. According to this procedure, 300 mg of sample was hydrolyzed in strong conditions with 3 mL of H_2SO_4 at 72% for 1 h at 30 °C in a water bath under stirring. Hydrolyzed sample was diluted with 84 mL of deionized water and the degradation continues in an autoclave at 121 °C for 21 min with the aim to guarantee the complete hydrolysis. Resulting liquid was filtered, neutralized and analyzed by HPLC with a refractive index detector to quantify the carbohydrates. Acid lignin content is obtained by analyzing same sample with HPLC using a UV–vis spectrometer. Details of both procedures are reported in the literature (Sluiter et al., 2008).

Cellulose content was determined by discounting from the whole glucose the fraction that takes part of glucomannans. According to the literature, the mannose (Man) to glucose (Glu) ratio in the pine hemicellulose is 4.15 (Yoon et al., 2008). No other monomers were detected in any analysis. Consequently, the glucose from cellulose (named Mono-C) and hemicellulose sugars (named Mono-H) in the original pine sawdust were calculated using the following equations:

$$Mono - C = Glu - \frac{Man}{4.15}$$

$$Mono - H = Ara + Xyl + Gal + Man + \frac{Man}{4.15}$$

The crystallographic structure of the solids (both fresh and after the hydrolysis) were determined by X-ray diffraction (XRD) using a Philips X'Pert Pro diffractometer, working with the Cu-Kα line (λ = 0.154 nm) in the 2θ range of 5–30° at a scanning rate of 0.02°/min. The crystalline structures were determined by comparison with the polymeric databases of ICDD PDF-2, PAN ICSD and PAN COD. The crystallinity index was calculated by the methodology proposed by Thygesen and coworkers (Thygessen et al., 2005) based on the Segal equation, where I_c is the intensity of the maximum crystalline peak and I_a is the minimum intensity between two crystalline peaks:

$$CI = \frac{I_c - I_a}{I_c}$$

The solid morphology was analyzed by scanning emission microscopy (SEM) using a JEOL JSM-6100 instrument. Aluminum support was used and samples were pretreated by sputtering with gold for 40 s. Micrographs showed correspond to 7500 magnifications.

The analyses of the solid phase were carried out to characterize both, the raw material and the remaining solids obtained after each reaction.

Table 1
Contents on cellulose, hemicellulose, lignin and ash of the fresh sawdust and the solids obtained after each hydrolytic treatment.

Entry	Sample	Moisture	Sawdust division (dry basis, %)			
			Cellulose	Hemicellulose	Lignin	Ash
1	Original sawdust	9.6	40.2	31.4	27.2	1.1
2	60 °C, 3%, 4 h	*n.m.*	42.3	28.4	36.0	1.5
3	65 °C, 3%, 4 h	*n.m.*	42.5	26.2	37.2	1.6
4	70 °C, 3%, 4 h	*n.m.*	42.6	25.4	38.1	1.6
5	80 °C, 3%, 4 h	*n.m.*	43.7	21.3	39.1	1.8
6	65 °C, 1%, 18 h	*n.m.*	42.9	25.4	28.9	2.7
7	65 °C, 2%, 18 h	*n.m.*	43.4	22.4	31.1	2.8
8	65 °C, 3%, 18 h	*n.m.*	46.2	18.5	32.1	3.0
9	2nd c. 60 °C, 3%, 4 h	*n.m.*	40.9	20.5	33.9	1.7
10	2nd c. 70 °C, 3%, 4 h	*n.m.*	40.6	17.8	37.8	2.9
11	2nd c. 80 °C, 3%, 4 h	*n.m.*	37.8	13.9	42.5	3.1

n.m. = not measured.

