Fernández-Bautista *et al.*
Journal of Wood Science (2024) 70:15
https://doi.org/10.1186/s10086-024-02128-2

Journal of Wood Science

ORIGINAL ARTICLE

Open Access

One-pot *Eucalyptus globulus* wood fractionation in acid-catalyzed mixtures of water and 1-pentanol: an optimization study

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Abstract

Samples of *Eucalyptus globulus* wood were processed in a reaction media made up of 1-pentanol, water, and diluted acid catalyst. The effects of selected variables (proportion of 1-pentanol in the reaction media, catalyst charge and temperature) on wood solubilization and on the composition of the various phases from treatments (solids containing cellulose, pentanol-rich phase containing depolymerized lignin, and the aqueous phase concentrating the hemicellulose-derived products) were assessed by Response Surface Methodology. Numerical optimization based on the statistical models allowed the identification of the best operational conditions, under which extensive removal of both lignin and hemicelluloses was reached at good selectivity toward cellulose solubilization. The treated solids achieved near stoichiometric conversion upon enzymatic hydrolysis performed under diverse operational conditions, and the lignin structural analysis provided data typical for hardwoods. The experimental results confirmed the potential of 1-pentanol as a solvent for *Eucalyptus globulus* wood-based biorefineries.

Keywords Wood, *Eucalyptus globulus*, One-pot fractionation, Pentanol, Biphasic media

Introduction

The economic growth based on an intense utilization of dwindling fossil resources presents important issues related to availability, price volatility, security of supplies, and environmental problems (namely the rapid increase in greenhouse gas emissions) [1]. Over the past few decades, a great effort has been conducted to switch the current economy into a sustainable, circular bioeconomy based on renewable resources [2, 3]. Lignocellulosic biomass (LB) is a promising renewable source for energy and petroleum-based drop-in chemicals [4], including biochemicals, platform chemicals, and biopolymers [5, 6].

LB is produced at *ca.* 10 metric tons per hectare per year [7], making it an abundant and widespread resource [8] that shows potential to solve the problems posed by the limited availability of fossil resources and global warming [9]. LB does not compete with food applications [10], and has been considered as an abundant, sustainable, and economically profitable type of biomass [11].

The diverse types of LB (for example, from woods, agriculture, or industry) are mainly made up of polysaccharides (cellulose and hemicelluloses) and lignin. Cellulose is a linear polymer made up of D-anhydroglucose units; whereas, hemicelluloses are branched heteropolysaccharides that may be composed of a variety of anhydrosugars (including anhydropentoses such as anhydroxylose and anhydroarabinose, and anhydrohexoses such as anhydroglucose, anhydromannose, and anhydrogalactose), which can be substituted (for example, with acetyl or

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uronyl groups). In most lignocellulosic materials (including hardwoods), anhydropentoses are the predominant hemicellulose structural units [3]. In contrast, lignin is a phenolic polymer made up of methoxylated *p*-hydroxyphenyl propanoid units (*p*-hydroxyphenyl, guaiacyl and syringyl).

These polymers are intertwined in a heterogeneous, tridimensional matrix, joined by hydrogen bonds, ester–ether bridges, and intermolecular interactions, that constitute a substrate recalcitrant to chemical modification [12].

In comparison with other LB types, woods show advantages as raw materials for the industry, such as high polysaccharide content, round-year availability, and ability to grow in lands not suitable for food crops. *Eucalyptus* is one of the most widely planted type of hardwoods [13]. *E. globulus* is one of the preferred species, because of its productivity, adaptability, limited requirements of water and nutrients, and current industrial utilization [13]. Moreover, the *E. globulus* hemicelluloses are largely dominated by a single polymer (acetylated heteroxylan), a feature facilitating its integral valorization [13].

Among the diverse methods employed to extract value from LB, the separation of the structural components ("fractionation") into individual streams is attracting increased interest. This goal can be achieved in lignocellulose biorefineries, which are based on integrated technologies for fractionation and sequential conversion [14, 15], and are expected to play a central role in the transition of the chemical industry towards a bio-based circular economy [3], providing an alternative for the sustainable manufacture of biochemicals and biofuels [14].

LB fractionation entails the disruption of the polymeric matrix by physicochemical processing [12]. Besides being a major contributor to the operational costs, the fractionation stage determines the reaction products. The usual fractionation methods cause the solubilization of lignin and at least a part of hemicelluloses, whereas the cellulose remains in solid phase with some structural alteration (particularly, in crystallinity) [14].

