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Research paper

Evaluation of hardwood and softwood fractionation using autohydrolysis and ionic liquid microwave pretreatment

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ABSTRACT

Differences in hardwood and softwood, elucidates their behaviour against pretreatments varies. In this work, microwave ionic liquid (IL) and autohydrolysis (AH) pretreatments were applied to *Eucalyptus globulus* (as a model of hardwood) and *Pinus radiata* (as a model of softwood). The comparison between hardwood and softwood of microwave ionic liquid (IL) and autohydrolysis (AH) were evaluated in terms of chemical composition of pretreated solids, liquid by streams composition (hemicellulose and lignin extraction) and, substrates enzymatic digestibility. Furthermore, micrographs using scanning electron microscopy (SEM) and confocal fluorescence microscopy supported results obtained. In this study, it has been demonstrated that autohydrolysis pretreatment effectiveness, through maximizing enzymatic digestibility, is opposite in hardwood (73 g glucan/100 g glucan introduced at severe conditions) and softwood (10 g/100 g glucan). IL pretreatment has been especially effective in softwood with higher digestibilities (78 g glucan/100 g glucan introduced) than those obtained in hardwood (68 g glucan/100 g glucan introduced). Confocal fluorescence microscopy images, together with SEM images have resulted to be a clarifying technique to explain enzymatic digestibility results. Final sugars yields after the whole process have shown that low solid yields recoveries obtained in AH treatments have considerably worsened final glucose production, mainly in softwood. IL microwave pretreatment have resulted in higher glucose yields in softwood than in hardwood.

1. Introduction

Despite recent advances, the biorefinery of lignocellulosic biomass is still a challenge [1]. There is a bottle-neck of the process in the conversion of complex carbohydrates to fermentable sugars [2]. Lignocellulose is a recalcitrance non-uniform three-dimensional structure, that requires pretreatment processes to deconstruct the linkages and disrupt the structure [3]. Pretreatment technologies can constitute up to 40% of the total processing costs of lignocellulosic biomass conversion [4].

Autohydrolysis (AH) has been described as an inexpensive, environmentally friendly and easy-handle process to selectively remove hemicellulose with low cellulose and lignin degradation [5,6]. AH only uses water as reactive, which results in the water autoionization towards acid hydronium ions (H_3O^+). Oligosaccharides obtained in the liquid phase are value added products used in food and pharmaceutical industries [7]. Ionic liquids (ILs) are effective biomass solvents that reduce recalcitrance, enabling deconstruction, and disruption of the lignin and hemicellulose network [8]. The non-flammability, high chemical and thermal stability, and negligible vapour pressure are some of the advantages against other pretreatment processes [9]. ILs also reduce cellulose crystallinity, increasing its accessibility and favouring high glucose conversions [10–12]. ILs are good microwave absorbers, enhancing biomass conversion processes and fastening the heating rate [13,14] However, the use of microwave must be assessed to avoid very severe conditions that may produce degradation [15].

Some studies have already been reported comparing softwoods and hardwoods pretreatments [16–19]. Pretreatments are, in general, more effective in hardwoods than in softwoods [19].

In this work, a comparison between hardwood and softwood pre-

Abbreviations: IL, Ionic liquid; [Emim][OAc], 1-ethyl-3-methylimidazolium acetate; AH, autohydrolysis; OS, oligosaccharides

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treatment efficiency are performed. Autohydrolysis and IL microwave pretreatments at different severity conditions are carried out. Chemical compositions of solid and liquid fractions for each pretreatment were determined. Solids enzymatic hydrolysis digestibilities were evaluated, compared and, supported with morphological structures obtained from scanning electron microscopy and confocal fluorescence laser microscopy techniques, in each case. In this study, identical enzyme cocktails, loading levels, and analytical methods were employed in order to offer an overview of the sugars and by-products obtained.

2. Material and methods

2.1. Materials and reagents

The ionic liquid 1-ethyl-3-methylimidazolium acetate ([Emim][OAc], > 95%, Iolitec GmbH) was employed for wood dissolution. *Eucalyptus globulus* and *Pinus radiata* sawdust were provided by the National Institute for Agronomic Research (CIFOR-INIA). Organosolv lignin from *E. globulus* and *P. radiata* wood were used as reference materials, for UV/VIS measurements (explained below). Sulfuric acid was used to precipitate lignin from the mixture. The enzymatic cocktail Accellerase 1500®, containing 70 mg of protein/mL of dissolution was donated by Dupont Industrial BioSciences and used in the enzymatic hydrolysis step.

2.2. Experimental

2.2.1. Autohydrolysis (AH) pretreatment

AH pretreatments were developed in a 450 mL stainless steel pressure reactor (Parr Instrument Company, model 4567). The reactor was fitted with a four blade turbine impeller working at 150 rpm. Before pretreatment, wood chips were milled and sieved to obtain a particle size between 0.3 and 2 mm. Eucalyptus and pine wood sawdust were mixed with deionized water in a liquid:solid ratio of 8:1 and 10:1 (g water: g dry biomass), respectively [20,21]. Mild autohydrolysis were developed at 150 °C for 30 min for both woods (AH150E for eucalyptus wood and AH150P for pine wood); intermediate, at 175 °C for 30 and 60 min in the case of eucalyptus and pine wood (AH175E and AH175P); and severe autohydrolysis conditions were performed at 200 °C for 30 and 90 min (AH200E and AH200P). The AH severity factor (S_0) was calculated [22].