3. Results and discussion

3.1. Sawdust characterization

As it is detailed in Table 1, the pine sawdust presents the typical chemical composition of softwood, with high content of insoluble lignin (17.9%) and hexoses (55.9%). These results were obtained considering the dry wood, after evaporating the water (9.4% of moisture). The ash content is 1.1%. According to the previous equations, the monomeric sugar distribution corresponds to 40.2% and 31.4% of Mono-C and Mono-H, respectively. An individual analyses of the hemicellulose fraction conclude that this fraction corresponds to 7.7% of xylose, 8% of arabinose; 10.8% of manose; 2.3% of galactose and 2.6% of glucose, being the two first sugars the main responsible of the inhibition of yeasts activity during sugar fermentation. These values are congruent the XRD spectrum observed in Fig. 1(a), that corresponds with a crystallinity index of 46.6%. This index is calculated from the height ratio between the intensity of the crystalline peak, I_{002}–I_{am}, (at $2\theta = 22.6°$ and 18°, respectively) and total intensity (I_{002}). The physical structure of the untreated sawdust sample was studied by SEM, being the most representative micrograph plotted in Fig. 1(b). At low resolution (60 magnifications), a clear fibrous structure with high and random porosity is observed. Increasing the magnifications to 7500, the cellulose well-defined structure is clearly detected, without evidences of lignin in the external structure, usually observed as spheres (Sannigrahi et al., 2011).

3.2. Influence of the hydrolysis temperature on the products distribution

The first step of the proposed process implies a hydrolysis of the pine sawdust using dilute hydrochloric acid as homogeneous catalyst. Previous references in the literature suggest this acid when reaction is carried out at high temperatures, from 100 °C to 190 °C with 0.5% of HCl, recovering almost 90% of the initial sugars after 20 min (Ertas et al., 2014). In our first tests, the HCl concentration is increased to 3% and four mild temperatures were studied (from 60 to 80 °C), increasing the reaction time to 4 h with the aim to compensate the predictable lower hydrolysis rate when working at low temperatures. Considering the reaction conditions, these experiments correspond to CSF values of 1.20 (60 °C), 1.35 (65 °C), 1.5 (70 °C) and 1.8 (80 °C). These values are slightly lower than those suggested in the literature as the optimum for the complete sugar hydrolysis (hemicellulose and cellulose) (Gaur et al., 2016; Gonzales et al., 2016); so it is expected that hemicellulose would be the most affected fraction, whereas cellulose would be almost unaltered. Initial experiments at same temperatures without any HCl in the reaction medium confirm the absence of any non-catalytic reaction (autohydrolysis), with no monomers detected by HPLC after 4 h at same reaction conditions. The sawdust autohydrolysis has been previously reported but at temperatures higher than 100 °C, usually close to 200 °C (Sidiras et al., 2011; Cara et al., 2012).

The temporal evolutions of arabinose (pentose selected as the main product because of its high concentration), the other hemicellulosic sugars (xylose, mannose and galactose considered together) and glucose are shown in Fig. 2. The arabinose evolution (Fig. 2a) has a continuous increasing trend, reaching concentrations higher than 3.2 g/L of this pentose in the liquid when temperature is fixed at 80 °C. This value corresponds to the removal of 43.7% of initial arabinose in the sawdust. Besides that lower conversions are obtained at lower temperatures, no equilibrium state is reached in any test, suggesting that longer reaction times could improve these values. Profiles obtained at 65 and 70 °C are quite similar, with final values of 1.4 and 1.6 g/L of arabinose in liquid phase, respectively. These values correspond to 17.5 and 20% of global arabinose recovery, respectively.

Concerning to the other sugars of the hemicellulosic fraction (Fig. 2b), results obtained at temperatures lower than 70 °C are very similar, with less than 0.7 g/L after 4 h of reaction in all the cases. On the contrary, the hydrolysis of the whole hemicellulose is very relevant at the highest temperature, with almost 18% of the initial galactose, xylose and mannose recovered in the liquid phase. These results suggest that 20% of the arabinose in the pine sawdust is located in the external structure of the hemicellulose polymers, whereas the other 80% takes part of the internal and more complex hemicellulosic fraction. As consequence, only the first 20% can be selectively hydrolyzed at these conditions. When this percentage is already hydrolyzed, the acidity is enough to attack the other sugar bonds and the selectivity of the

