Industrial Crops & Products 206 (2023) 117581

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Valorization of *Pinus pinaster* wood by one-pot treatments in butanol/water biphasic mixtures

Carlos Vila[a,b], Remedios Yáñez[b,c,*], José L. Alonso[a,b]

[a] *Universidade de Vigo, Facultade de Ciencias, As Lagoas s/n, 32004 Ourense, Spain*
[b] *CINBIO, Universidade de Vigo, 32004 Ourense, Spain*
[c] *Universidade de Vigo, Escola de Enxeñaría Industrial, Campus Lagoas-Marcosende, 36310 Vigo, Spain*

ARTICLE INFO

Keywords:
Pinus pinaster wood
One-pot fractionation
Butanol/water
Biphasic organosolv

ABSTRACT

Biphasic butanol/water treatments have been proposed for the efficient fractionation of *Pinus pinaster* wood, with the objective of recovering glucan enriched solid phases, and two liquid streams, one organic and one aqueous, mainly made up of lignin-solubilized products and hemicellulosic compounds, respectively. Under selected operating conditions (190ºC, 40% of butanol, 1% of catalyst, and 10 min), high lignin solubilization (77.82%) and glucan recovery (86.93%) were achieved and an aqueous phase containing 15.8 g/L of oligosaccharides was also obtained. Further processing of the organic phase allowed the recovery of 62.69% of the initial lignin with a high purity (close to 94%). Finally, the enzymatic digestibility of the treated solid was evaluated achieving a saccharification yield of about 95%. Therefore, a promising softwood biorefinery scheme based on the use of a hydrophobic green solvent is proposed.

1. Introduction

The problems caused by current oil consumption are driving the search for renewable and sustainable alternatives to reduce this dependence (Culaba et al., 2022; Romaní et al., 2019). In this line, lignocellulosic materials (LCM) are considered promising and attractive raw materials for the production of second generation platform biochemicals, since they are highly available, inexpensive, and avoid the competition with food (Isikgor and Becer, 2015).

P. pinaster is one of the main coniferous in the Iberian Peninsula, whose stock in Spain exceeds 162 million cubic meters (MITECO, 2020). It is a fast-growing species used mainly as sawn timber, for the production of wood panels and in the pulp and paper industry (Alonso-Esteban et al., 2022).

The LCM are mainly constituted of cellulose, hemicelluloses, and lignin, all of them with a great economical potential. Hence, the efficient use of LCM as feedstock through a biorefinery approach implies the selective separation and recovery of these fractions (Madadi et al., 2022). The selection of appropriate methods for the fractionation of LCM will depend on the recalcitrant nature and on the key components characteristics. For instance, hemicelluloses, given their low resistance, are susceptible to hydrolysis under mild conditions, whereas the lignin can be dissolved in organic solvents or in aqueous media with the appropriate chemicals (Penín et al., 2019).

For the integral valorization of LCM, several sequential fractionation strategies have been evaluated, including aqueous and organosolv treatments or technologies based on certain green solvents, for instance, ionic liquids, or more recently, deep eutectic solvents (DES) (Guo et al., 2022; del Río et al., 2022; Moreira et al., 2020; Penín et al., 2022, 2020; Santos et al., 2019; Wang et al., 2021). However, nowadays, some authors are involved in the search of cost-effective and single pot technologies for the valorization of LCM. In this context, biphasic systems, formulated with water and hydrophobic solvents (pentanol or butanol), can act as effective media for the efficiently upgrading of the main fractions of LCM into three distinct phases (Haldar et al., 2022; Madadi et al., 2022; Schmetz et al., 2019): spent solids rich in cellulose and aqueous and organic streams containing mainly hemicelluloses and lignin derived compounds, respectively. Teramura et al. (2016) demonstrated the efficacy of low concentrations of butanol or pentanol for the catalytic fractionation of sorghum bagasse and Rivas et al. (2021) addressed the upgrading of vine shoots in media formulated with water/butanol/H_2SO_4. Renders et al. (2018) evaluated a one-pot reductive catalytic process with equivolumetric water/n-butanol mixtures for the fractionation of eucalyptus wood. However, Panovic et al.

---

* Corresponding author at: CINBIO, Universidade de Vigo, 32004 Ourense, Spain.
E-mail address: reme@uvigo.es (R. Yáñez).

https://doi.org/10.1016/j.indcrop.2023.117581
Received 19 May 2023; Received in revised form 15 September 2023; Accepted 2 October 2023
0926-6690/© 2023 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

(2018) and Lancefield et al. (2017) tested higher butanol contents (95%) in HCl-catalyzed organosolv treatments at reflux (118ºC) to fractionate certain biomass (beech and Douglas fir woods, walnut shells and rice husks). Schmetz et al. (2019) compared diluted acids and H_2SO_4/n-butanol biphasic systems for the pretreatment of different feedstocks: sugar beet pulp, tall fescue, sugar cane bagasse, beech, eucalyptus, and Japanese cedar. In this later study, the biphasic organosolv treatments allowed to obtain purer and higher quality cellulose and lignin than the diluted acid ones from all the raw materials tested, with the exception of Japanese cedar. Kawamata et al. (2021) evaluated several molar ratios of water/butanol for the isolation of lignin from Japanese cedar and willow woods. In recent studies, Madadi et al. (2022) assessed one-step treatments of aspen wood with diluted acid/pentanol media and Brienza et al. (2022) optimized the dithionite-assisted organosolv in n-butanol/water with birch and spruce woods, miscanthus, and wheat straw.

However, to date, the biphasic delignification of pine wood with butanol/water has only been previously assessed by del Rio et al. (2010). In this study, the butanol processing of *P. contorta* wood was evaluated under fixed operational conditions with several acid and basic catalysts (H_2SO_4, SO_2, NaOH, or $MgCl_2$).

The results obtained in the aforementioned studies have showed the great future prospect of this technology in the scope of biomass biorefinery. However, given the limited information available in this area of research, especially with softwoods, further optimization work is needed to advance in the knowledge and feasibility of catalyzed biorefineries in hydrophobic biphasic media, which would facilitate the separation of the main products. In this way, certain limitations of organosolv processes could be overcome, thus contributing to improve their economic profitability.

In this work, the one single-stage processing of *P. pinaster* wood in catalyzed biphasic media (water/butanol/H_2SO_4) is addressed. With this purpose, an experimental design (with treatment temperature, butanol concentration and catalyst loading as independent variables) is proposed to maximize the glucan recovery in the solid streams while reaching a high delignification degree and preserving the generated oligosaccharides in the aqueous phases.

2. Materials and methods

2.1. Raw material and chemicals

P. pinaster samples were kindly provided by a company dedicated to the fiberboard production (FINSA OREMBER, Ourense, Spain). To obtain a particle size of 1 mm, dried samples were ground (Retsch SM1 74075 instrument, Germany).

The following chemicals and solvents were employed: 1-butanol (Scharlau, ≥ 99.5% purity), sulfuric acid (Fisher, ≥ 95% purity), glucose (Panreac, ≥ 99% purity), xylose (Sigma, ≥ 99% purity), arabinose (Sigma, ≥ 99% purity), galactose (Sigma, ≥ 99% purity), mannose (Panreac, 99%), levulinic acid (Alfa Aesar, 98% purity), formic acid (Scharlau, ≥ 95% purity), acetic acid (Sigma, 99.7% purity), hydroxymethylfurfural (HMF; Sigma-Aldrich, 99% purity), and furfural (Fluka, ≥ 99% purity).

2.2. Biphasic processing of P. pinaster samples

Isothermal biphasic treatments were performed in a 100 mL Parr 5500 Series reactors (Parr, Illinois, USA) using butanol-water solutions acidified with sulfuric acid.

Table 1 summarizes the three-level Box-Behnken factorial experimental design proposed in this study to optimize the wood fractionation. The selected independent variables were: catalyst loading, denoted as x1 (in the range 0.5 and 1.5 g H_2SO_4/ 100 g dry wood), the butanol concentration or x2 (20–60%, as weight percentage in liquor) and the temperature or x3 (160–190 ºC). Values for the normalized, dimensionless variables (x1, x2, and x3) were also included in the same table.

Table 1
Experimental design expressed in terms of the dimensional variables catalyst load (expressed as weight percentage with respect to dry raw material), butanol concentration (BC, as weight percentage in liquor) and temperature and dimensionless variables x1, x2 and x3.

Experiment	Catalyst (%)	BC (%)	Temperature (ºC)	x_1	x_2	x_3
1	0.5	20	175	-1	-1	0
2	1.5	20	175	1	-1	0
3	0.5	60	175	-1	1	0
4	1.5	60	175	1	1	0
5	0.5	40	160	-1	0	-1
6	1.5	40	160	1	0	-1
7	0.5	40	190	-1	0	1
8	1.5	40	190	1	0	1
9	1	20	160	0	-1	-1
10	1	60	160	0	1	-1
11	1	20	190	0	-1	1
12	1	60	190	0	1	1
13	1	40	175	0	0	0
14	1	40	175	0	0	0
15	1	40	175	0	0	0

In this first set of experiments (denoted as experiments 1–15), the liquor/ solid ratio (LSR), the reaction time, and the mechanical stirring speed were fixed (according to preliminary assays) in 10 g/g, 30 min, and 150 rpm, respectively. To assess the influence of decreasing the reaction time, a second set of 3 experiments was performed at 30, 20, and 10 min (denoted as experiments 16, 17, and 18, respectively), keeping constant the other operational parameters under selected conditions (LSR of 10 g/g, 1 g of H_2SO_4/100 g dried wood, 40% of butanol, and 190 ºC).