Among the diverse fractionation methods, the organosolv technologies (based on reactions performed in reaction media composed of water and an organic solvent, frequently in the presence of a catalyst) have been considered as a promising alternative for LB processing [2, 4, 16–18]. In organosolv media, the fractionation goes through lignin depolymerization by breakage of the α-*O*-aryl and β-*O*-aryl bonds in lignin [17, 19].

The literature reported on organosolv fractionation deals with a wide variety of solvents. The solvent choice represents a tool for a tailored transformation of the considered feedstock into defined target products. The organic solvents used in LB fractionation include aliphatic alcohols (methanol, ethanol, butanol, pentanol), polyhydric alcohols (glycerol, ethylene glycol, propylene glycol), acids (formic, acetic and oxalic acid acids), ketones, ethers, dichloromethane, gamma-valerolactone, tetrahydrofuran and 2-methyltetrahydrofuran [10, 12, 15, 17, 19, 20].

In general, the organosolv methods provide an efficient separation of high-purity cellulose with little solubilization [10, 17], facilitate the recovery of components, and may reach high delignification rate [17]. Interestingly, the soluble lignin fragments obtained are sulfur-free and show few structural changes [18], facilitating their use as intermediates for the manufacture of aromatics and fine chemicals [12].

The utilization of catalysts in organosolv fractionation enhances the rate and extent of hemicellulose and lignin solubilization, allows operation under mild conditions, and may result in improved selectivity [12, 17]. Particularly, acidic catalysts facilitate the cleavage of *β*-aryl ether linkages, enabling lignin breakdown into smaller fragments [14, 17].

Most organosolvents employed for LB fractionation are water-soluble, and lead to a cellulose-enriched solid phase and a liquid phase containing products derived from both hemicelluloses and lignin. A complete fractionation requires the further separation of hemicellulose-derived products from the lignin-derived ones, a goal that is generally achieved by adding a co-solvent (typically, water) able to cause the precipitation of hydrophobic compounds [10, 14, 15, 18]. Since lignin valorization is essential to develop viable strategies for LB utilization [8], alternative processing schemes enabling an efficient lignin separation are receiving increased attention. In this context, the utilization of biphasic media, obtained by mixing a partially miscible solvent with water (usually in the presence of a catalyst), are receiving special attention. With this approach, a complete LB fractionation can be reached in a single step: cellulose remains in the processed solid, lignin-derived products are concentrated in the organic solvent, and hemicellulose-derived products in the aqueous one [2, 14, 15, 18, 21]. Depending on the operational conditions, the water-soluble, hemicellulose-derived products may be dominated by higher saccharides, monosaccharides or monosaccharide-dehydration products, which appear together with the acetic acid generated from acetyl groups [5, 21, 22].

Typically, biphasic media for LB fractionation are obtained by mixing acidic water with solvents such as 2-methyltetrahydrofuran [6, 15], 2-phenoxyethanol [6] or 1-butanol [2, 9, 22–25]. In comparison, little attention has been paid to LB fractionation in media containing 1-pentanol, an innovative and promising technology [15]. Considered as a solvent for fractionation, 1-pentanol shows favorable features related to its low water solubility (1.7–2%, that makes the solvent recovery easier), and to a normal boiling temperature (137 °C) which allows an easy separation of the solvent by distillation. The biphasic nature of the liquor from fractionation also facilitates the solvent recovery, and limits the energy demand. On the other hand, the lignin fragments soluble in the 1-pentanol-rich phase present little contamination by polysaccharides, a high phenolic hydroxyl content, and a limited structural alteration (preserving a significant part of the β-*O*-4 linkages responsible for reactivity). These are features favorable for the further production of lignin-based biomaterials [14, 15, 26]. Moreover, delignification is fast, selective and extensive, and the lignin solubility is facilitated by the decreased value of the Hildebrand solubility parameter respect to other alcohols with shorter chains [27]. Additionally, mixtures of 1-pentanol and dilute acidic solutions may cleave the inter-unit ester and ether linkages of hemicellulose and lignin [14], resulting in polysaccharide hydrolysis simultaneous to lignin solubilization.

This study deals with the fractionation of *Eucalyptus globulus* wood in media containing 1-pentanol, water, and sulfuric acid (acting as a catalyst). Treatments were carried out under diverse operational conditions to obtain data suitable for a quantitative assessment on the *Eucalyptus* wood fractionation, including the amount of the dissolved mass (measured in terms of solid recovery) and composition of phases resulting from fractionation. The effects of treatments were interpreted by means of a Response Surface Methodology analysis, which allowed a generalized assessment of the amount and selectivity of the component separation. For this purpose, the cellulose solubilization was measured in terms of the mass of cellulose lost from the solid phase upon processing. A numerical optimization was performed using an objective function measuring the overall separation of phases and the recovery of valuable products. As additional aspects, the treated solids were employed as substrates for enzymatic hydrolysis, and the major structural features of lignin were identified.