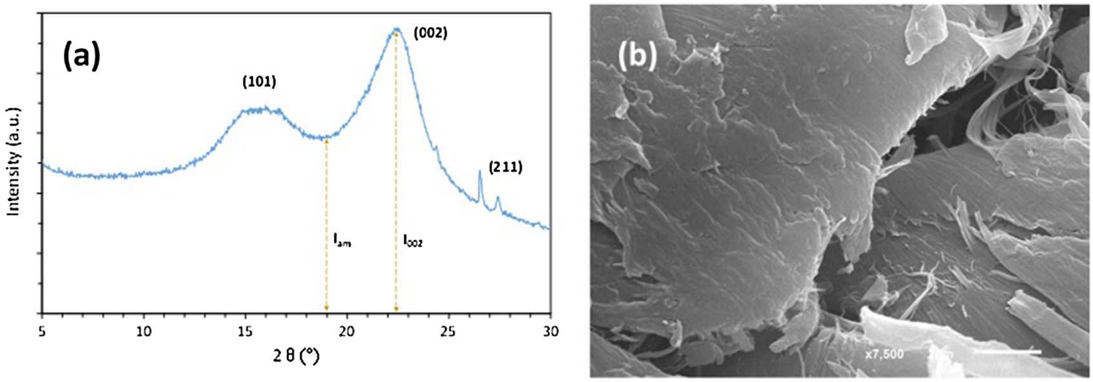

Fig. 1. (a) X-ray diffraction (XRD) spectrum of fresh pine sawdust. The codes of the crystalline peaks are shown between brackets. The positions needed to calculate the crystallinity index are also pointed: the cellulose crystalline peak (I_{002}) and the minimum between the two crystalline peaks (I_{am}). (b) SEM image of untreated pine sawdust with a scale bar equivalent to 2 μm.

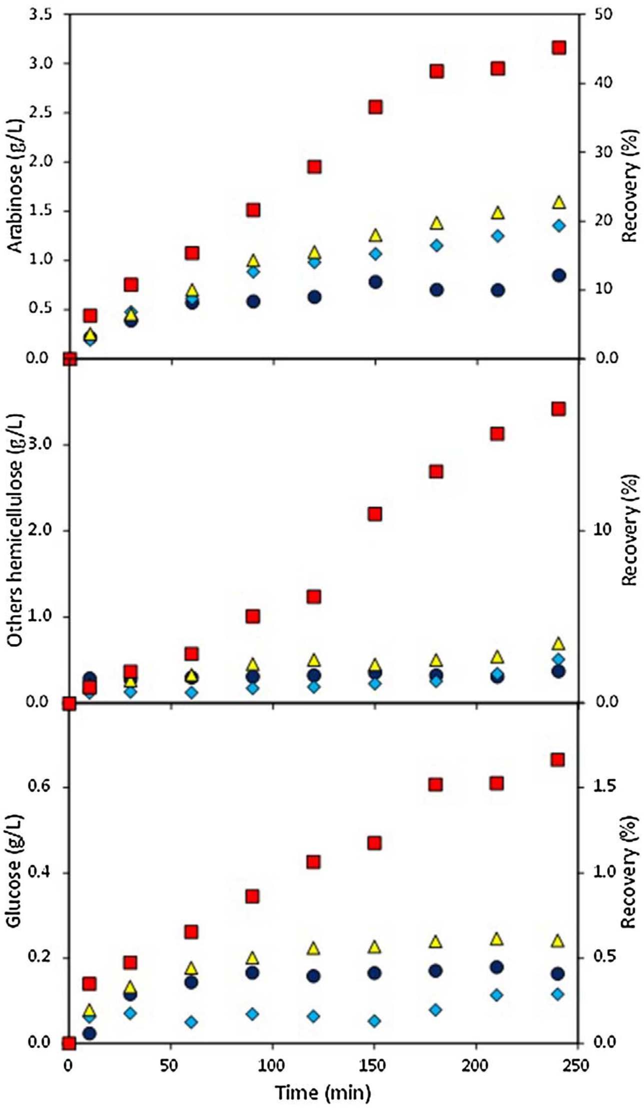

Fig. 2. Temporal evolution of the different monomeric sugars detected in the hydrolysis of pine sawdust catalyzed by 3% of HCl. Results correspond to reaction at: (●) 60 °C; (◆) 65 °C; (▲) 70 °C and (■) 80 °C.

reaction decreases quickly.

Similar behavior is observed for glucose (Fig. 2c), although less markedly. In this case, less than 1% of the whole glucose is obtained in the liquid phase, being much more relevant at 80 °C. According to sawdust composition summarized in Table 1, only 2.6% of the glucose is part of the hemicellulosic fraction (mainly as glucomannan polymers in this kind of woods). Then, the glucose percentage in the liquid phase is congruent with the selective degradation of the hemicellulose, without modifying the most of the glucose of the cellulose fraction. As conclusion, 66% of the glucose from hemicellulose are obtained in the liquid phase at 80 °C, whereas this value decreases to 13.7% at 60 °C (higher selectivity to arabinose).