Once the reaction times elapsed, the media were quickly cooled, and the solid phases were separated by filtration and washed with two aliquots of butanol followed by distilled water. Some aliquots were analyzed by the methodology described below for the quantification of glucan, hemicellulosic compounds, and Klason lignin. The aqueous and organic phases were separated by decantation and collected. Analysis for the quantitative determination of oligosaccharides, organic acids, and sugars, among others, were made by liquid chromatography (HPLC, see below). Afterwards, lignin was isolated from the organic liquors. With this purpose, firstly the butanol was recovered by vacuum distillation, in order to be reused to wash the pulp. After that, the lignin-enriched phase was dissolved in acetone and precipitated in water acidified with sulfuric acid. Finally, the organosolv-isolated lignin was washed with abundant hot water and vacuum dried at 40 °C for subsequent characterization.

Under the selected operational conditions, some experiments were conducted in a 600 mL reactor (Parr Instruments Company, Moline, Illinois, USA) to assess the effect of increasing the reactor size (scale up) on the results.

The pulp yield (PY), the percentage of glucan recovery (PGR), the percentage of lignin removal (PLR), and percentage of hemicelluloses solubilized (PHS) were calculated following the Eqs. 1 to 4:

$$PY = \frac{M_{TS}}{M_{rm}} * 100 \tag{1}$$

$$PGR = \frac{Gn_{TS} * PY/100}{Gn_{rm}} * 100 \tag{2}$$

$$PR = \frac{KL_{TS} * PY/100}{KL_{rm}} * 100 \tag{3}$$

$$PHS = \frac{(HC_{rm} - HC_{TS} * PY/100)}{HC_{rm}} * 100 \tag{4}$$

Where M_{TS} and M_{rm} are the mass weight of treated solids and raw material, Gn_{TS}, KL_{TS}, and HC_{TS} correspond to the percentages of glucan, Klason lignin and hemicelluloses in the treated solids and Gn_{rm}, KL_{rm}, and HC_{rm} are the percentages in raw material, and PY is the pulp yield achieved in the treatment.

2.3. Enzymatic hydrolysis

The solid recovered in the optimum was tested as substrate in enzymatic saccharification assays performed with a commercial blend (Cellic® Ctec3), kindly provide by Novozymes (Madrid, Spain). The experimental conditions of the enzymatic experiments were as follows: orbital agitation of 150 rpm, T = 52.5 ºC, pH= 5.0, and 0.05 N citrate buffer. The cellulasic activity was determined by Filter Paper assays according to Adney and Baker (2008), and defined in Filter Paper Units (FPU) per mL of blend. In these preliminary experiments two enzyme to substrate ratios (10 and 20 FPU/g solid) were evaluated at a LSR of 10 g/g. The analysis of the samples taken to follow the saccharification kinetic were made by HPLC as described below.

2.4. Analytical procedure

2.4.1. Chemical composition of the raw material, treated solids, and liquid fractions

Ground samples of feedstock and treated solids (particle sizes < 0.5 mm, using a Kinematica™ polymix PX-MFC 90 D) and liquid phases were characterized according to the methodology proposed by Portela-Grandío et al. (2021) and Rivas et al. (2021).

2.4.2. Characterization of lignin

Aliquots of lignin were analyzed as previously described López et al. (2020).

2.5. Data analysis

R software (RSM package) was used for the fitting of data.

3. Results

3.1. Determination of the Chemical composition of P. pinaster wood

The chemical composition of the *P. pinaster* wood samples (g/ 100 g oven-dry wood ± standard deviation) was the following: glucan, 40.64 ± 1.30; Klason lignin, 26.16 ± 0.89; mannan, 12.36 ± 0.48; xylan, 5.55 ± 0.22; galactan, 3.79 ± 0.04; acetyl groups, 1.94 ± 0.77; arabinan, 1.84 ± 0.10; extractives, 4.69 ± 0.27 and ash, 0.19 ± 0.01. It should be noted the glucan and Klason lignin contents of this raw material, as well as the relevant presence of mannan and xylan, as it is often the case in softwood species (Nitsos et al., 2018). Unlike other lignocellulosic materials and hardwoods, the main fractions present in softwood hemicelluloses are partially acetylated glucomannans or galactoglucomannans (Nitsos et al., 2018).

Similar results were reported in literature for *P. pinaster* wood (Penín et al., 2019) and for *Pinus radiata* wood (Nitsos et al., 2018; Santos et al., 2022).

3.2. Experimental design: optimization of the operational conditions

Although to the potential and availability of softwoods, their valorization by organosolv processes is not as developed as for hardwoods, due probably, to their strong recalcitrance associated to the higher lignin content, as well as their different structure with more guaiacyl units, more prone to condensation reactions, and lower methoxy content (Nitsos et al., 2018; Pan et al., 2007). Other authors also noted that their less porous morphology could reduce the mass transfer thus limiting the lignin extraction (Brienza et al., 2022). These factors make their processing more difficult, and because of that, they usually require higher severities of treatment. To address this challenge, in this study, based on previous literature (Rivas et al., 2021; Schmetz et al., 2019; Teramura et al., 2018, 2016), a biphasic delignification using butanol/water mixtures acidified with sulfuric acid was selected for the efficient one-pot fractionation of *P. pinaster* wood. Butanol was selected because is a renewable solvent with a high boiling point, which presents the following advantages over ethanol: i) higher energy density; ii) minor water absorption capacity, and iii) better blending ability (Lancefield et al., 2019). Moreover, the considerably advances achieved in the biotechnological production of biobutanol in the last decades (Riaz et al., 2022) open new application perspectives as next-generation biofuel and promote its future interest as a biomass fractionation solvent.

The key compounds pursued by the biorefinery scheme evaluated in this work are the following: glucan, lignin derived compounds, and hemicellulosic oligosaccharides. The effectiveness of butanol organosolv in the recovery of hemicellulose-derived saccharides in aqueous phase has been also reported by Del Rio et al. (2010), since the poor miscibility between water and butanol could favor the content increase in aqueous phases. Moreover, the delignification potential of butanol has been demonstrated, even at low concentrations (Teramura et al., 2016). Other authors also highlighted the lower energy requirements for the separation and recycling of two-phase systems compared with single-phase ones (Zimmermann et al., 2018), as well as the high purity and quality of the cellulose and lignin obtained when this solvent is used for the fractionation of several raw materials (Schmetz et al., 2019).

After a set of preliminary assays, a three-level Box-Behnken factorial experimental design was proposed for developing the experimental plan (see Table 1). Table 2 summarizes certain results obtained in the 15 experiments of the design, including the contents of glucan and lignin of the treated solids as well as information relative to C5- and C6-monosaccharides and the main oligosaccharides present in the aqueous phases. On the other hand, Table 2 also shows the calculated values for the PY or y1 and for the dependent variables: the PLR or y2, the PGR or y3, and the production of galactoglucomannan oligosaccharides (GGMOS) or y4. These variables were selected in order to give the maximum information about the effects of the treatments on the main fractions of pine wood samples and on the treatment selectivity, taking into account the objectives of the process evaluated.

3.2.1. Pulp yield

As seen in Table 2, the PY varied in the range 67.28–35.49 g treated solid/ 100 g dried raw material. Pan et al. (2007) reported lower PY (between 28.63% and 42.55%) for the sulfuric acid-catalyzed organosolv of *P. contorta* and Santos et al. (2019) found a PY of 28.9% when treated *P. radiata* at 185 ºC, for 75 min, with 1% of H_2SO_4 w/w, and using an ethanol concentration of 50%. However, as might be expected, high yields (between 44.6% and 79.6%) were obtained when *P. radiata* wood was processed in ethanol media in the absence of catalyst (Santos et al., 2022).

In general lines, it was found that increasing the severity of treatments (temperature and catalyst loading) and the butanol concentration resulted in decreasing of PY, due to higher hemicelluloses and/or lignin solubilization, as described previously in other organosolv treatments (Romaní et al., 2019; Santos et al., 2022, 2019). In fact, the low value of PY (35.49%) was obtained in experiment 12, performed at the highest temperature and butanol concentration and intermediate concentration of catalyst, which correspond with one of the higher PLR (87.65%).

3.2.2. Composition of the treated solids

The main components found in the processed solids were glucan, Klason lignin, and mannan although minor amounts of xylan, galactan, and arabinan, as well as acetyl groups were also detected. The glucan content varied in the range 55.71–85.50%, whereas the lignin between 5.90% and 36.10%, which correspond to PGR of 73–92% and PLR of 13–91%. These values compare well with those obtained in acid-catalyzed ethanol organosolv treatments, 75% and 85% for *P. contorta* (Pan et al., 2007), and 59% and 89% for *P. radiata* (Santos et al., 2019),

Table 2
Chemical composition of the solid and aqueous phases and values obtained for the pulp yield (PY) and the selectivity dependent variables (percentages of lignin removal and glucan recovery, and galactoglucomannan oligosaccharides denoted as PLR, PGR, and GGMOS, respectively).