2.2.2. IL microwave pretreatment

Eucalyptus and pine were milled and sieved to obtain particles with sizes < 150 μm. Extractives were removed using acetone and water to avoid foam formation that may cause dissolution problems [23,24]. 0.8 g extractives free samples of eucalyptus and pine wood were mixed with 20 g of [Emim][OAc]. Samples were heated under microwave irradiation in a Berghof SpeedWave Four microwave oven, using a two-step programme detailed in a previous work [25]. Operation temperatures were 80 °C (IL80E for eucalyptus and IL80P for pine wood) and 120 °C (IL120E and IL120P) in a total time of 50 min.

Afterwards, 50 mL of deionized water was added to precipitate the wood dissolved in the process. The solution was stirred for 10 min in a water bath at 40 °C, and was subsequently filtered under a vacuum to obtain the pretreated wood. Pretreated samples were washed 5-fold with 70 mL of deionized water.

2.2.3. Enzymatic hydrolysis

The enzymatic hydrolysis was carried out in an orbital incubator at 150 rpm and at 50 °C. 1 % (w/w) pretreated wood (< 150 μm) was suspended in 50 mM citrate buffer (pH 5.0) containing 0.002% of sodium azide in a working volume of 8 mL. Accellerase 1500 enzymatic cocktail in a dosage of 0.25 mL/g glucan was added. Aliquots of 150 μL were periodically taken at 3, 6, 12, 24, 48 and 72 h, and centrifuged to stop the enzymatic reaction.

2.3. Analytical methods

2.3.1. Chemical characterization of biomass samples

Biomass compositions, before and after pretreatments, were determined according to the NREL/TP-510-42618 methodology adapted to small quantities of samples [26,27]. The acid-soluble lignin amount was determined using a Varian Cary 50 UV-VIS spectrometer at 205 and 240 nm, with an absorptivity of 110 and 12 $L\,g^{-1}\,cm^{-1}$ for eucalyptus and pine wood, respectively. Sugars in the hydrolysate were determined by HPLC, neutralizing with $CaCO_3$ and, filtering under 0.45 μm before the analysis, using a 300 × 7.8 mm Casbosep-CHO 682 column with Micro-Guard cartridges (BioRad, Life Science Group Hercules, Ca) at 80 °C, using water as mobile phase, and a flow rate of 0.4 mL/min.

2.3.2. Morphology of biomass samples

A Jeol JSM 6400 scanning electron microscope (SEM) was employed to observe the surfaces of untreated and pretreated samples. A Gold sputtering onto the sample surface was used to impart electrical conductivity. The operation voltage of the SEM was 20 kV. Analysis were developed in the technical facilities of the Spanish National Centre for Electron Microscopy.

A Leica SP-2 AOBS confocal laser microscope was used to visualize supramolecular structure changes. A laser at 405 nm was used to excite samples for fluorescence visualization. Wavelength emission ranges were 428–480 nm and 547–658 nm for hollocellulose and lignin (autofluorescent) respectively. β-1-4 polysaccharides linkages were dyed using 0.1% Calcofluor white stain [3]. Images were acquired at a step size of 2 μm and were combined into a z-axis max projection using Image-J software. Analysis were developed in the technical facilities of the Centre for Cytometry and Fluorescence Microscopy of the Complutense University of Madrid (UCM).

2.3.3. Chemical composition of autohydrolysis liquors

Monomeric sugars, organic acids and furans obtained from autohydrolysis liquors were directly measured by HPLC using a refractive index detector, according to the NREL/TP-510-42623 procedure [28]. The above mentioned operating conditions were used for sugars determination. Organic acids and furans were determined, using a Rezex ROA-Organic Acid H+ (8%) 300 × 7.8 mm column at 60 °C, with a mobile phase (0.005 M H_2SO_4) eluted at 0.6 mL/min.

2.3.4. Chemical composition of liquid by-stream obtained after microwave IL pretreatment

Recovered IL in the washing fractions was quantified by HPLC equipped with a UV detector measuring at 235 nm. The Eclipse Plus C18 4.6 × 100 mm column was operated using a mixture of acetonitrile/water 50/50 % (v/v) as mobile phase with a flow rate of 1 mL/min and at 30 °C.

The lignin content accumulated in the IL was analyzed by UV/VIS spectroscopy using a Varian Cary 50 scan UV/VIS spectrophotometer. A rotary evaporator was used to remove the water and recover the IL. Samples were diluted in 0.1 N NaOH and filtered, to measure the absorbance at 280 nm [29]. Water content in the recovered ionic liquid was determined using a thermobalance and mass was corrected. The total dissolved lignin concentration was obtained from the reference curve of eucalyptus and pine wood organosolv lignin samples. Reference samples used were prepared. The absorbance measurement of

each sample was subtracted of a blank sample prepared with the microwave heated ionic liquid at the working temperature.