Materials and methods

Raw material

Eucalyptus globulus wood chips were kindly provided by a pulp producer (ENCE, Pontevedra, Spain), and milled in a Wiley instrument fitted with a 1-mm screen. The grounded material was air dried, homogenized and stored.

Fractionation

Milled wood samples were mixed with the desired amounts of 1-pentanol, water, and sulfuric acid, and processed in a stirred Parr Instrument reactor (Moline, IL, USA). The operational conditions employed in treatments are listed below (Table 1). The solid phase was recovered by filtration, and the liquid phase was allowed to separate into aqueous and organic layers using a separation funnel. The treated solids were washed with 1-pentanol and hot water, air dried and weighted to measure the solid yield.

Analysis of wood, processed solids and aqueous phases

Samples from the wood lot and processed solids, and aqueous phases were analyzed for composition on basis to the methodology reported by Rivas et al. [21, 24].

Response Surface Methodology assessment

The Response Surface Methodology was based on an incomplete, factorial, centered, second-order experimental design with minimal dead volume, involving 3 operational variables measured at 3 levels. The experimental plan included 15 experiments, all of which were performed for the same isothermal reaction time (30 min) in media containing the same proportion of dry wood to liquid phase (10 g liquid/g solid). The operational variables considered, their variation ranges, and the nomenclature were as follows:

- catalyst (H_2SO_4) charge (0.5–1.5 g/100 g wood), denoted CatCh,
- volume percent of 1-pentanol in the liquid phase (20–60), denoted %Pent,
- Isothermal reaction temperature (160–190 °C), denoted T.

The effects of treatments were assessed in terms of variables measuring:

- the solid yield, defined as:
  - mass of treated solid/100 g oven-dry wood, denoted y_1
- the composition of the treated solids, defined by:
  - cellulose content, measured as wt% in oven-dry basis, and denoted y_2

Table 1 Structure of the experimental plan, expressed in terms of dimensional and dimensionless operational variables

Exp	Dimensional independent variables			Dimensionless independent variables		
	CatCh (g/100 g dry wood)	%Pent (mL/100 mL)	*T* (°C)	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

  - hemicellulose content (including xylan and xylan substituents), measured as wt% in oven-dry basis, and denoted y_3
  - lignin content, measured as wt% in oven-dry basis, and denoted y_4

- the composition of target products in the aqueous phases, defined by:

  - concentration of xylooligosaccharides (XOS) of the aqueous phase (including constituent sugars and acetyl substituents), measured in g/L, and denoted y_5
  - Concentration of xylan-derived saccharides (including oligosaccharides and pentoses) in the aqueous phase, measured in g/L, and denoted y_6

For calculation purposes, new dimensionless, normalized, independent variables (x_1: dimensionless catalyst charge, x_2: dimensionless 1-pentanol volume percent; x_3: dimensionless reaction temperature) were defined. These dimensionless, independent variables varied in the range (− 1, 1), and were linearly related to the dimensional ones. Table 1 lists the operational conditions corresponding to the experiments of the experimental design, expressed as dimensional and dimensionless independent variables.

The interrelationships between dependent and dimensionless independent variables were established by a second order model with interaction terms:

$$y_j = b_{0j} + \Sigma_i b_{ij} x_i + \Sigma_i \Sigma_k b_{ikj} x_i x_k, \tag{1}$$

where y_j (j: 1–6) stand for the dependent variables, x_i and x_k (i or k: 1–3, $k \geq i$) represent the dimensionless independent variables, and $b_{0j} \ldots b_{ikj}$ are the regression coefficients, which were calculated from the experimental data using the least-squares method. The correlation of models has been measured by the coefficients of determination R^2, which indicates which part of the variation in a given dependent variable is predictable from the independent variables. Assuming homogeneity of the variance along the experimental domain, the Fischer's *F* test was employed to measure the goodness of data prediction from the various models (i.e., the statistical significance of the models). The significance of the contributions of defined model terms to the variation of the dependent variables was measured by a *t* test, which provides a given level of significance (usually, the significance is reported at the levels of 95 or 90%, corresponding to *p* values of 0.05 and 0.1, respectively, where *p* is the probability of the null hypothesis, which assumes that the considered term does not contribute significantly to the dependent variable).