	Main components of solid phases		Main components of aqueous phases						Selectivity dependent variables		
	Glucan	Klason lignin	Hexoses	Pentoses	GOS[a]	MOS[b]	GALOS[c]	PY (y_1)	PLR (y_2)	PGR (y_3)	GGMOS (y_4)
Expt.	g/100 g of treated solid		g/100 g of raw material					%	%	%	g/100 g of raw material
1	59.26	32.73	7.80	5.12	1.05	4.09	1.49	63.02	21.16	91.89	6.63
2	60.32	29.59	13.50	4.58	1.22	1.29	0.60	53.64	39.34	79.60	3.10
3	63.38	24.20	4.42	3.12	0.26	3.02	0.22	57.47	46.84	89.61	3.50
4	76.20	5.90	7.07	2.02	1.34	1.77	0.52	41.57	90.62	77.95	3.62
5	55.71	33.85	3.68	4.60	2.77	8.82	2.84	67.28	12.95	92.24	14.43
6	63.35	19.45	11.05	4.43	1.38	3.29	0.88	53.42	60.28	83.27	5.55
7	76.53	17.07	10.25	4.82	1.67	4.53	1.25	44.15	71.20	83.14	7.46
8	75.38	15.41	5.46	0.46	4.22	1.91	0.96	41.73	75.42	77.40	7.09
9	58.41	36.10	13.71	6.78	1.30	3.93	1.06	60.09	17.08	86.37	6.29
10	66.89	26.80	10.44	4.95	1.88	3.20	1.56	49.00	49.80	80.66	6.64
11	68.32	20.13	10.39	5.25	1.42	1.76	1.34	43.63	66.44	73.34	4.53
12	85.50	9.10	10.06	2.51	2.75	2.57	0.74	35.49	87.65	74.67	6.06
13	73.59	19.94	11.88	4.75	1.29	2.16	1.14	46.12	64.86	83.51	4.60
14	72.50	20.40	9.96	5.84	0.58	3.47	1.58	46.79	63.52	83.47	5.63
15	73.74	20.45	10.61	4.41	1.01	2.15	0.54	46.00	64.05	83.46	3.71

[a] GOS- glucooligosaccharides
[b] MOS- mannooligosaccharides
[c] GALOS- galactooligosaccharides

as well as with those reported for butanol-based organosolv process, 74% and 67% for *P. contorta* (Del Rio et al., 2010) or values close to 80% for eucalyptus wood (Schmetz et al., 2019).

As seen in Table 2, high glucan and low Klason lignin contents (85.50 and 9.10 g/100 g of treated solid, respectively) were observed in experiment 12 (performed at 1% of catalyst, butanol concentration of 60%, and 190 °C). However, it seems that delignification could be favored by the catalyst loading, since the lowest experimental Klason lignin value (5.90 g/100 g of treated solid) was achieved in experiment 4 (1.5% of catalyst, butanol concentration of 60%, and 175°C). On the other hand, as expected, the experiment 9, carried out at the lowest butanol concentration and the lowest temperature (20% and 160 °C), resulted in a treated solid with the highest Klason lignin.

3.2.3. Liquid phases composition

The analysis of the aqueous phases showed the presence of hemicellulose-derived compounds (mainly mono- and oligosaccharides, with high contents of mannose), and minor amounts of sugar degradation products. The PHS varied between 83.16% and 97.98% in the entire interval evaluated. In particular, the experiment 9 (with PHS of 94.59%) led to the aqueous phase with the highest sugar content, 20.49 g monosaccharides/100 g of dried raw material, being mannose and xylose the main components (7.37 and 5.17 g/100 g of dried raw material, respectively).

However, the highest content of oligosaccharides in aqueous phase was found in experiment 5 (T = 160 °C, catalyst loading= 0.5%, and butanol content= 40%) in which a yield of 15.99 g oligomers/100 g of dried raw material was achieved. Unfortunately, these operational conditions resulted in a very poor delignification extent (see GGMOS and PLR columns in Table 2).

Interestingly, HPLC analysis also revealed low concentrations of furfural, HMF, and acetic acid in the liquors (data not shown), a fact that facilitates further oligosaccharides purification steps, necessary for food and fermentation applications.

To reach a balance between glucan recovery and Klason lignin solubilization, preserving the maximum oligosaccharides, special attention should be focus on experiments 7 and 12 performed both at 190 °C, catalyst loading of 0.5% and 1%, and butanol concentration of 40% and 60%, respectively. Under these conditions, solids with glucan contents of 76.53 and 85.50 g/100 g of treated solid, and Klason lignin contents of 17.07 and 9.10 g/100 g of treated solid were found, respectively, corresponding to PGR of 83.14% and 74.67% and PLR of 71.20% and 87.65%. Concerning the liquors, remarkable productions of GGMOS (7.46 and 6.06 g/100 g of dried wood, respectively) and monosaccharides (15.07 and 12.57 g/100 g of dried wood, respectively) were also obtained under the mentioned operational conditions. Moreover, low amounts of sugar derived products (HMF, furfural and formic acid) were observed in both liquid phases, although their content in the organic liquors was slightly higher. Rivas et al. (2021) noted that the transference of furans to the organic phase could limit the side reactions in the aqueous stream. Schmetz et al. (2019) also reported that the partial solubilization of some fermentation inhibitors (organic acids and sugar degradation compounds) in the organic phase could favor further fermentation stages of the aqueous fraction.

Del Rio et al. (2010) reported a content of oligomeric sugars close to 28 g/L (highlighting the presence of hexoses such as mannose, glucose, and galactose) for the water-soluble fractions obtained in the sulfuric acid-catalyzed butanol treatments of *P. contorta* (65% of butanol, 170 °C, 60 min, and 1.1% of H_2SO_4). These experimental conditions also led to 24.45 g/L of monosaccharides, and to minor formation of furans (1.04 g/L of furfural and 0.32 g/L of HMF). In the first set of experiments, higher monosaccharide concentrations (27.45 g/L) were detected in experiment 9, whereas the oligosaccharide contents were lower, reaching the highest concentration in experiment 5 (21.69 g/L).

3.2.4. Selectivity dependent variables: empirical modelling and response surface methodology

To evaluate the process selectivity and to facilitate data interpretation, response surface methodology (RSM) tool was selected. With this purpose, PY and three selectivity dependent variables (PGR, PLR and GGMOS) were correlated with the independent variables (catalyst loading, butanol concentration, and temperature) by empirical models, as follows:

$$y_j = b_0 + \sum_{i=1}^{3} b_i x_i + \sum \sum_{i<j=1}^{3} b_i j x_i x_j + \sum_{i=j}^{3} b_{ii} i x_i^2 \tag{5}$$

where $\boldsymbol{y_j}$ ($\boldsymbol{j} = 1$–4) are the dependent variables; $\boldsymbol{x_i}$ or $\boldsymbol{x_j}$ ($\boldsymbol{i}$ or $\boldsymbol{j}$: 1–3, $\boldsymbol{j} \geq \boldsymbol{i}$) represent the normalized, dimensionless independent variables (see Table 1); and $\boldsymbol{b_{0j}}\ldots\boldsymbol{b_{ij}}$ correspond to regression coefficients obtained fitting the experimental data to the proposed models by multiple regression.

Table 3 summarizes the regression coefficients and some relevant statistical parameters. The correlation parameters (R2) shown in Table 3

Table 3

Regression coefficients and statistical parameters measuring the correlation and significance of the models, obtained for pulp yield (y_1), percentages of lignin removal and glucan recovery (y_2 and y_3, respectively) in the solid fractions, and galactoglucomannan oligosaccharides (y4) obtained in aqueous phase.

Coefficients	PY or y_1 (%)		PLR or y_2 (%)		PGR or y_3 (%)		GGMOS or y_4 (g/100 g of raw material)	
(Intercept)	46.303	[a]	64.141	[a]	83.477	[a]	4.64604	[a]
x1	-5.194	[a]	14.189	[a]	-4.831	[a]	-1.58063	[a]
x2	-4.605	[a]	16.363	[a]	-1.04		-0.09206	
x3	-8.1	[a]	20.074	[a]	-4.249	[a]	-0.97195	
x1:x2	-1.627		6.398	[a]	0.157		0.91307	
x1:x3	2.862	[a]	-10.775	[a]	0.81	[a]	2.12792	[a]
x2:x3	0.739		-2.877		1.761	[b]	0.29668	
x1ˆ2	6.106	[a]	-7.465	[a]	3.272	[a]	1.15866	
x2ˆ2	1.515		-7.187	[a]	-1.984	[b]	-1.59214	[b]
x3ˆ2	-0.764		-1.712		-2.736	[a]	2.82608	[a]
Statistical parameters								
R2	*0.988*		*0.987*		*0.972*		*0.906*	
F experimental	*44.65*		*42.5*		*19.26*		*5.38*	
Significance level (%)	*99.86*		*99.90*		*91.02*		*96.07*	

[a] Significant coefficients at 95% confidence level

[b] Significant coefficients at 90% confidence level

for the variables y_1 to y_4 varied in the range *0.906–0.988*, indicating a high degree of fit between dependent and independent variables according to the proposed models.