2.3.5. Enzymatic digestibility

Glucose and xylose were determined by HPLC as explained above (section 2.3.3). Glucan and xylan digestibilities were defined as the percentage of glucan and xylan converted into glucose or xylose during enzymatic hydrolysis, according to the NREL/TP-5100-63351 procedure [30].

3. Results and discussion

3.1. Autohydrolysis of eucalyptus and pine wood

Solid recovery and compositional analysis are shown in Table 1. A decrease of the solid recovery is produced from mild conditions to severe conditions. The relative composition of both woods as compared to the untreated sample, varies in most experiments, indicating specific biomass fractions are selectively removed [31]. Tendencies followed in eucalyptus and pine wood were similar. Lignin content is increased between untreated and AH wood as a consequence of hemicelluloses extraction in the case of AH150 and AH175 samples, and as a result of hemicellulose and cellulose degradation in AH200 samples.

oligosaccharides have not been largely obtained in any of the woods although higher values are observed in pine wood due to the higher content of non-structural glucan in softwood (due to glucomannan composition) [35,36]. This statement confirms the limited removal of structural glucan by AH [31]. Eucalyptus at AH mild conditions (AH150E), yields 7.5% of total xylan recovered in form of xylo-oligosaccharides, while pine at AH mild conditions, yields 15.8% of total mannan recovered in form of mannan-oligosaccharides. At intermediate AH conditions of eucalyptus wood 69.7% of total xylan is recovered in form of xylo-oligosaccharides, while 68.8% of total mannan coming from pine wood is obtained as mannan-oligosaccharides. Severe operating conditions of eucalyptus and pine wood (AH200E and AH200P) leads to nor xylo-oligosaccharides, in the case of eucalyptus, and 2.0% of total mannan in form of mannan-oligosaccharides, in the case of pine, obtained. Monomeric sugars, mainly glucose are increased at severe operating conditions as a result of cellulose extraction in the liquid phase [18].

The main acids and furans detected were formic acid, acetic acid, furfural and hydroxymetylfurfural (HMF). As acetic acid depends on the acetylation level of hemicellulose and treatment severity (mainly temperature), acetic acid production of eucalyptus wood is superior at severe conditions. Furfural (formed via xylose/arabinose dehydration) production is also higher in eucalyptus due to the higher content of

Table 1
Solid recovery and compositional analysis of untreated and pretreated wood samples. ± Standard deviations values.

Sample name	*Severity factor*	*Solid Yield*	COMPOSITION (g/100 g oven dry weight pretreated biomass)						
			Lignin	*Glucan*	*Xylan*	*Galactan*	*Arabinan*	*Mannan*	*Acetate*
		%	%	%	%	%	%	%	%
Untreated eucalyptus	0	100	26.17 ± 0.19	49.13 ± 1.27	17.45 ± 0.34	0.60 ± 0.10	0.35 ± 0.18	0.80 ± 0.40	3.96 ± 0.11
AH150E	*2.95*	92.95	29.05 ± 1.70	51.94 ± 1.29	16.85 ± 0.42	0.81 ± 0.15	0.20 ± 0.20	0.70 ± 0.70	5.66 ± 0.13
AH175E	*3.69*	74.73	37.22 ± 0.58	60.96 ± 1.75	4.77 ± 0.02	0	0	0	1.90 ± 1.59
AH200E	*4.42*	68.72	62.16 ± 0.75	37.45 ± 1.06	0	0	0	0	0
Untreated pine	*0*	100	33.00 ± 0.76	41.52 ± 0.21	4.20 ± 0.25	2.10 ± 0.30	0.82 ± 0.15	11.37 ± 0.05	2.53 ± 0.40
AH150P	*2.95*	91.66	34.72 ± 0.18	44.29 ± 1.09	6.78 ± 0.24	3.67 ± 0.42	0.13 ± 0.01	10.40 ± 0.11	1.61 ± 0.01
AH175P	*3.99*	76.80	36.45 ± 1.59	54.12 ± 0.48	2.49 ± 0.00	0.26 ± 0.02	0	4.30 ± 0.12	0.62 ± 0.14
AH200P	*4.90*	66.58	46.60 ± 0.43	49.72 ± 0.63	0.44 ± 0.03	0.13 ± 0.13	0	0.11 ± 0.11	0.34 ± 0.02
Untreated eucalyptus		100	27.21 ± 0.20	51.08 ± 1.32	18.14 ± 0.35	0.62 ± 0.10	0.18 ± 0.18	0.41 ± 0.41	4.12 ± 0.11
IL80E		88.46	30.54 ± 0.41	45.44 ± 0.20	13.25 ± 0.53	1.35 ± 0.22	0	3.52 ± 1.76	6.62 ± 0.04
IL120E		86.67	28.14 ± 0.89	45.89 ± 1.43	14.22 ± 0.67	0.87 ± 0.17	0	3.79 ± 1.90	4.62 ± 0.99
Untreated pine		100	34.09 ± 0.79	42.89 ± 0.22	4.34 ± 0.26	2.17 ± 0.31	0.85 ± 0.16	11.75 ± 0.05	2.61 ± 0.41
IL80P		90.88	33.16 ± 0.54	44.31 ± 0.67	5.80 ± 0.44	2.61 ± 0.43	0.94 ± 0.05	11.36 ± 0.71	1.65 ± 0.24
IL120P		89.81	31.55 ± 0.59	45.22 ± 0.12	5.52 ± 0.31	2.33 ± 0.52	1.20 ± 0.01	11.70 ± 0.64	1.15 ± 0.00