No aromatics were detected in the liquid analyses, suggesting that lignin is unaltered after the reaction. In the same way, no furfural or 5-hydroxymethylfurfural (5-HMF) were detected, discarding the degradation of sugar monomers. A relevant concentration in sugar oligomers, mainly dimmers, was detected in all the cases, reaching final values of 1.62, 3.12, 2.70 and 2.99 g/L at 60, 65, 70 and 80 °C, respectively. All these compounds were considered together, being their temporal evolution included in the Supplementary information (Fig. S1). At short reaction times, the concentration of these oligomers is directly proportional to the reaction temperature. After one-two hours, profiles suggest an equilibrium situation in which the partial hydrolysis of hemicellulose is in equilibrium with the complete hydrolysis of these oligomers, yielding the sugars. Despite the temperature, the oligomer concentrations indicate that the hydrolysis is not complete after 4 h, with a significant amount of intermediates. Considering all the sugars detected in the liquid phase (monomers and oligomers together), the final concentrations where 3, 5.1, 5.3 and 10.1 g/L (in increasing temperature order), which correspond to values of initial hemicellulose recovered of 9.6, 16.25, 16.7 and 32.1%, at 60, 65, 70 and 80 °C, respectively. On the other hand, small amounts of acetic and formic acid were also detected (never reaching 5% of the total concentration), suggesting the presence of acetyl groups in terminal positions of hemicellulose polymers. The presence of these acids after these pretreatments has been previously reported in the literature (Moniz et al., 2014). Considering the weak character of these acids and the initial pH of the reaction (0.09), these acids do not play any relevant role in the process.

Considering that the hemicellulose hydrolysis is not the only aim of this work but also to get higher arabinose selectivities, results obtained must be analyzed in terms to selectivity in order to set the best operation conditions. The final distribution of the main products after 4 h of reaction is plotted in Fig. S2 in terms of selectivity. As it could be expected, the whole selectivity to pentoses is always over 85%, in good agreement with the soft character of this pretreatment. The highest arabinose selectivity is reached at 65 °C, obtaining a liquid phase in which arabinose is almost 70% of the whole sugars, with more than 94% of pentoses at these conditions. This value corresponds to 20% of the arabinose in the initial pine sawdust recovered after 4 h and profiles suggest that this value can be improved with longer times or by introducing a second step at higher temperature in which the reaction conditions make easier this hydrolysis.

Solids obtained after all the reactions were recovered by filtration, dried in the oven at 105 °C and analyzed in order to determine the evolution of their structure. XRD analyses, shown in Fig. 3, are congruent with results in the liquid phase, observing a slight and progressive increase in the crystallinity. This fact is justified by the selective hydrolysis of hemicellulose phase, keeping unaltered the cellulose. These spectra correspond to crystallinity index (CI) values of 51.8, 52.7, 53.6 and 54.2% after reaction at 60, 65, 70 and 80 °C, respectively. NREL procedure was also applied to these samples, obtaining the structural sugar fractionation summarized in Table 1 (entries 2–4). The decrease in the hemicellulose fraction is the main conclusion of these analyses, with the subsequent increase in the other fractions, mainly the lignin one. This result is congruent with the absence of aromatic compounds in the liquid phase, and suggests that

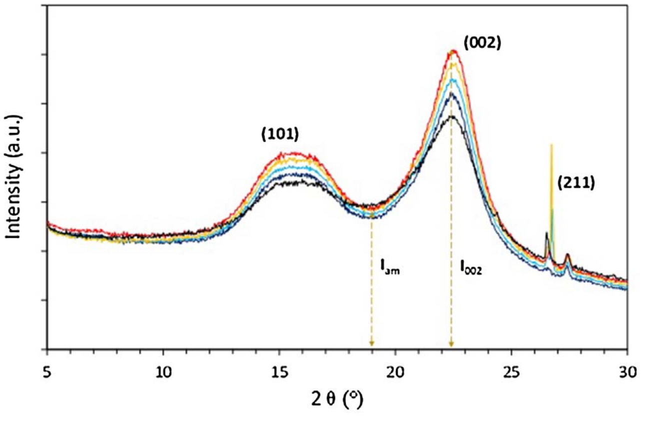

Fig. 3. X-ray diffraction (XRD) spectra of fresh pine sawdust (black) and after the hydrolysis at different temperatures: 60 °C (dark blue); 65 °C (light blue); 70 °C (yellow) and 80 °C (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