As seen in Table 3, the lineal terms of the three independent variables had a negative effect on the PY (y1), with temperature being the most influential. Fig. 1 shows the calculated response surfaces for the PY, the PLR, and the PGR as a function of the catalyst and butanol concentration at three temperature levels. As can be expected (see Fig. 1a), the PY decreased with the catalyst loading and temperature, being the effect of this variable more pronounced at low catalyst loadings. In a previous study dealing with the sulfuric acid-catalyzed ethanol treatment of *P. contorta*, Pan et al. (2007) reported a negative effect of the sulfuric acid on PY at temperatures higher than the turning point (175 °C), associated with the enhanced solubilization of carbohydrate.

Concerning the variable y2 (PLR), temperature was the most influential variable on PLR, followed by the butanol concentration and the catalyst loading, exerting all of them a positive effect (see Table 3). A positive effect of organic solvent concentration and temperature on delignification degree has also been previously reported by Santos et al. (2022) with *P. radiata* wood during ethanol-based organosolv process in absence of catalyst, achieving the highest PLR values (60–75%) at ethanol contents of 45–50 wt%, temperatures higher than 185 °C, and prolonged times (>90 min). Pan et al. (2007) also found substantial effects of the temperature and ethanol concentration on delignification of *P. contorta* wood in the acid catalyzed pretreatment, whereas time and sulfuric acid content did not.

As observed in Fig. 1b, PLR has been favored by the butanol concentration and the temperature. As happened with the PY, the effect of the temperature was less pronounced at intermediate to high catalyst loadings. A similar pattern was observed in other treatments in organic media with acacia wood, wheat straw or sugarcane bagasse (Sun and Chen, 2007; Novo et al., 2011; Portela-Grandío et al., 2021). However, contrary to what occurs in several acid-catalyzed organosolv (Melati et al., 2019; Pan et al., 2006), in this study no lignin repolymerization or formation of pseudo-lignin (associated to carbohydrate degradation products) was found, probably due to the relatively short reaction times. In this line, the effective role of butanol in preventing the deposition of pseudo-lignin was also confirmed over several feedstock's surfaces (Schmetz et al., 2019).

Although the highest PLR value predicted by the model (89.97%) was found at 190 °C, butanol concentration of 60%, and catalyst loading of 1% (experiment 12), it seems that the close values attained at medium butanol concentration would not justify the use of higher percentages of organic solvent.

On the contrary, temperature and catalyst loading exerted a similar and negative influence on the PGR, whereas the butanol concentration had a little and not significant effect. In this case, it should be noted the positive influence of the quadratic term of catalyst loading, and the interaction of butanol concentration and temperature. Fig. 1c also confirms the opposite behavior pattern exhibited by the PGR, which reached high values at the lowest temperature and catalyst loading evaluated. Thus, the maximum PGR predicted by the model was 94.01%, reached at 160 °C, butanol concentration of 40%, and catalyst loading of 0.5% (experiment 5).

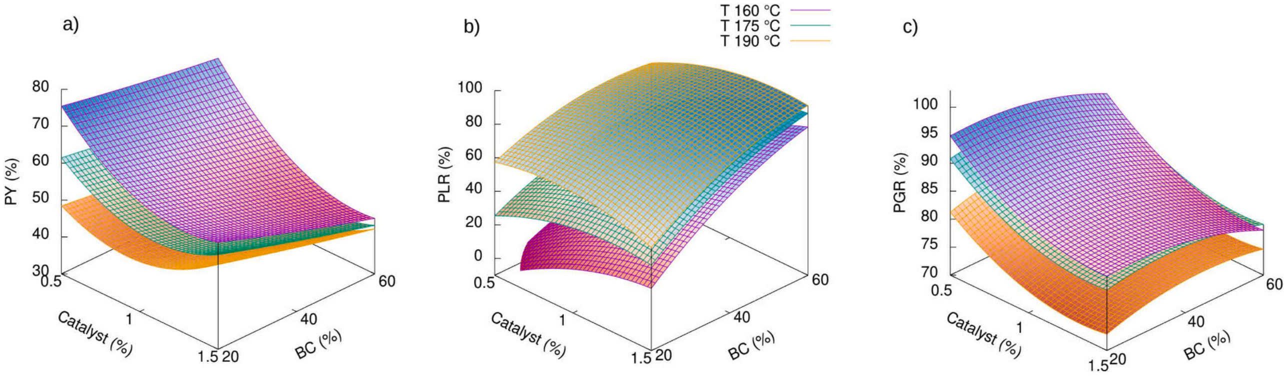

Fig. 1. Response surfaces of: a) pulp yield (PY), b) percentage of lignin removal (PLR), and c) percentage of glucan recovery (PGR) as a function of the catalyst load (expressed as weight percentage with respect to dry raw material) and butanol concentration (BC, expressed as weight percentage in liquor), at three temperature levels (160, 175, and 190°C).

The maximum PGR value compares favorably with the ones reported by Santos et al. (2022) with *P. radiata* wood in autocatalyzed ethanol organosolv pretreatment (70–90%), as well as with those obtained in catalyzed organosolv by Pan et al. (2007) with *P. contorta* (75%) and by Pan et al. (2006) with hybrid poplar (88%).

On the other hand, the PHS varied between 83.16% and 97.97% (data not shown), reaching the maximum solubilization at the highest temperature and catalyst concentration tested (190 ºC, 1.5% of catalyst, and 40% of butanol) and being the catalyst concentration the most influential variable. In other organosolv treatments, the favorable effect of high temperatures on the solubilization of hemicelluloses and glucan was also observed (Cybulska et al., 2017; Nitsos et al., 2018; Santos et al., 2022; Cheng et al., 2019). In line with previous studies (Teramura et al., 2016; Liu et al., 2019) performed with bamboo and sorghum bagasse, recently, Madadi et al. (2022) also found that the pentanol concentration and temperature promoted the solubilization of lignin and hemicelluloses of aspen wood in biphasic treatments, preserving the cellulose fraction in the spent solid.

The other selectivity dependent variable fitted to the model was the GGMOS, as a representative fraction of the oligosaccharides present in the aqueous phases derived from pine wood. The regression parameters obtained showed (see Table 3), as it happened with the PGR, that the three lineal terms exerted a negative effect on GGMOS but more limited, only being significant the catalyst loading. Fig. 2 shows the calculated response surface for the GGMOS with respect to the catalyst and temperature at three butanol concentrations. Interestingly, the model predicted the highest values of GGMOS (in the range 1.81–13.37 g/ 100 g of dried raw material) at butanol concentrations of 40%. Moreover, as it can also be seen in Fig. 2, the operation at 190 ºC resulted in significant GGMOS productions (for instance, values in the interval of 6.43–8.20 g/ 100 g of dried raw material with a butanol concentration of 40%). Although the model predicted the maximum of GGMOS (13.31 g/ 100 g of dried raw material) at the minimum temperature and catalyst loading evaluated, conditions of the experiment 5 that also lead to the maximum PGR (as stated before), under these conditions the degree of delignification was very low (PLR of 9.72%).

Taking into account the response surfaces of Figs. 1 and 2, in order to improve the PLR value, preserving the glucan fraction in the treated solids and oligosaccharides in the aqueous phase, it would be necessary to increase the temperature to 190 ºC, maintaining the catalyst and butanol concentration at intermediate values. The optimization of the organic solvent content is desirable since it would help to reduce treatment costs (Teramura et al., 2016). Moreover, its presence in the aqueous phase could inhibit further microbial processing. On the other hand, the employment of low catalyst loading would allow limiting the formation of sugar degradation compounds in the aqueous phase, which would hinder further oligosaccharide purification steps and even could act as microbial inhibitors (Teramura et al., 2016).

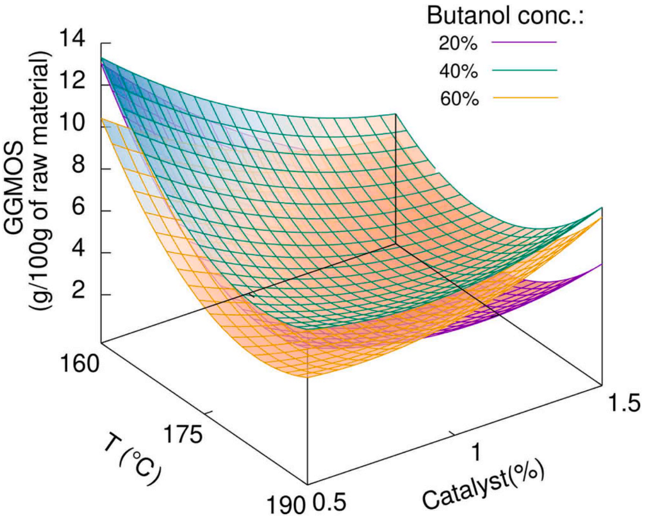

Fig. 2. Response surface of the GGMOS in the aqueous phase, as a function of the catalyst load (expressed as weight percentage with respect to the raw material) and temperature, at three butanol concentrations (20%, 40%, and 60%, as weight percentage in liquor).