Enzymatic hydrolysis

Samples of treated solids obtained under selected conditions were used as substrates for enzymatic hydrolysis experiments, which were carried at 50 °C in 50 mL Erlenmeyer flasks with orbital stirring (150 rpm) in media containing 0.05 M citrate buffer (pH = 4.80). Three types of cellulolytic enzymes (Celluclast 1.5 L, Cellic Ctec 2 and

Cellic Ctec 3), kindly provided by Novozymes (Spain), were used in experiments. As Celluclast is known to be in deficit of cellobiase activity, the media containing this enzymatic complex was supplemented with Novozyme 188 β-glucosidase, at a fixed ratio (β -glucosidase/cellulase activity ratio = 5 IU/FPU). The activities of the stock solutions were as follows: Cellic Ctec 2, 109 FPU/mL; Cellic Ctec 3, 224 FPU/mL; Celluclast 1.5 L, 64 FPU/mL; and Novozyme 188, 630 IU/mL. The considered operational variables were reaction time, cellulase to substrate ratio (CSR), and liquid to solid ratio (LSR). The experimental plan is detailed below. Sample aliquots were collected at the desired reaction times, kept in boiling water for 5 min to deactivate the enzymes, centrifuged, diluted, filtered and assayed by HPLC using the methodology indicated in the "Analysis of wood, processed solids and aqueous phases" section.

Table 2 Raw material composition

Component	Content, g/100 g o.d. raw material
Glucan	46.1 ± 0.08
Xylan	16.22 ± 0.07
Arabinan	0.31 ± 0.01
Acetyl groups	3.99 ± 0.11
Klason lignin	21.39 ± 0.25
Acid soluble lignin	1.38 ± 0.05
Ashes	0.23 ± 0.06
Extractives	2.92 ± 0.08
Other	7.46 ± 0.61

Lignin isolation and characterization

The solid resulting from vacuum-evaporation of the 1-pentanol-rich phase from the treatment performed under optimal conditions was redissolved in acetone, supplemented with acidic water, an allowed to precipitate overnight at 4 °C. The precipitate (lignin) was recovered by filtration, and dried in a vacuum oven at 40 °C for 48 h. For characterization, Fourier Transform Infrared Spectroscopy (FTIR) of lignin was performed as per Rivas et al. [24].

Results and discussion

Eucalyptus globulus wood composition

The composition of the wood lot used in experiments is summarized in Table 2. The data obtained for the various fractions are within the range reported for *Eucalyptus globulus* [13] which is known to present a high cellulose content and hemicelluloses constituted by heteroxylan [28]. It can be noted that the target fractions considered in this study (cellulose, xylan, arabinosyl units, acetyl substituents, and Klason lignin) accounted for 88% of the dry wood weight.

Experimental results obtained in fractionation experiments and model development

Table 3 shows the results determined for the diverse experimental variables in the experimental plan depicted in Table 1.

As explained above, the complete fractionation of the raw material is expected to result in a solid phase retaining as much cellulose as possible, in an aqueous

Table 3 Results obtained for the dependent variables y_1–y_6 in experiments 1–15 (see Table 1 for operational conditions)

Exp	y_1	y_2	y_3	y_4	y_5	y_6
1	58.70	72.20	3.09	20.48	2.14	18.50
2	41.36	82.17	1.47	15.31	0.06	14.57
3	49.43	86.98	2.33	9.67	5.12	28.35
4	40.87	86.87	1.08	8.90	0.00	16.50
5	67.69	64.79	5.55	18.51	8.61	22.16
6	35.19	91.27	0.43	8.89	0.00	22.44
7	49.66	83.74	1.58	12.26	0.17	14.94
8	36.26	87.65	0.26	10.03	0.58	4.68
9	61.88	66.49	4.62	17.64	0.05	18.79
10	54.61	81.22	4.19	14.18	1.26	30.59
11	42.87	79.42	1.62	14.70	0.00	7.81
12	39.25	94.96	1.63	8.21	0.93	5.90
13	48.95	88.78	1.47	9.98	0.07	21.43
14	50.74	89.86	1.92	10.70	0.44	20.44
15	48.84	87.99	1.42	9.53	0.52	20.74

y_1, solid yield; y_2, cellulose content of treated solids in wt%, y_3: hemicellulose content of treated solids in wt%; y_4: lignin content of treated solids in wt%; y_5, XOS concentration in g/L aqueous phase; y_6, concentration of xylan-derived saccharides, in g/L aqueous phase

phase containing high amounts of saccharides, and in an organic phase containing the products as much depolymerized as possible. Typically, achieving a high delignification degree entails an important hemicellulose conversion into humins (resulting from condensation of monosaccharide-dehydration products), and may cause significant cellulose losses. Looking at the compositional results in Table 2, it can be seen that the above conditions were better achieved when the solid yields (y_1) were in the vicinity of 40–50% (near the cellulose percentage in wood). With this idea in mind, the ranges considered for variables x_1, x_2 and x_3, which provided a wide range of solid yields, covered the conditions of practical interest.