this pretreatment at these conditions is selective to sugars. Considering these compositions, with all the main and side products in the liquid phase, final carbon balances were always higher than 85% (88.4, 91.1, 86.4 and 95.5%, in the temperature order). Analyzing the individual composition of the hemicellulose fraction (the remaining solids), less than 1.5% of arabinose was obtained in all the cases (1.4, 0.97, 0.48 and 0.36%), indicating that these treatments are good alternatives to remove this sugar. However, the concentration of the other main pentose (xylose) is still relevant, with values higher than 2% in all the cases (5.2, 4.1, 3.2 and 2.1%).

The physical structure of the treated sawdust samples was studied by SEM, being the most representative micrographs plotted in Fig. S3. The high similarity among all the images corroborates that main structure of pine sawdust keeps almost unaltered during the treatment. At low resolution (60 magnifications), a clear fibrous structure with high and random porosity is observed. Increasing the magnifications to 7500, the cellulose well-defined structure is clearly detected, observing fewer imperfections after treatment at highest temperatures (related to the hemicellulose), but with no evidences of strong degradation. According to the literature, the presence of discrete spherical balls or droplets on the surface would be the first sign of a too severe pretreatment in which hemicellulosic derivatives condense, obtaining different compounds generally called pseudo-lignin (Sannigrahi et al., 2011). These droplets were not detected in any of these micrographs.

3.3. Influence of the HCl concentration in the arabinose selective hydrolysis

Considering that acidity could promote other hydrolysis, and trying to prevent or minimize the hydrolysis of the other hemicellulosic sugars, data obtained with 3% of HCl were compared with results at lower acidity (1 and 2% of HCl). These experiments were performed at 65 °C during 18 h. Temporal evolution of main products is plotted in Fig. 4, whereas Fig. S4 summarizes the final products distribution after these 18 h in terms of selectivities. Concerning to the arabinose, no significant differences in the profiles were observed among the different experiments, with similar tendencies and higher values at increasing acidity. However, the concentrations of the other sugars is almost negligible in the first 10 h reaction time in the experiments performed with 1 and 2% of HCl, whereas these concentrations are significant working at 3% HCl. At 1% and 2% profiles are very similar, with a fast increase in these sugars concentrations during the last four hours and reaching similar values. Similar trends are obtained in the analyses of glucose, being even more evident the differences between 3% of HCl and softer conditions.

As to the final values, more than 52% of the initial arabinose is recovered after 18 h working with 3% of HCl, with a final concentration of 3.81 g/L of this sugar in the liquid phase; whereas less than 17% of the other hemicellulosic sugars were hydrolyzed, obtaining a final concentration lower than 0.5 g/L of cellulose. These data correspond to an arabinose selectivity of 52%, whereas all the hemicellulosic sugars correspond to almost 95% of the sugars in the liquid phase (Fig. S4). The selectivity to arabinose is improved when HCl concentration decreases, reaching the maximum value at 2% of HCl, with 60%. However, the final concentrations obtained at these conditions are considerably lower (1.8 and 3 g/L with 1 and 2% of HCl, respectively). As in previous cases, no aromatic compounds were detected in any of these experiments, suggesting that cellulose and lignin are unaltered after the treatment. On the other hand, the oligomer concentrations increase from 2.3 to 5.4 g/L at increasing acidity. Taking into account that cellulose remains in the solid phase, these results indicate that 19.1, 28.6 and 41.3% of the initial hemicellulose (at 1, 2 and 3% of HCl, respectively) is recovered in the liquid phase (as monomers or oligomers).

After 18 h of reaction, the solids were recovered by filtration, dried in an oven and analyzed following the NREL procedure and data obtained were added to Table 1 (entries 6–8). The increases in the lignin and glucose percentages (mainly in the lignin) are congruent with the fact that these fractions keep unaltered despite of the treatment and only the hemicellulose is attacked by the acid. Analyzing the individual composition of the hemicellulose fraction, less than 1% of arabinose was obtained in all the cases (0.97, 0.26 and 0%), indicating that these treatments are good alternatives to remove this sugar. However, the concentration of the other main pentose (xylose) is still relevant, with values higher than 2% in all the cases. Considering these sugars remained in the solid phase as well as the oligomers and monomers in the liquid phase, the final carbon balance of results obtained were always higher than 92%.