Therefore, the experimental conditions selected to continue the study were as follows: 190 ºC, butanol concentration of 40%, and catalyst loading of 1%. Under these conditions, the model predicted a PY= 37.13%, a PLR= 82.98%, and a PGR= 76.28% whereas the GGMOS achieved a value of 6.50 g/ 100 g of dried raw material.

3.3. Validation of the model and evaluation of the effect of the reaction time

In order to validate the model and to evaluate the influence of the time, three additional experiments were performed at 30, 20, and 10 min (experiments 16, 17, and 18, respectively), while maintaining the other parameters under the previously selected values (see above).

The treated solid of experiment 16, showed the following composition: glucan, 84.43%; xylan, 0.49%, and Klason lignin, 13.70%. On the other hand, the values determined for the selected dependent variables were: PY= 36.13%, PLR= 81.08%, PGR= 75.05%, and GGMOS= 6.51 g/100 g of dried raw material, demonstrating a close agreement between experimental results and the values predicted by the mathematical models (see data outlined in the previous section).

Fig. 3 shows the time dependence of the PY, and PLR and PGR, as well as total monosaccharides and total oligosaccharides contents in the aqueous phases. As can be expected, a decrease in the reaction time resulted in improved PGR, PY, and oligosaccharides production but lower PLR values.

Therefore, the results would not justify the increase of the reaction time, and experiment 18 performed at a shorter treatment reaction time was selected to continue the work.

3.4. Process scale-up and material balance in the selected conditions

To evaluate the effect of the reactor size on the selected variables, a new run was carried out under the conditions of experiment 18 in a Parr reactor of 0.6 L (see Materials and Methods for details). The experimental conditions assayed resulted in a PY of 43.98 g/g and PGR, PLR, and PHS values of 86.93%, 77.82%, and 90.91%, respectively.

Fig. 4 presents a schematic diagram of the process including information about the material balance under the selected operational conditions (190 ºC, butanol concentration of 40%, catalyst loading of 1%, 10 min, and 150 rpm).

The isothermal biphasic delignification of 100 g of dry *P. pinaster* wood resulted in the recovery of 43.98 g of solid by filtration, containing 35.33 g of glucan, 2.29 g of xylan, and 5.84 g of Klason lignin. Afterwards, the funnel separation of the biphasic reaction liquors yielded an aqueous phase with a total monosaccharide content of 16.10 g (mainly, glucose and mannose) and a total oligosaccharide content of 9.89 g (of which 8.92 g were GGMOS). On the other hand, 16.4 g of lignin were also recovered from the organic phase by precipitation and filtration, which represents 62.69% of the initial lignin.

These results compare favorably with the reported by Del Río et al. (2010) when treated *P. contorta* wood with sulfuric acid-catalyzed butanol. Under fixed operational conditions (butanol content of 65%, H_2SO_4 loading of 1.1%, 170 °C, and 60 min), the authors obtained a spent solid with 33 g of glucan and 8.1 g of acid insoluble lignin/ 100 g of raw material and minor contents of xylan, arabinan, mannan, and galactan (< 0.5 g/ 100 g of raw material). Simultaneously, a water-soluble fraction containing about 28 g/L of oligomeric sugars (mainly made up of mannose, glucose, and galactose), 24.45 g/L of monosaccharides, 1.04 g/L furfural, and 0.32 g/L HMF was also recovered

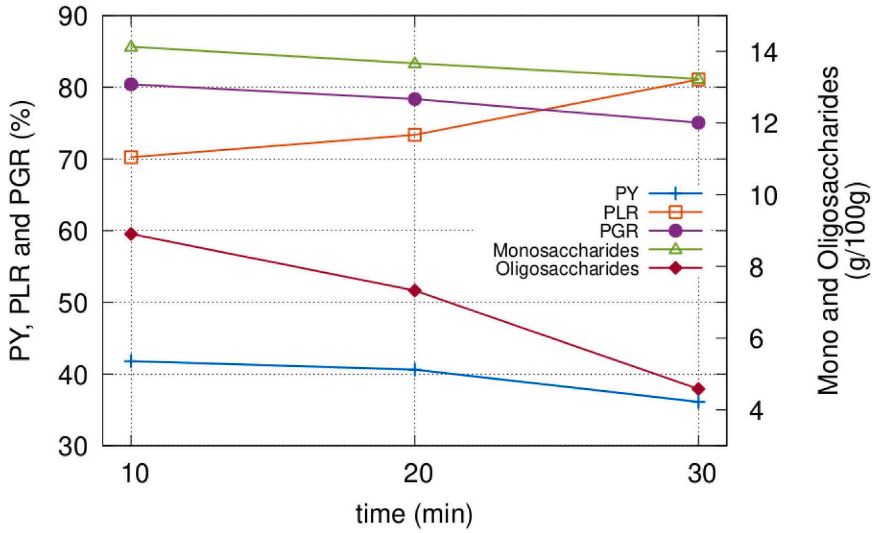

Fig. 3. Time courses of pulp yield (PY), percentage of lignin removal (PLR) and percentage of glucan recovery (PGR) in pretreated solids, and total oligosaccharide and total monosaccharide contents in aqueous fractions in experiments 16, 17, and 18 (carried out at 30, 20, and 10 min, respectively).

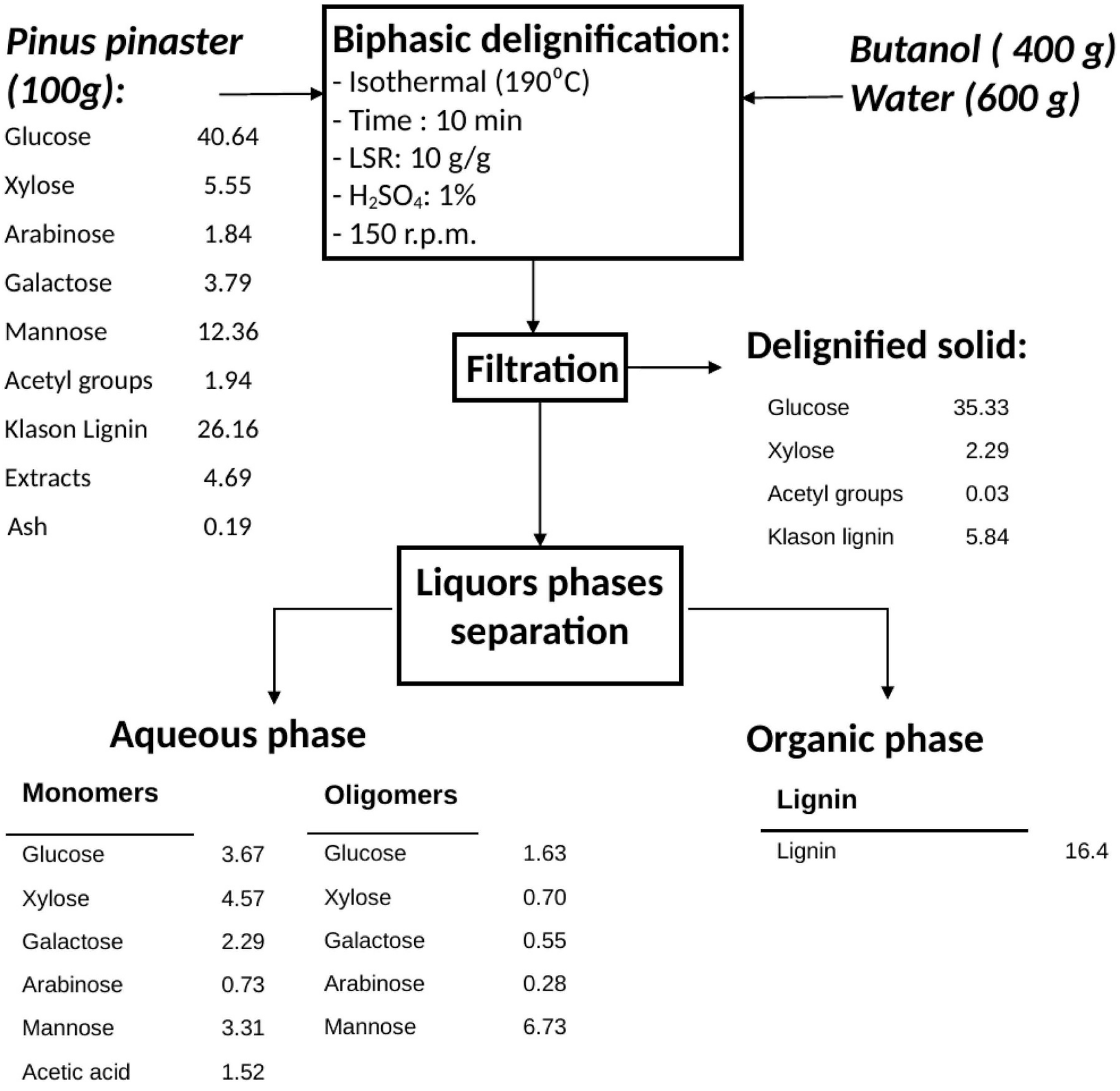

Fig. 4. Diagram of the process and the material balance under the selected operational conditions.