The data in Table 3 indicated values of y_1 from 35.2 up to 67.7%, a range that includes the optimal data reported in literature (see below). Moreover, wide ranges were observed for the cellulose and lignin content of treated solids (y_2: 64.8–94.96%; y_4: 20.5–8.2%); whereas, hemicelluloses (including substituents) were extensively solubilized in all the treatments, decreasing from 20.52% in wood to y_3 in the range 0.26–5.55%. Regarding the hemicellulose-derived products, scarce generation of XOS took place, with volumetric concentrations (y_5) within the range 0–8.61 g/L aqueous phase; whereas, the concentrations of total saccharides (measured by variable y_6) were in the range 4.68–30.6 g/L aqueous phase.

In order to provide a quantitative assessment on the fractionation by means of a Response Surface Methodology analysis, the experimental data in Table 3 were fitted to Eq. 1. The set of regression coefficients, as well as the significance of the model terms, the significance of the equations and the R^2 coefficient measuring the regression are listed in Table 4. Since the models developed for the diverse dependent variables were statistically significant, they were employed in the further discussion.

Pulp yield, composition of treated solids and selectivity

Figure 1a illustrates the calculated dependence of the solid yield y_1 on the most influential variables (CatCh and T) for media containing 20, 40 or 60% 1-pentanol. As expected, y_1 decreased strongly with T in the three reaction media considered, whereas CatCh showed effects dependent on temperature: when CatCh increased, pronounced y_1 drops were predicted at low or moderate temperatures, but little variations were obtained at 190 °C. This fact was ascribed to the compositional changes undergone by the feedstock: under conditions of mild or intermediate severity, the solubilization of hemicelluloses and lignin took place progressively, resulting in decreased solid yields; but at 190 °C, the solid obtained at the lowest CatCh was mainly made up of cellulose, which was not substantially degraded by further increases in the catalyst charge On the other hand, the substrate solubilization was promoted in media of high 1-pentanol content, particularly at low and intermediate temperatures. This finding was justified by the hydrophobic character of 1-pentanol, which improved the ability of the reaction medium to dissolve lignin, facilitating the deconstruction of the lignocellulosic matrix, and so the hydrolysis of residual hemicelluloses [14]. Figure 1a shows that

Table 4 Regression coefficients and statistical parameters measuring the correlation and significance of models and model terms

Coefficient	y_1	y_2	y_3	y_4	y_5	y_6
a. Regression coefficients and significance of model terms						
b_0	49.51*	88.87*	1.60*	10.07*	0.35	20.87*
b_1	− 8.98*	5.03**	− 1.16*	− 2.22*	− 1.93*	− 3.22*
b_2	− 2.55	6.21*	− 0.20	− 3.40*	0.63	2.71**
b_3	− 6.45*	5.25**	− 1.21*	− 1.75*	− 1.03	− 7.58*
b_{12}	2.20	− 2.52	0.09	1.10	− 0.76	− 1.98
b_{13}	4.78	− 5.64**	0.95**	1.85	2.26**	− 2.64**
b_{23}	0.85	0.20	0.11	− 0.76	− 0.07	− 3.43**
b_{11}	− 2.22	− 2.74	− 0.34	1.13	1.63**	− 0.55
b_{22}	0.30	− 4.08	0.72	2.39**	− 0.15	− 0.84
b_{33}	− 0.09	− 4.27	0.69	1.22	0.36	− 4.26*
b. Statistical parameters measuring correlation and significance of models						
R^2	0.927	0.923	0.937	0.938	0.911	0.975
Fischer´s *F* parameter	7.0	6.5	8.0	8.5	5.5	22.0
Significance of models (based on the *F* parameter)	> 97	> 97	> 98	> 98	> 96	> 99