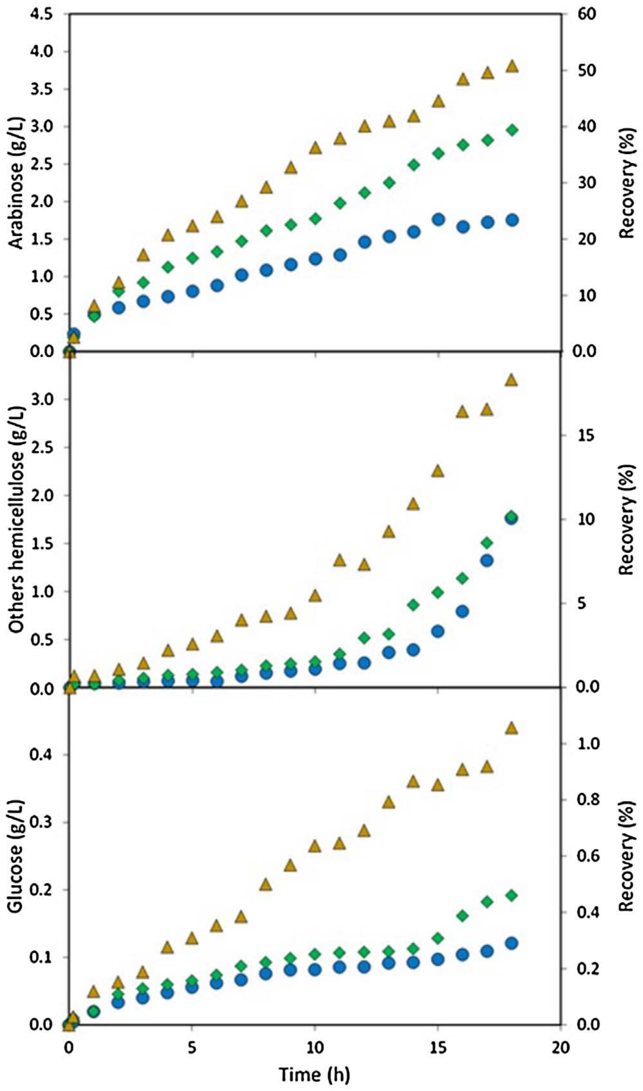

Fig. 4. Temporal evolution of the different monomeric sugars detected in the hydrolysis of pine sawdust at 65 °C as function of the HCl concentration. Symbols: (●) 1%; (◆) 2%; (▲) 3%.

In parallel with previous experiments, solids were also analyzed by XRD and SEM, being the main results summarized in Figs. S5 and S6, respectively, of the supplementary information. As it could be expected, very similar profiles were obtained with both techniques. Concerning to the XRD, very similar profiles were obtained at 1 and 2% with less intensity in the case of 3%. Results obtained correspond to a slight and successive increase in the crystallinity for the two main reactions (56.6 and 56.8%) and a slight decrease to 54.8% in the reaction at 3%. These values suggest that reaction at 3% slightly attack the cellulose fraction, whereas reactions at 1 and 2% only affect to the hemicellulose one. On the other hand, no evidences of pseudo-lignin were detected by SEM,

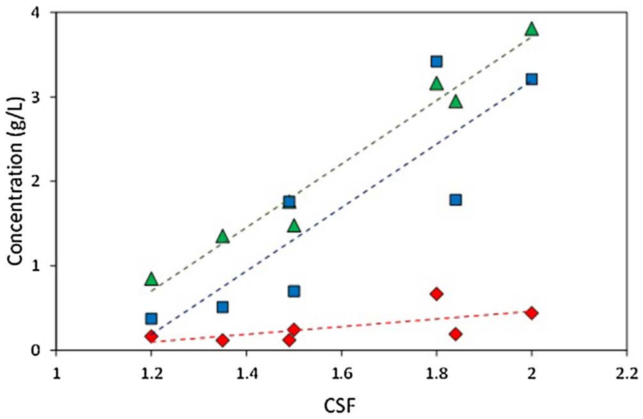

Fig. 5. Correlation between the coefficient of severity factor and the final sugar concentrations obtained by the hydrolysis of pine sawdust. Results corresponding to: (▲) arabinose; (■) other pentoses and (♦) glucose.

observing a higher degradation in the initial laminar structure after treatments at strongest conditions.