(Del Rio et al., 2010).

Concerning previous ethanol organosolv studies carried out with several species of pine, Pan et al. (2007) in the center-point of the design proposed for the fractionation of *P. contorta* (170 ºC, 65% ethanol, 60 min, and 1.1% H_2SO_4 w/w oven-dried wood), obtained a PY of 41%, a PLR of 93%, and a PGR of 75%. The delignification results are higher than those obtained in this study, but the treatment required much longer reaction time, higher organic solvent concentration, and catalyst loading. However, the PGR reached in this study was higher. The chemical analysis of the water soluble fraction revealed significant percentages of saccharides recovery (galactose, 78%; xylose, 45%; mannose, 41% and arabinose, 55%), mainly in oligomeric form, as well as a significant amount of levulinic acid and minor quantities of HMF, furfural, and formic acid (Pan et al., 2007). In this work, the recoveries of galactose, xylose, mannose, and arabinose were 74.9%, 94.9%, 81%, and 54.8%, respectively, whereas smaller contents of furfural, HMF, and levulinic acid were also found. Santos et al. (2019) studied the catalyzed organosolv of *P. radiata* wood at much longer reaction times (185 ºC, 75 min, 1% H_2SO_4 w/w, and ethanol concentration of 50%), resulting in a treated solid with an 89.9% of cellulose and a PY of 28.9%. However, it should be highlighted the poor recovery of lignin from the black liquors (only 8.1 g per 100 g of wood), which contained 39.01 g/L of sugars and 16.38 g/L of sugar degradation products. In the same study, the isothermal autohydrolysis (150 ºC and 30 min) followed by organosolv ($T = 185$ ºC, 50% of ethanol, 1% of H_2SO_4 and, $t = 75$ min) were also assayed, without important improvements despite the prolonged treatment. This alternative led to the complete solubilization of hemicelluloses but without oligomers production (Santos et al., 2019).

Moreover, it can be stated that there is additional scientific evidence showing better outcomes for biphasic treatments with butanol/water than for monophasic water/ethanol media, even with several softwood species. For instance, the biphasic treatment (with 95% butanol/ 0.2 M aqueous HCl, at reflux for 6 h) of beech and Douglas fir woods improved considerably the ethanosolv delignification yields (Lancefield et al., 2017). Similar findings were observed by Kawamata et al. (2021) during the pretreatment of Japanese cedar and willow woods at 200 ºC for 2 h, using a water/1-butanol molar ratio of 4. The authors reported the softwood lignin repolymerization in water-ethanol monophasic treatments, contrary to what happened in water-butanol biphasic media, which promoted the lignin depolymerization in both softwood and hardwood.

Although the lower delignification degrees reported for softwoods, the promising results obtained in this study for the one-pot single-step treatment of *P. pinaster* wood in biphasic media, demonstrate the feasibility of this technology and its potential application to softwoods, which contributes to open up new market prospects for these raw materials and for the biobutanol. Moreover, the encouraging results obtained with other softwoods, with respect to monophasic water/ethanol organosolv, support the hypothesis that more research is needed on biphasic solvents area in order to optimize the process parameters, improve extraction yields, and thus design efficient strategies for these abundant feedstocks.

3.5. Lignin recovery and characterization

The lignin of the organic phase obtained under the selected conditions was collected by sequential precipitation and filtration steps. In this way, an 80.7% of the solubilized lignin was isolated from the butanol-enriched phase, representing 62.7% of the raw material lignin.

Concerning the lignin purity, the results of the acid hydrolysis revealed the following chemical composition (g /100 g lignin o.d.b.): Klason lignin, 92.4 ± 1.0%; sugars content, 3.78 ± 0.4%; and acid-soluble lignin, 1.3 ± 0.1%. Moreover, the presence of ash was less than 1 ± 0.1%. The purity of the lignin was also confirmed by the 2D NMR assay, where an intense signal for the methoxyl groups and signals corresponding to the G-units are depicted in the aromatic region of the NMR spectrum (data not shown). The purity degree is in range with those achieved by Mendes et al. (2021) in the alkaline organosolv lignin isolated from *P. pinaster* and by Parot et al. (2022) in catalyzed organosolv lignin obtained from spruce woods.

In the biomass biorefinery scope, lignin upgrading is a crucial issue to improve the global process economy (Lancefield et al., 2017). In this context, the high-purity lignin obtained in this study would be suitable for high-value applications, including bio-based polymers and chemicals, among others (Santos et al., 2022, 2019).

The FTIR spectrum (Fig. 5a) showed the typical pattern of a softwood lignin with a predominance of guaiacyl groups (G-groups) (Schmetz et al., 2020). A broad band corresponding to phenolic and aliphatic hydroxyls is shown around 3404 cm^{-1}. Bands corresponding to methoxyl and methylene groups in aliphatic chains are shown at 2933 and 2871 cm^{-1}, respectively. In the fingerprint region, no significant peaks corresponding to ester groups were observed (1702 and 1667 cm^{-1}), whereas peaks corresponding to the aromatic compounds, typical of a lignin with predominantly G-groups, were observed at 1596, 1510, and 1459 cm^{-1}, highlighting the higher intensity of the 1510 peak. The dominant peak of this region was found at 1265 cm^{-1}, which corresponds to G-groups and the C=O stretch. Moreover, a peak associated to an in-plane aromatic C-H deformation typical of G units was observed at 1140 cm^{-1} . On the other hand, the bands corresponding to S units (1327 cm^{-1}) were very small and the separation of the peaks at 854 and 819 cm^{-1}, associated to C-H out of plane of G units, can be clearly observed.

The values obtained for the weight-average molecular weight (Mw) and number-average molecular weight (Mn) were 11270 and 1457 g/mol, respectively, thus resulting in a calculated polydispersity index (Ð=Mw/Mn) of 7.74. These values are higher than those found in previous literature (Khan et al., 2022; Mendes et al., 2021; Parot et al., 2022).

The analysis of TGA and dTGA data (see Fig. 5b) showed that the total mass loss was less than 5% up to 200 ºC, therefore the lignin decomposition takes place in several stages at higher temperatures. In a first stage (up to 300 °C) an 11.7% of the lignin mass was lost, showing the dTGA curve a peak at 224 °C. Between 300 and 450 °C the mass loss was 27.2% with a peak on the dTGA curve at 352 °C. However, the main degradation took place from 450 °C, with a mass loss of 56.8% between 450 and 600 °C, and a relevant peak at 470 °C. Main lignin degradation occurred at higher temperatures than those reported in literature for this raw material (Khan et al., 2022; Mendes et al., 2021) and for commercial softwood-Kraft lignin (Amit et al., 2021). At 600 °C and above, less than 2% of the char remains.

3.6. Enzymatic hydrolysis of the treated solids

To evaluate the fermentation potential of the treated solids, some preliminary enzymatic hydrolysis assays were carried out, using two enzyme/substrate ratios (10 and 20 FPU/g of solid) at fixed LSR of 20 g/g. In the experiment performed with 20 FPU/g, a high glucan to glucose conversion (94.7%) was reached after 48 h of saccharification, resulting in hydrolysates containing 40.49 g/L of glucose.

Similar saccharification yields were found by Madadi et al. (2022) for the aspen wood treated with butanol (94%). The results compare well with those obtained in previous studies focused on the saccharification of organosolv spent solids derived from other feedstocks. For instance, Santos et al. (2019) published a yield of 63% using *P. radiata* treated under two steps treatments. Teramura et al. (2016) achieved a conversion of 84% with sorghum bagasse and Liu et al. (2019) an 80% with bamboo treated with pentanol.