[*] Significant coefficients at the 99% confidence level

[**] Significant coefficients at the 95% confidence level

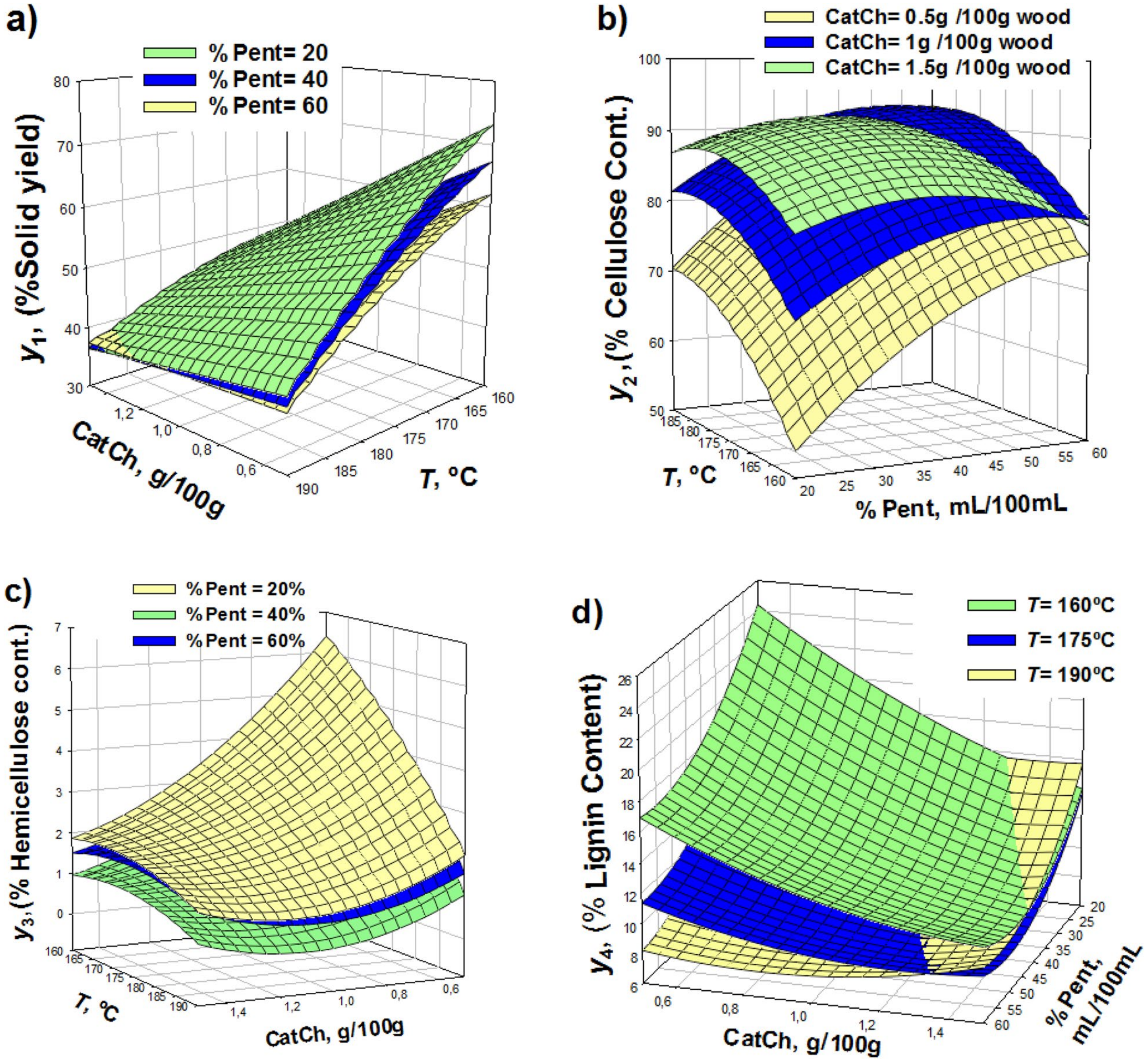

Fig. 1 Response surfaces calculated for variables y_1 to y_4 measuring the solid yield and the composition of the processed solids

practical pulp yields (roughly in the range 40–50%) could be achieved in a wide zone of the experimental domain.

These findings are supported by the behavior predicted for the cellulose content of samples (variable y_2): Fig. 1b shows that the surface responses became almost horizontal in the vicinity of 190 °C, with marked slope increases at lower temperatures. In contrast, at low temperatures and low or intermediate catalyst charges, the cellulose content of samples increased remarkably, revealing the conditions under which intense compositional changes took place. Figure 1b shows that solids containing more than 80% cellulose could be produced at 175–190 °C in media containing 40–60% 1-pentanol, no matter of the catalyst charge.