In order to analyze the influence of hydrolysis conditions globally (temperature, pH and time) in the results, the final concentration of arabinose, the other hemicellulosic sugars and glucose in the liquid phase as function of the CSF factor is plotted in Fig. 5. A linear trend is observed in the evolution of the arabinose, concluding that all the reaction parameters studied (pH, temperature and time) have a similar and positive effect in its hydrolysis. Different behaviors were obtained for the other sugars. Concerning to the other pentoses, data are adjusted also to a linear tendency with similar slope as the arabinose. However, there are relevant differences between similar CSF obtained by changing different parameters, in such a way that temperature seems to be the key parameter. Comparing similar CSF's, such as 1.49 and 1.5 or 1.8 and 1.84, a higher amount of these sugars was recovered at temperatures over 70 °C, whereas similar CSF's obtained at lower temperatures by combining with lower acidity or longer times are not so effective. Finally, as to the glucose, the logical increasing tendency at increasing severe conditions was also observed, but with a significant lower slope, being congruent with the refractory character of the cellulose. In this case, the key role of the reaction temperature was also detected, being the relevance much lower than in the case of pentoses.

3.4. Successive cycles at different temperatures

Second alternative proposed consists on trying to improve the hemicellulose recovery (not only the selective arabinose) by combining the results obtained in a first cycle at 65 °C (the most selective one, shown in Fig. S2) with second cycles in which the solid (after filtered and dried) is subjected to a secondary hydrolytic step at different temperatures (60, 70 and 80 °C). The evolution of these second cycles is shown in Fig. 6. Similar trends than in the first cycle were obtained, with very similar profiles at 60 and 70 °C and significant differences in the case of the highest temperature tested. Considering that the selectivity to pentose sugars in the first cycle at 65 °C was higher than 94%, the main objective of this second cycle is to increase the amount of sugars recovered trying to keep constant this selectivity. According to this premise, best results are obtained with the second cycle at 80 °C, with almost 23% of hemicellulosic sugars recovered as monomers after the two cycles. This value increases significantly when the amount of oligomers is also considered, reaching a final hemicellulose removal of 53% (final oligomers concentrations of 6.49 g/L at these conditions). Besides, more than 54% of the initial arabinose was recovered as monomers. The increasing tendency of profiles obtained at 80 °C suggests that longer times can increment this value to close to 100%, whereas profiles at lower temperatures seem to reach a maximum and the stationary state at lower conversions (mainly in the case of arabinose with removal ratios of 33 and 35% after the second cycle at 60 and 70 °C). On the contrary of the behavior in the first cycles, the evolution of the glucose in the liquid phase was very similar in all the cases, with differences lower than 0.1 g/L between the lowest and the highest temperature tested. All these values correspond to the selectivities distributions showed in Fig. S7. In all the cases, the glucose selectivity was lower than 18%, reaching global pentose selectivities of 83.2, 82.5 and 88.2%, at increasing temperatures.