3.2. Eucalyptus and pine autohydrolyzed liquors

Liquid fractions composition obtained after AH are shown in Table 2. Due to the presence of extractives, small amounts of phenolics, resin acids, fatty acids, juvabiones, alcohols, alkanes and esters may be present [32–34]. As a consequence of the higher liquid: solid ratio employed in AH of pine, concentrations are slightly lower than in eucalyptus. Xylo-oligosaccharides are the OS present at higher concentration in eucalyptus liquors, resulting from the solubilisation of xylan, the major component of the *E. globulus* hemicelluloses. Mannan-oligosaccharides are the OS present at higher concentration in *P. radiata* liquors, resulting from the solubilisation of mannan. In contrast, gluco-

xylose and arabinose. As HMF is formed via glucose/galactose/mannose dehydration, the high HMF production in eucalyptus may be attributed to *gluco*-oligosaccharides dehydration and the already mentioned differences used in the liquid-to-solid-ratio. Finally, formic acid, produced via furfural and HMF degradation, is also formed in both woods at severe operating conditions [18].

From a biorefinery perspective, intermediate and severe conditions would generate value added products. Although mild conditions may elucidate behaviours in softwood similar to hardwood, the need of higher severity factors to obtain comparable results between soft and hardwood evince the more recalcitrance structure of softwood, which requires stronger pretreatment conditions [17].

Table 2
Composition of the hydrolysates obtained from autohydrolysis.

Sample name	AH150E	AH175E	AH200E	AH150P	AH175P	AH200P
Severity factor	*2.95*	*3.69*	*4.42*	*2.95*	*3.99*	*4.90*
Gluco-oligomers (g/L)	0.27	0.30	0.34	0.29	0.93	0.11
Xylo-oligomers (g/L)	0.69	6.38	0	0.36	0,68	0
Galacto-oligomers (g/L)	0.27	0.51	0	0.00	0.57	0.01
Arabino-oligomers (g/L)	0.02	0	0	0.22	0.13	0
Mannan-oligomers (g/L)	0.05	0.26	0	0.85	3.43	0.26
Glucose (g/L)	0.02	0.17	0.59	0	0.03	0.57
Xylose (g/L)	0	0.75	0	0	0.55	0.13
Galactose (g/L)	0.03	0.30	0	0	0.22	0.22
Arabinose (g/L)	0	0	0	0.37	0.33	0
Mannose (g/L)	0	0	0	0	0.09	0.27
Formic acid (g/L)	0.01	0.11	0.63	0.02	0.05	0.37
Acetic acid (g/L)	0.04	0.44	2.40	0.04	0.18	0.84
Hydroxymethylfurfural (g/L)	0	0	2.15	0	0.08	1.99
Furfural (g/L)	0	0.09	1.31	0	0.15	0.92
Formic acid (g/L)	0.01	0.11	0.63	0.02	0.05	0.37
Acetic acid (g/L)	0.04	0.44	2.40	0.04	0.18	0.84
Hydroxymethylfurfural (g/L)	0	0	2.15	0	0.08	1.99
Furfural (g/L)	0	0.09	1.31	0	0.15	0.92

3.3. Microwave IL pretreatment of eucalyptus and pine wood

Solid recovery values after microwave ionic liquid treatment are from 86 to 90%, as shown in Table 1. Chemical composition of the pretreated solid is partially altered with the IL microwave treatment. Lignin content slightly decreases with the increase of the temperature. This happens because of biomass dissolution without degrading components and a subsequent precipitation step without fractional separation. Additionally, while the glucan present in untreated pine almost completely remain in the pretreated solid (around 94%) there is a considerable decrease (around 22%) of the glucan present in eucalyptus. Partial cellulose degradation has been produced in hardwood (supported by the detection of sugars in the IL stream by HPLC). The partial degradation of the cellulose may be attributed to the effect of the IL itself, or the employment of microwave heating that accelerate process but also may produce partial degradation under harsher conditions [15].

3.3.1. Liquid by-stream of microwave IL pretreatment

Washing fractions analysis showed that at least 3 washing steps are needed to recover the IL impregnated in the solid. Most of the IL (62–85 g of IL/100 g of initial IL introduced) was recovered in the regeneration step. No IL was detected in the 4th and 5th wash. That, would sum 13 mL of water needed per g of IL used in the process. At least, 96% of the initial IL introduced in the process was located in the washing steps, which is in accordance with other authors results in similar processes and our previous work [36,37].