4. Conclusions

The selective fractionation of *P. pinaster* in a biphasic butanol/water media acidified with sulfuric acid was successfully addressed. The

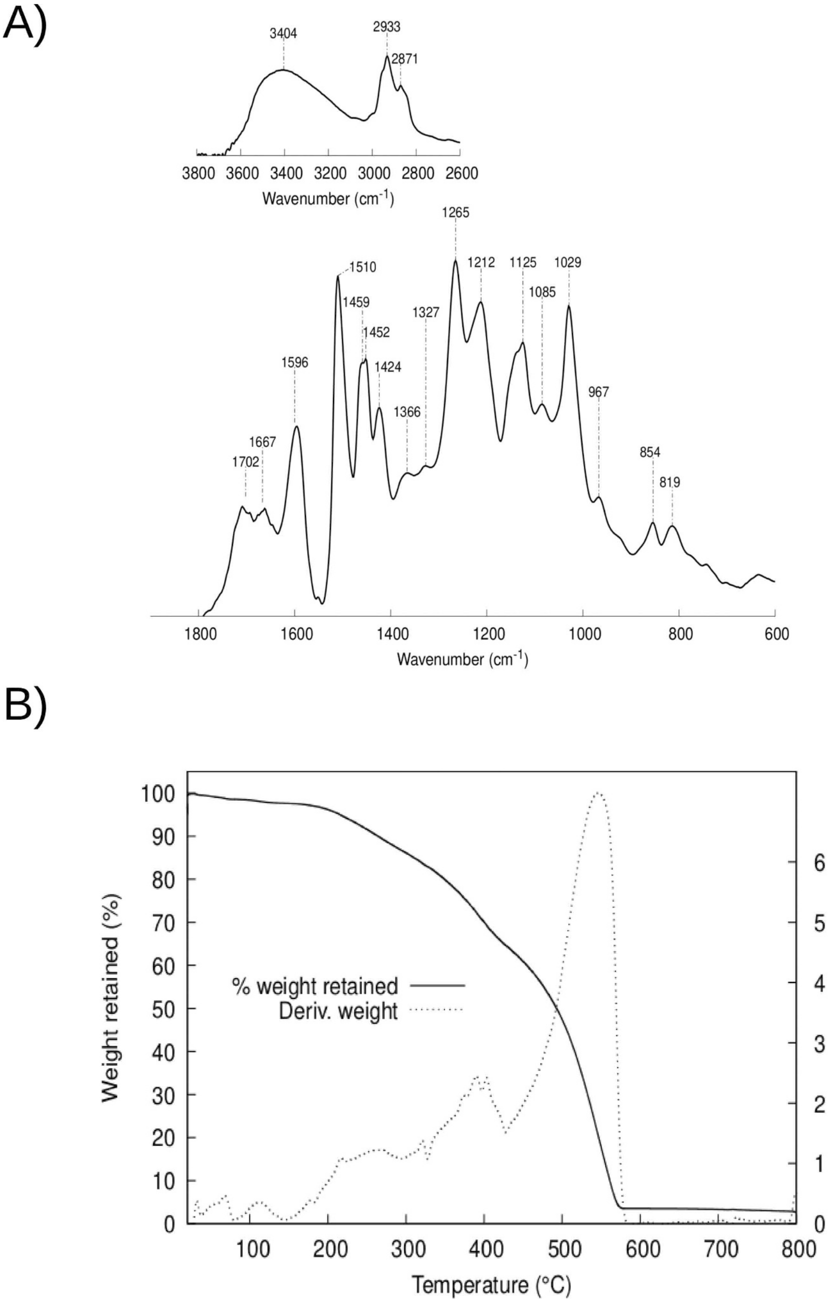

Fig. 5. FTIR (A) and TGA (B) of the lignin obtained from experiment 18.

approach proposed allowed the recovery in the treated solid of almost 87% of the raw material glucan, as well as an 62.7% of the initial lignin from the resulting organic phase, with a purity of 92.4%. Moreover, an aqueous phase containing 15.8 g/L of oligosaccharides and 24.54 g/L of monosaccharides was simultaneously obtained. Therefore, this study upgrades the three main fractions of pine wood by a one-single stage processing, yielding glucan-rich solids with high enzymatic digestibility (saccharification yield, 94.7%), oligosaccharides mixtures with prebiotic potential, and a phenolic biopolymer with multiple high value applications, thus contributing to the development of emerging softwood biorefinery processes.

CRediT authorship contribution statement

José Luis Alonso: Supervision, Writing – review & editing, Project administration, Funding acquisition. **Carlos Vila:** Investigation, Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Remedios Yáñez:** Conceptualization, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgements

The authors are grateful for the financial support of MCIN/ AEI /10.13039/501100011033 (Project PID2020-116717RB-I00) and Xunta de Galicia (Project GRC-ED431C 2022/08). We also express our gratitude to C.A.C.T.I. for carrying out NMR, FTIR and TGA assays.

References

Adney, B., Baker, J., 2008. Measurement of cellulase activities laboratory. Tech. Rep. NREL/TP 510–42628.

Alonso-Esteban, J.I., Carocho, M., Barros, D., Velho, M.V., Heleno, S., Barros, L., 2022. Chemical composition and industrial applications of Maritime pine (Pinus pinaster Ait.) bark and other non-wood parts. Rev. Environ. Sci. Biotechnol. 21, 583–633. https://doi.org/10.1007/s11157-022-09624-1.

Amit, T.A., Roy, R., Raynie, D.E., 2021. Thermal and structural characterization of two commercially available technical lignins for potential depolymerization via hydrothermal liquefaction. Curr. Res. Green. Sustain. Chem. 4. https://doi.org/10.1016/j.crgsc.2021.100106.

Brienza, F., Van Aelst, K., Devred, F., Magnin, D., Tschulkow, M., Nimmegeers, P., Van Passel, S., Sels, B.F., Gerin, P., Debecker, D.P., Cybulska, I., 2022. Unleashing lignin potential through the dithionite-assisted organosolv fractionation of lignocellulosic biomass. Chem. Eng. J. 450, 138179 https://doi.org/10.1016/j.cej.2022.138179.

Cheng, F., Sun, J., Wang, Z., Zhao, X., Hu, Y., 2019. Organosolv fractionation and simultaneous conversion of lignocellulosic biomass in aqueous 1, 4-butanediol/ acidic ionic-liquids solution. Ind. Crops Prod. 138, 111573 https://doi.org/10.1016/j.indcrop.2019.111573.

Culaba, A.B., Mayol, A.P., San Juan, J.L.G., Vinoya, C.L., Concepcion, R.S., Bandala, A. A., Vicerra, R.R.P., Ubando, A.T., Chen, W.-H., Chang, J.-S., 2022. Smart sustainable biorefineries for lignocellulosic biomass. Bioresour. Technol. 344, 126215 https://doi.org/10.1016/j.biortech.2021.126215.

Cybulska, I., Brudecki, G.P., Zembrzuska, J., Schmidt, J.E., Lopez, C.G.-B., Thomsen, M. H., 2017. Organosolv delignification of agricultural residues (date palm fronds, Phoenix dactylifera L.) of the United Arab Emirates. Appl. Energy 185, 1040–1050. https://doi.org/10.1016/j.apenergy.2016.01.094.

del Rio, L.F., Chandra, R.P., Saddler, J.N., 2010. The effect of varying organosolv pretreatment chemicals on the physicochemical properties and cellulolytic hydrolysis of mountain pine beetle-killed lodgepole pine. Appl. Biochem. Biotechnol. 161, 1–21. https://doi.org/10.1007/s12010-009-8786-6.

del Río, P.G., Gullón, B., Romaní, A., 2022. Current breakthroughs in the hardwood biorefineries: hydrothermal processing for the co-production of xylooligosaccharides and bioethanol. Bioresour. Technol. 343. https://doi.org/10.1016/j.biortech.2021.126100.

Guo, K.-N., Zhang, C., Xu, L.-H., Sun, S.-C., Wen, J.-L., Yuan, T.-Q., 2022. Efficient fractionation of bamboo residue by autohydrolysis and deep eutectic solvents pretreatment. Bioresour. Technol. 354. https://doi.org/10.1016/j.biortech.2022.127225.

Haldar, D., Dey, P., Thomas, J., Singhania, R.R., Patel, A.K., 2022. One pot bioprocessing in lignocellulosic biorefinery: a review. Bioresour. Technol. 365. https://doi.org/10.1016/j.biortech.2022.128180.

Isikgor, F.H., Becer, C.R., 2015. Lignocellulosic biomass: a sustainable platform for the production of bio-based chemicals and polymers. Polym. Chem. 6, 4497–4559. https://doi.org/10.1039/c5py00263j.

Kawamata, Y., Yoshikawa, T., Koyama, Y., Ishimaru, H., Ohtsuki, S., Fumoto, E., Sato, S., Nakasaka, Y., Masuda, T., 2021. Uniqueness of biphasic organosolv treatment of soft-and hardwood using water/1-butanol co-solvent. Ind. Crops Prod. 159, 113078 https://doi.org/10.1016/j.indcrop.2020.113078.

Khan, S., Rauber, D., Shanmugam, S., Kay, C.W.M., Konist, A., Kikas, T., 2022. Efficient lignin fractionation from scots pine (pinus sylvestris) using ammonium-based protic ionic liquid: process optimization and characterization of recovered lignin. Polymers 14. https://doi.org/10.3390/polym14214637.

Lancefield, C.S., Panovic, I., Deuss, P.J., Barta, K., Westwood, N.J., 2017. Pre-treatment of lignocellulosic feedstocks using biorenewable alcohols: towards complete biomass valorisation. Green. Chem. 19, 202–214. https://doi.org/10.1039/C6GC02739C.

Liu, J., Hu, H., Gong, Z., Yang, G., Li, R., Chen, L., Huang, L., Luo, X., 2019. Near-complete removal of non-cellulosic components from bamboo by 1-pentanol induced organosolv pretreatment under mild conditions for robust cellulose enzymatic hydrolysis. Cellulose 26, 3801–3814. https://doi.org/10.1007/s10570-019-02334-y.

López, M., Santos, V., del Río, J.C., Rencoret, J., Parajó, J.C., 2020. One-pot processing of eucalyptus globulus wood under microwave heating: simultaneous delignification and polysaccharide conversion into platform chemicals. ACS Sustain. Chem. Eng. 27, 10115–10124. https://doi.org/10.1021/acssuschemeng.0c02023.