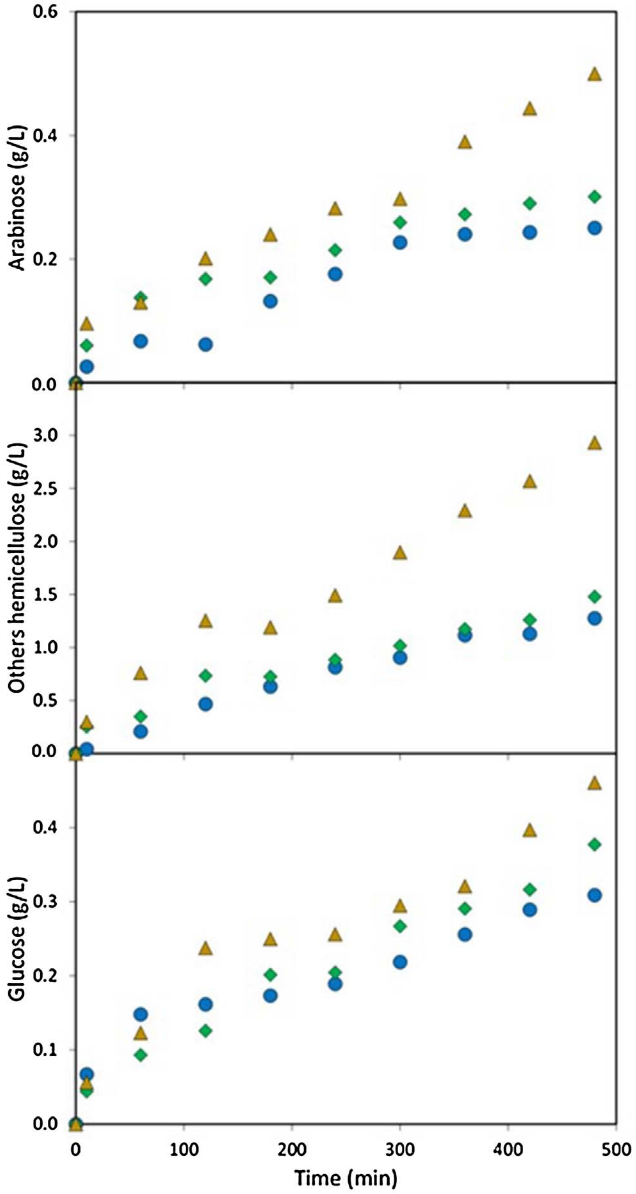

Fig. 6. Temporal evolution of the different monomeric sugars detected in the second cycle of hydrolysis catalyzed by 3% of HCl as function of the temperature. Symbols: (●) 60 °C; (♦) 70 °C; (▲) 80 °C.

Concerning to the solids obtained after the treatments, the sawdust fractions are included in Table 1 (entries 9–11), highlighting the proportional increase of lignin and cellulose ones, and the subsequent decrease in the hemicellulose. Analyzing the individual sugar composition of this last fraction, no arabinose or xylose were detected after any second cycle, considering that this procedure is effective to remove the pentoses that can produce inhibition in fermentation reactions. As consequence, solids obtained after these two-cycle hydrolysis can be better transformed into bioethanol and the combination of 65 and 80 °C were chosen as the optimum configuration based on its higher conversion and hemicellulose removal. XRD and SEM analyses are reported in the supplementary information (Figs. S8 and S9) are congruent with results in the liquid phase, observing an increase in the crystallinity in experiments that only attack to the hemicellulose fraction (51.2 and 55.9% after treatments at 60 and 70 °C) and a slight decrease to 52.8% after the treatment at highest temperature, in which the cellulose is also affected. SEM images also show the higher degradation of main structure at these conditions.

These results are slightly lower than other previously reported in the literature using stronger pretreatment conditions. For example, the 14.9% of arabinose final yield observed by Yu et al. These results cannot be directly compared because they are conditioned by the higher amount of arabinose in the raw material as well as the relevant hydrolysis of glucose because of the more severe conditions that reaches selectivities higher than 30% (Yu et al., 2017). Other relevant results are summarized in the review of Peng et al., suggesting the arabinose separation by different extractions and precipitations (Peng et al., 2012). Despite that good results are reported, arabinose is obtained as a salt and not as monomers, and these salts must be treated before their direct upgrading. The higher selectivity and its recovery as monomer is the main advantage in comparison to other works related to the hydrolysis of similar type of woods but using more severe conditions (Ertas et al., 2014; Gaur et al., 2016; Gonzales et al., 2016; Sidiras et al., 2011)

4. Conclusions

The diluted-acid treatment of *pine* sp. sawdust has shown to be effective for the selective extraction of arabinose monomers from the hemicellulose fraction, keeping intact the lignin and cellulose fractions. As it was expected, higher hemicellulose hydrolysis was reached at highest temperatures (80 °C) with 9.57 g/L of pentose monomers in the liquid phase after 4 h. However, these conditions are too aggressive and a relevant amount of glucose was also detected (almost 1 g/L), suggesting that the cellulose fraction is also damaged. At softer temperatures (65 °C) 7 g/L of pentoses were obtained after 18 h, recovering more than 50% of the initial arabinose without detecting glucose. In order to improve these results, a process in two steps is suggested, recovering all arabinose in liquid phase after a first cycle of 4 h at 65 °C and a second one at 80 °C for 8 h (54% as monomers and the rest as different soluble oligomers, not arabinose detected in the solid phase). At these conditions, the amount of xylose in the solid phase is also negligible, considering this procedure as a good alternative to removal the pentoses from the sawdust, obtaining a pre-treated material with only six-carbon sugars.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.indcrop.2017.04.027.

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