The recovered IL after the vacuum distillation step was 91–95%. The pH of the diluted IL was in the range 7.38–7.69, and the lowest pH corresponded with the lowest solid yield obtained (86.67%). The amount of lignin present in the recovered IL (Figure S1) is noticeable compared to the initial lignin introduced in the process (1.20–10.81 g of lignin per 100 g of lignin introduced). The effect of microwave heating, has been found to enhance lignin extraction in other materials such as rice straw [38]. Eucalyptus lignin content in the IL strongly increases with the increase of the temperature in the IL microwave pretreatment. However, lignin content in the recovered IL in pine remains almost unaltered. Differences between both woods may indicate that [Emim][OAc] delignification capacity depends on the kind of lignin to extract.

3.3.2. Enzymatic digestibility and pretreated solids morphology

Glucan digestibilities during enzymatic hydrolysis of samples subjected to AH or IL treatment is shown in Fig. 1.

Untreated eucalyptus wood obtains the lowest glucan digestibility (15 g hydrolysed glucan/100 g glucan introduced). There is a gradual increment for AH150E, AH175E and AH200E, justified by the physical disruption of lignocellulose and reduction in hemicellulose content [5,39].

Tendencies in autohydrolyzed pine wood are the opposite to eucalyptus, resulting in a decrease of glucan digestibility when the autohydrolysis severity factor increases. Softwood is commonly known as a “worst-case scenario” for biomass pretreatment. In this case, autohydrolysis worsen glucan digestibility in comparison to untreated pine wood. Partial solubilisation and depolymerisation of lignin and its recondensation and deposition on the external surface is one of the main causes observed for enzymes inhibition after autohydrolysis treatments [18].

Evaluating the effect of temperature variations on IL microwave pretreatments, glucan digestibility of eucalyptus pretreated wood is enhanced to 55 g/100 g and 68 g/100 g with the addition of the IL pretreatment for experiments IL80E and IL120E, respectively. In the case of *P. radiata* wood, the increase of temperature does not cause a relevant increase in glucan digestibility that is only enhanced to 72 g/100 g and 78 g/100 g for experiments IL80P and IL120P, respectively. These results are attributed to the decrease of DP of cellulose, due to the effect of IL and microwave heating. Delignification of softwood has not been as high as in hardwood (when the IL pretreatment temperature increases), so the effect of the IL has mainly affected cellulose dissolution. The highest digestibility is obtained in IL120P sample. These results contrast to the observed by Li et al., where

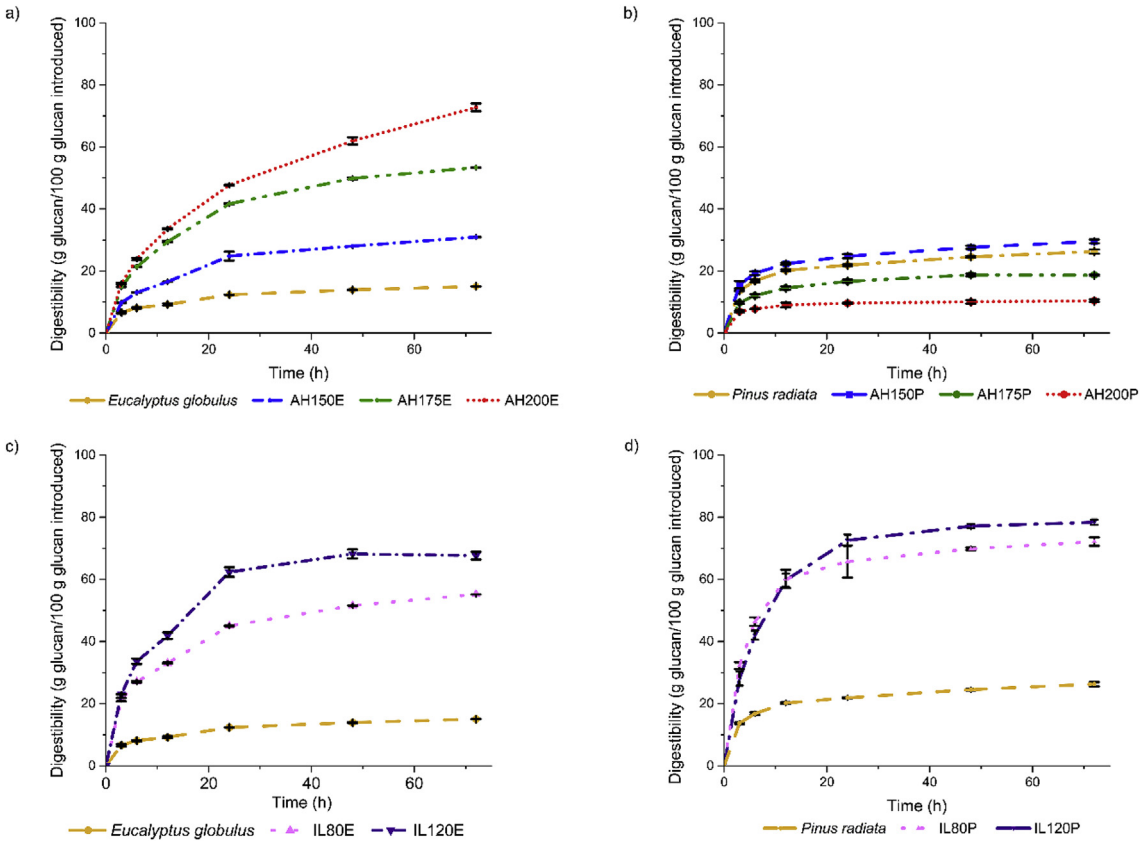

Fig. 1. Glucan digestibility in a) untreated eucalyptus and eucalyptus AH samples; b) untreated pine and pine AH samples; c) untreated eucalyptus and eucalyptus IL microwave pretreated samples; d) untreated pine and pines IL microwave pretreated samples. Bars denote standard deviations.