Madadi, M., Zahoor, Song, G., Karimi, K., Zhu, D., Elsayed, M., Sun, F., Abomohra, A., 2022. One-step lignocellulose fractionation using acid/pentanol pretreatment for enhanced fermentable sugar and reactive lignin production with efficient pentanol retrievability. Bioresour. Technol. 359, 127503 https://doi.org/10.1016/j.biortech.2022.127503.

Melati, R.B., Shimizu, F.L., Oliveira, G., Pagnocca, F.C., de Souza, W., Sant'Anna, C., Brienzo, M., 2019. Key factors affecting the recalcitrance and conversion process of biomass. BioEnergy Res 12, 1–20. https://doi.org/10.1007/s12155-018-9941-0.

Mendes, C.V.T., Moreira, R., Portugal, A., Graça, V.S., Carvalho, M., 2021. Biorefining of pinus pinaster stump wood for ethanol production and lignin recovery. Chem. Eng. Technol. 44, 1043–1050. https://doi.org/10.1002/ceat.202000447.

MITECO (Spanish Ministry for the Ecological Transition). Anuario de estadística forestal 2020. ⟨https://www.miteco.gob.es/es/biodiversidad/estadisticas/forestal_anuario_2020.html⟩.

Moreira, R., Mendes, C.V.T., Banaco, M.B.F., Carvalho, M.G.V.S., Portugal, A., 2020. New insights in the fractionation of Pinus pinaster wood: sequential autohydrolysis, soda ethanol organosolv and acidic precipitation. Ind. Crops Prod. 152. https://doi.org/10.1016/j.indcrop.2020.112499.

Nitsos, C., Rova, U., Christakopoulos, P., 2018. Organosolv fractionation of softwood biomass for biofuel and biorefinery applications. Energies 11, 50. https://doi.org/10.3390/en11010050.

Novo, L.P., Gurgel, L.V.A., Marabezi, K., Curvelo, A.A., da, S., 2011. Delignification of sugarcane bagasse using glycerol–water mixtures to produce pulps for saccharification. Bioresour. Technol. 102, 10040–10046. https://doi.org/10.1016/j.biortech.2011.08.050.

Pan, X., Gilkes, N., Kadla, J., Pye, K., Saka, S., Gregg, D., Ehara, K., Xie, D., Lam, D., Saddler, J., 2006. Bioconversion of hybrid poplar to ethanol and co-products using an organosolv fractionation process: optimization of process yields. Biotechnol. Bioeng. 94, 851–861. https://doi.org/10.1002/bit.20905.

Pan, X., Xie, D., Yu, R.W., Lam, D., Saddler, J.N., 2007. Pretreatment of lodgepole pine killed by mountain pine beetle using the ethanol organosolv process: fractionation and process optimization. Ind. \ Eng. Chem. Res 46, 2609–2617. https://doi.org/10.1021/ie061576l.

Panovic, I., Lancefield, C.S., Phillips, D., Gronnow, M., Westwood, N.J., 2018. Selective primary oxidation of lignin streams from butanol-pretreated agricultural waste biomass. ChemSusChem. https://doi.org/10.1002/cssc.201801971.

Parot, M., Rodrigue, D., Stevanovic, T., 2022. High purity softwood lignin obtained by an eco-friendly organosolv process. Bioresour. Technol. Rep. 17. https://doi.org/10.1016/j.biteb.2021.100880.

Penín, L., Peleteiro, S., Rivas, S., Santos, V., Parajó, J.C., 2019. Production of 5-hydroxymethylfurfural from pine wood via biorefinery technologies based on fractionation and reaction in ionic liquids. BioResources 14, 4733–4747. https://doi.org/10.15376/biores.14.2.4733-4747.

Penín, L., Santos, V., Parajó, J.C., 2020. Delignification of autohydrolyzed wood in media containing water and a protic ionic liquid. J. Wood Chem. Technol. 40, 235–247. https://doi.org/10.1080/02773813.2020.1746808.

Penín, L., López-Hortas, L., Vila, C., Santos, V., Parajó, J.C., 2022. Fractionation of Eucalyptus regnans wood: properties of the soluble products and reactivity of the treated solids. J. Wood Chem. Technol. 42, 46–57. https://doi.org/10.1080/02773813.2021.2015387.

Portela-Grandío, A., Peleteiro, S., Yáñez, R., Romaní, A., 2021. Integral valorization of Acacia dealbata wood in organic medium catalyzed by an acidic ionic liquid. Bioresour. Technol. 342, 126013 https://doi.org/10.1016/j.biortech.2021.126013.

Renders, T., Cooreman, E., Van den Bosch, S., Schutyser, W., Koelewijn, S.-F., Vangeel, T., Deneyer, A., Van den Bossche, G., Courtin, C.M., Sels, B.F., 2018. Catalytic lignocellulose biorefining in n-butanol/water: a one-pot approach toward phenolics, polyols, and cellulose. Green. Chem. 20, 4607–4619. https://doi.org/10.1039/C8GC01031E.

Riaz, S., Mazhar, S., Abidi, S.H., Syed, Q., Abbas, N., Saleem, Y., Nadeem, A.A., Maryam, M., Essa, R., Ashfaq, S., 2022. Biobutanol production from sustainable biomass process of anaerobic ABE fermentation for industrial applications. Arch. Microbiol. 204, 672. https://doi.org/10.1007/s00203-022-03284-z.

Rivas, S., López, L., Vila, C., Parajó, J.C., 2021. Organosolv processing of vine shoots: fractionation and conversion of hemicellulosic sugars into platform chemicals by microwave irradiation. Bioresour. Technol. 342, 125967 https://doi.org/10.1016/j.biortech.2021.125967.

Romaní, A., Larramendi, A., Yáñez, R., Cancela, Á., Sánchez, Á., Teixeira, J.A., Domingues, L., 2019. Valorization of Eucalyptus nitens bark by organosolv pretreatment for the production of advanced biofuels. Ind. Crops Prod. 132, 327–335. https://doi.org/10.1016/j.indcrop.2019.02.040.

Santos, T.M., Rigual, V., Oliet, M., Alonso, M.V., Domínguez, J.C., Rodriguez, F., 2019. Two-step fractionation of Pinus radiata by autohydrolysis and organosolv delignification for enzymatic hydrolysis. J. Chem. Technol. Biotechnol. 94, 3951–3959. https://doi.org/10.1002/jctb.6197.

Santos, T.M., Rigual, V., Domínguez, J.C., Alonso, M.V., Oliet, M., Rodriguez, F., 2022. Fractionation of Pinus radiata by ethanol-based organosolv process. Biomass-.-. Convers. Biorefinery. https://doi.org/10.1007/s13399-022-02329-z.

Schmetz, Q., Teramura, H., Morita, K., Oshima, T., Richel, A., Ogino, C., Kondo, A., 2019. Versatility of a dilute acid/butanol pretreatment investigated on various lignocellulosic biomasses to produce lignin, monosaccharides and cellulose in distinct phases. ACS Sustain. Chem. Eng. 7, 11069–11079. https://doi.org/10.1021/acssuschemeng.8b05841.

Schmetz, Q., Muzyka, C., Berchem, T., Richel, A., 2020. Monitoring technical lignin partition in aqueous/alcohol biphasic systems according to pH: influences of the molecular structure and solvent characteristics. Green. Chem. 22 (20), 7031–7046. https://doi.org/10.1039/d0gc01905d.

Sun, F., Chen, H., 2007. Evaluation of enzymatic hydrolysis of wheat straw pretreated by atmospheric glycerol autocatalysis. J. Chem. Technol. \ Biotechnol. 82, 1039–1044. https://doi.org/10.1002/jctb.1764.

Teramura, H., Sasaki, K., Oshima, T., Matsuda, F., Okamoto, M., Shirai, T., Kawaguchi, H., Ogino, C., Hirano, K., Sazuka, T., Kitano, H., Kikuchi, J., Kondo, A., 2016. Organosolv pretreatment of sorghum bagasse using a low concentration of hydrophobic solvents such as 1-butanol or 1-pentanol. Biotechnol. Biofuels 9, 1–11. https://doi.org/10.1186/s13068-016-0427-z.

Teramura, H., Sasaki, K., Oshima, T., Kawaguchi, H., Ogino, C., Sazuka, T., Kondo, A., 2018. Effective usage of sorghum bagasse: optimization of organosolv pretreatment using 25\% 1-butanol and subsequent nanofiltration membrane separation. Bioresour. Technol. 252, 157–164. https://doi.org/10.1016/j.biortech.2017.12.100.

Wang, J., Rajan, K., Annamraju, A., Chmely, S.C., Pingali, S.V., Carrier, D.J., Labbé, N., 2021. A sequential autohydrolysis-ionic liquid fractionation process for high quality lignin production. Energy Fuels 35, 2293–2302. https://doi.org/10.1021/acs.energyfuels.0c03849.

Zimmermann, C.J., Bollar, N.V., Wettstein, S.G., 2018. Liquid phase conversion of lignocellulosic biomass using biphasic systems. Biomass-.-. Bioenergy 118, 163–171. https://doi.org/10.1016/j.biombioe.2018.08.009.