The fractionation treatments were able to cause an extensive hemicellulose solubilization, as revealed by the surface response calculated for y_3 (see Fig. 1c). Operating at low catalyst charges, samples containing around 2% hemicelluloses could be obtained near the highest temperature considered, no matter the 1-pentanol content of the media. Under these conditions, limited effects were caused by the catalyst charge, which in turn was very influential at low temperatures. According to Fig. 1c, samples containing less than 3% of residual hemicelluloses could be obtained operating at 185–190 °C, no matter the catalyst and 1-pentanol content employed.

In fractionation treatments, delignification is one of the major operational objectives. Figure 1d shows that operating at 160 °C, the lignin content of samples (variable y_4) dropped markedly with the catalyst charge and when the 1-pentanol concentration increased (in accordance with the ideas discussed for y_1). This general trend was less important at 175 °C, whereas a slight y_4 increase was noticed at high catalyst charges operating at 190 °C, a fact ascribed to lignin repolymerization under harsh conditions. This behavior is in agreement with reported studies on organosolv fractionation, in which the reactions involving lignin have been modeled assuming two consecutive reactions (depolymerization and repolymerization), where the second one is promoted under harsh conditions [29]. Specifically, lignin repolymerization has been reported as an influential effect on acid-catalyzed fractionation in water/1-pentanol media [14, 16].

To assess the effects of the operational variables on the extent and selectivity of component separation, the following variables were defined:

- Cellulose recovery in solid phase, denoted %CelRec, measured as the g cellulose in treated solid per 100 g of cellulose in the wood subjected to treatment,
- Lignin removal from the solid phase, denoted %LigRem, measured as the g of lignin removed by processing per 100 g of lignin in the wood subjected to treatment.

Based on these definitions, it can be inferred that the interrelationships among these variables and the dependent ones are given by the equations:

$$\%\mathrm{CelRec} = y_1 \cdot y_2 / y_{2w}, \tag{2}$$

$$\%\mathrm{LigRem} = 100 \cdot [y_{4w} - (y_1 \cdot y_4/100)]/y_{4w}, \tag{3}$$

where y_{2w} and y_{4w}·are the wood contents of cellulose and lignin listed in Table 2.

Figure 2a to f shows the dependence of %CelRec and %LigRem on the operational variables. Operation in media containing 20% 1-pentanol resulted in limited cellulose recoveries, which reached maximum values (in the range 80–95%, see Fig. 2a) under conditions defined by medium–low catalyst charges and low temperatures (160–175 °C); whereas the extent of delignification was also poor, reaching values up to 70–75% at 186–190 °C independently from the catalyst charge employed (see Fig. 2d). The model predictions displayed in Fig. 2b, c, e and f indicated that operation with 40 or 60% 1-pentanol resulted in improved fractionation, both in terms of cellulose recovery (which increased up to 95–100% in wide zones of the experimental domain, for example the ones defined by medium–low temperatures and medium–low catalyst charges) and lignin removal, which reached 80–85% either at 190 °C (no matter of the catalyst charge) or at 160–170 °C (at the highest catalyst charge assayed). The dark brown area in Fig. 2b corresponded to calculated cellulose recoveries slightly higher than 100%, a fact ascribed to the joint contribution of both experimental and fitting errors. In practical terms, the experimental conditions within this experimental domain enabled near quantitative cellulose recovery.

Based on the data obtained for the treated solids, it can be seen that a reasonable fractionation compromise (extensive delignification at reasonable cellulose recovery rates) could be achieved operating in media containing intermediate or high 1-pentanol percentages at low or intermediate temperatures, with low or intermediate catalyst charges.

Generation of soluble saccharides

Since hemicelluloses can be significantly degraded upon fractionation, the hemicellulose removal from the solid phase may not be representative for the amount of saccharides present in the aqueous phase. Taking this in mind, variables y_5 and y_6 (measuring the concentrations of xylooligosaccharides and xylan-derived saccharides in the aqueous phase, respectively) were included in the experimental design. In particular, the concentration of xylooligosaccharides derived from *Eucalyptus globulus* wood was studied because these products show valuable biological properties [30], mainly derived from their prebiotic character [31, 32]. Although XOS have been considered as target products for 1-pentanol-based biorefineries [5], Fig. 3a shows that their production was limited, and promoted under conditions leading to poor fractionation. As a consequence, considered on the scope of an integrated utilization of the raw material, XOS show a questionable potential as target products for 1-pentanol-based fractionation of *Eucalyptus* wood under the conditions considered in this study; and the valorization of hemicellulose-derived products should be better focused on the total xylan-derived saccharides. These latter include xylose and XOS (which, in turn, can be easily converted into xylose by reactions catalyzed by acids or enzymes. Figure 3b shows that the volumetric concentration of the total xylan-derived saccharides, measured by variable y_6, reached remarkable values.