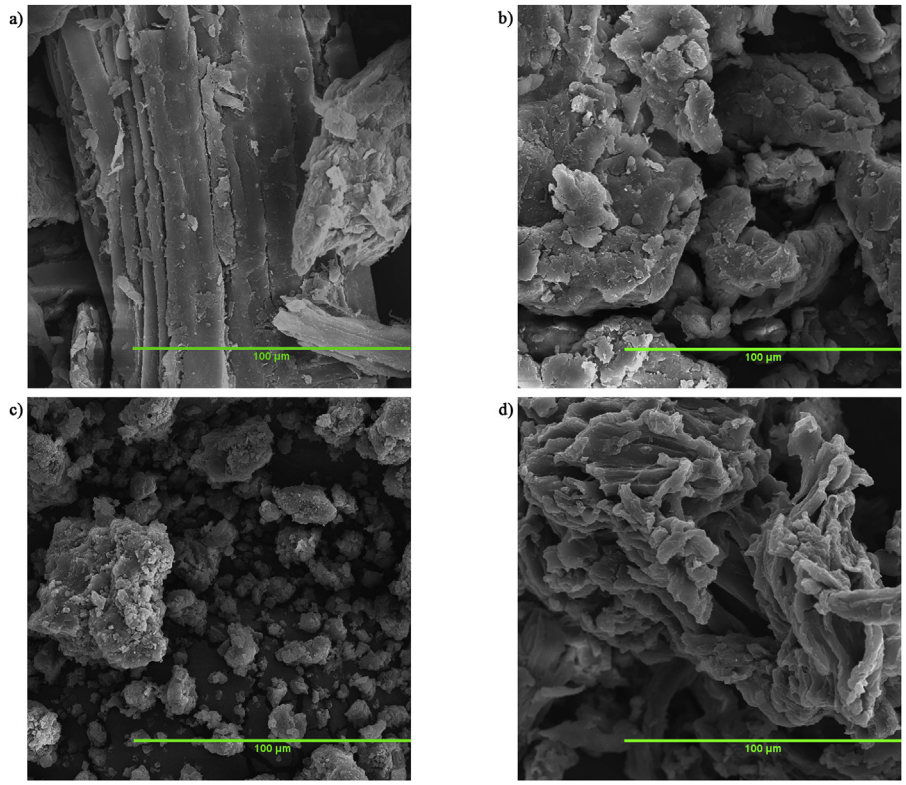

Fig. 2. SEM micrographs of a) Untreated eucalyptus, b) AH175E, c) AH200E and d) IL120E.

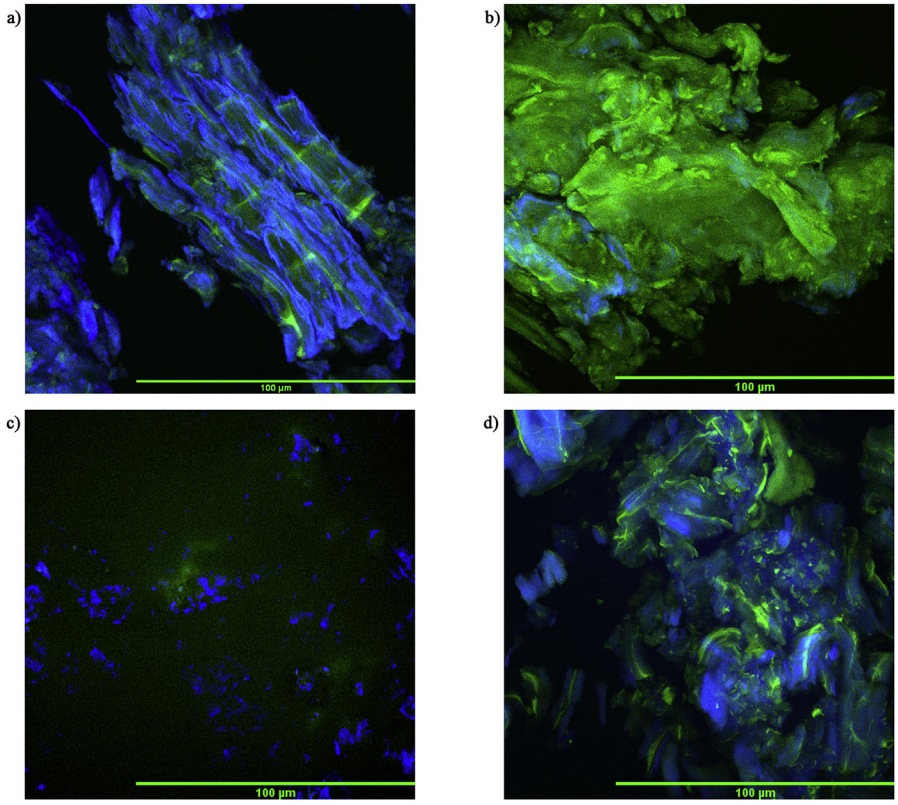

Fig. 3. Confocal fluorescence microscopy images of a) Untreated eucalyptus, b) AH175E, c) AH200E and d) IL120E.

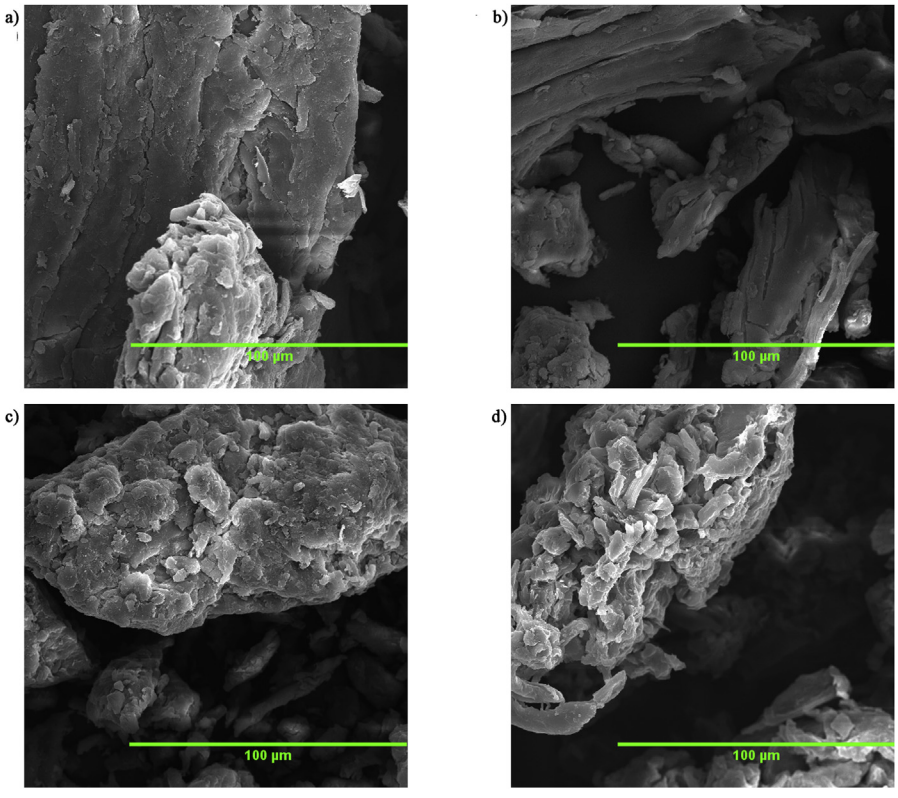

Fig. 4. SEM micrographs of a) Untreated pine, b) AH175P, c) AH200P and d) IL120P.

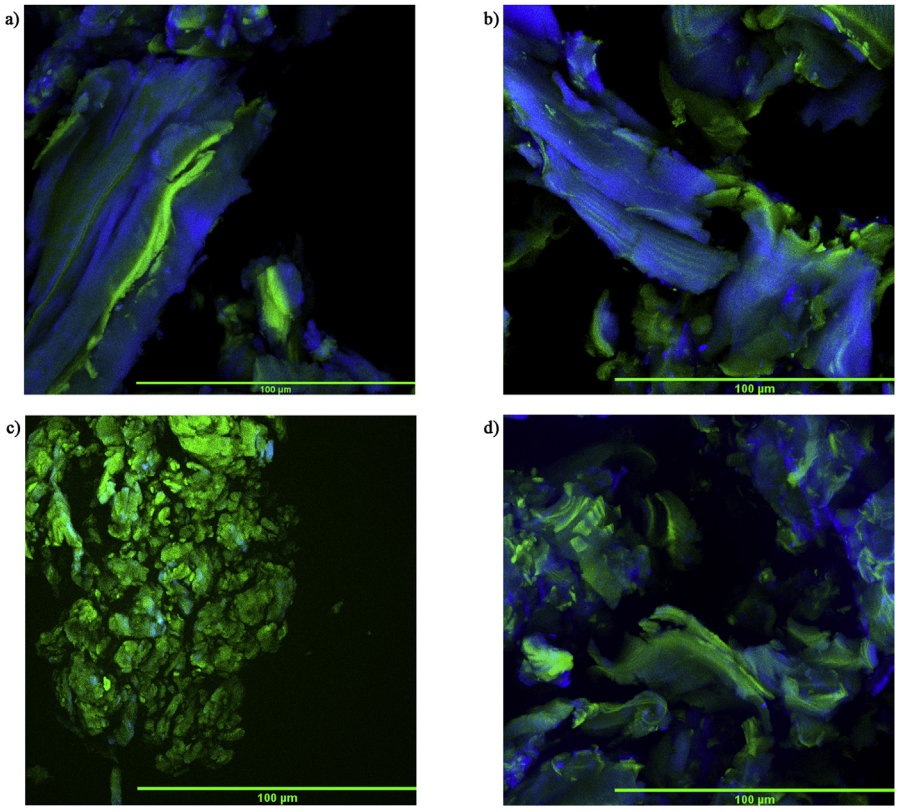

Fig. 5. Confocal fluorescence microscopy images of a) Untreated pine, b) AH175P, c) AH200P and d) IL120P.

eucalyptus yielded higher (93%) digestibility than pine (62%) (in that case using conventional heating) [17]. In a global comparison, eucalyptus highest digestibility is obtained at severe AH conditions, while the pine highest digestibility is obtained with IL120P treatment.

Xylan digestibility was also quantified in the case of eucalyptus (Figure S2 from the supplementary material), and the highest values were for IL80E and IL120E, respectively. In the case of pine wood, xylose and mannose were not obtained.

Figs. 2–5 show the most representatives SEM and confocal microscope samples. The overall surface of untreated eucalyptus (Fig. 2a) and pine wood (Fig. 4a) was smoothed with no visible pores in the structure. Hemicelluloses (in blue) dyed with calcofluor are observed (Figs. 3a and 5a) in the surface as well as lignin (in green). After intermediate autohydrolysis conditions (AH175E and AH175P), eucalyptus suffers an alteration of its native structures and hemicellulose is removed, remaining lignin in the surface. In the case of pine wood, morphology remains unaltered (Fig. 4b) and the decrease of hemicellulose composition is not clearly observed on the surface structure (Fig. 5b). At severe operating conditions (AH200P and AH200E), eucalyptus solids are observed as an agglomeration of smaller particles (Fig. 2c). The surface is observed to be rough and large number of pores are visualized. Due to the opacity, AH200 samples are more difficult to be observed in the confocal microscope (Fig. 3c). However, autofluorescent lignin (green) is barely observed and cellulose is only appreciated. This behaviour contrasts with the pine wood structures observed. In AH200P, cellulose is not visible and almost the complete surface of the particle is compound by lignin (Fig. 5c). The already mentioned lignin recondensation of softwood is in this way proved, probably causing enzymatic saccharification low yields [18].

IL microwave treated samples (Figs. 2d and 4d), in both cases, have a relatively homogeneous macrostructure with more porosity, due to partial dissolution and regeneration of wood fibres in a fusing structure, where structural modification of lignin and hollocellulose have been produced (Figs. 3d and 5d) [3,40].

3.4. Process mass balance

Figure S3 and Table S1 from the supplementary material summarizes the calculations developed in the whole process. The highest glucose yields are obtained with microwave ionic liquid pretreatments. It is highlighted that under severe AH conditions (specially AH200E), the partial degradation of wood results in strong decrease of glucose yields. Although glucan digestibility is in this case the highest of eucalyptus samples, the final glucose yield is lower (20.8 g of glucose/100 g of untreated eucalyptus wood) than in AH175E sample (27 g of glucose/100 g of untreated eucalyptus wood). The highest eucalyptus glucose yield is obtained in the sample IL120E. Despite of the recalcitrance of softwood and low cellulose content of the raw material in relation to hardwood, the highest glucose production is obtained with pine wood (IL120P) with 35.4 g of glucose/100 g of initial pine wood.

From a biorefinery perspective, autohydrolysis pretreatment is performed at higher particle sizes (experiments run at particle sizes between 0.3 and 2 mm); while the high viscosity of ILs forces this pretreatment efficacy to the operation at lower particle sizes (dust). The milling energy requirements before the pretreatment should be considered to determine the most appropriate pretreatment. Additionally, IL microwave pretreatment will require an extra pre-conditioning step to remove extractives. According to the quantity of solvent employed, consumption is considerably higher in IL pretreatments than the water employed in autohydrolysis due to the low solid:liquid ratios employed in IL pretreatments. Furthermore, water washing volumes are also much higher, as well as water washing employed.

Note that overall mass balance has evinced that lignin has been overestimated in the case of experiments developed with autohydrolized at 200 °C eucalyptus wood, probably due to “pseudo-lignin”. Thus, by-products such as lignin-furfural that cannot be distinguished from lignin increases the lignin value in the mass balance [41].

4. Conclusions

Microwave ionic liquid and autohydrolysis pretreatments of hardwood (*E. globulus*) *vs* softwood (*P. radiata*) have been studied. Autohydrolysis enhanced enzymatic digestibility of eucalyptus, while pine digestibility decreased due to lignin deposition in the external surface as observed in confocal fluorescence microscopy images. IL microwave pretreatment slightly affected biomass composition, although lignin extraction was considerably higher in eucalyptus (up to 11 g of lignin/100 g of initial lignin) than in softwood (less than 2 g/100 g). The high effectivity of IL microwave pretreatment in both biomasses was proved. The high effectivity of IL microwave pretreatment in softwood (digestibility of 78%, 35.4 g of glucose/100 g of pine) over hardwood become IL in an alternative technology for this kind of recalcitrant biomasses.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.biombioe.2018.07.014.

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