Optimization

The choice of optimal fractionation conditions from the results listed in Sects. "Generation of soluble

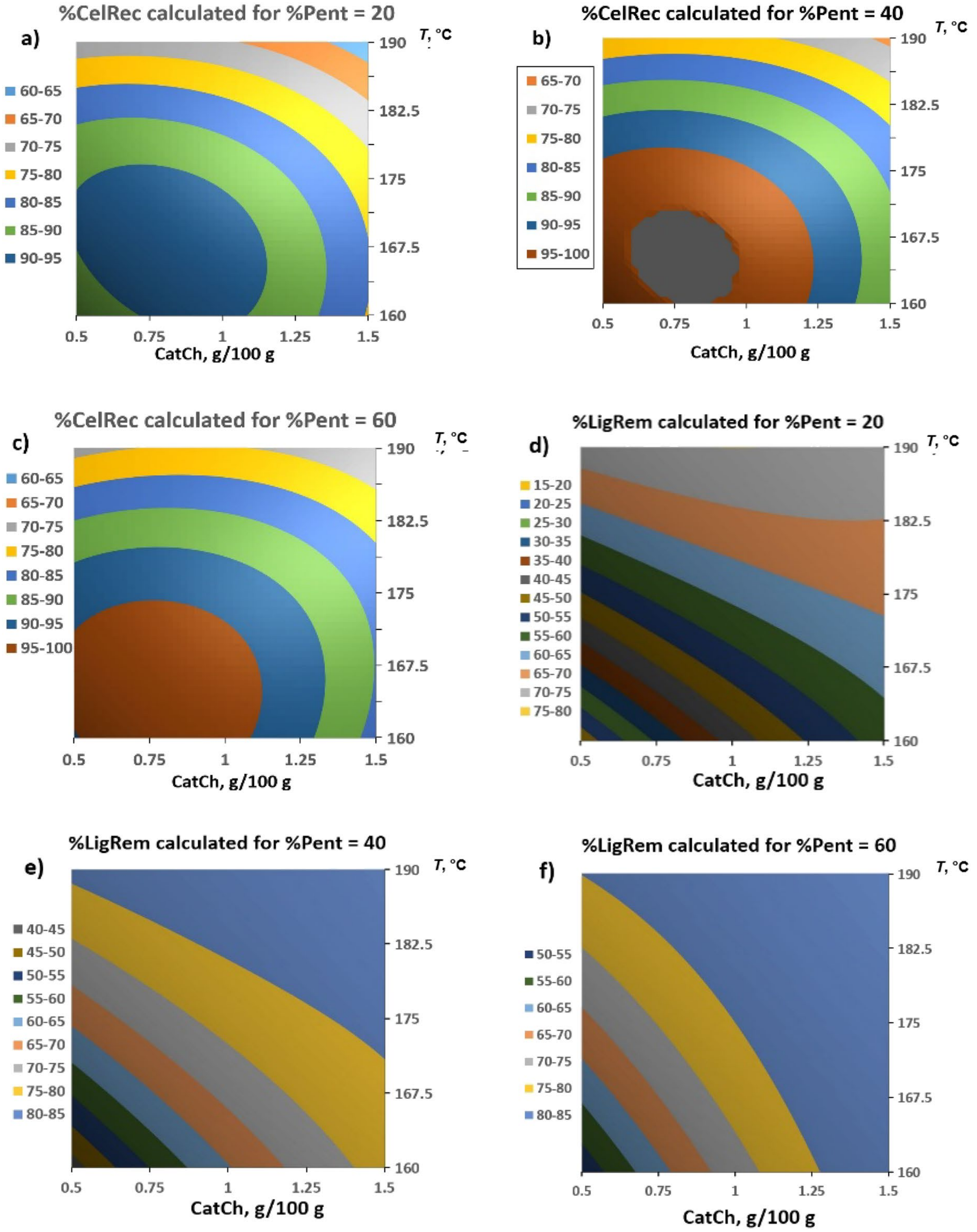

Fig. 2 Contour plots calculated for variables %CelRec and %LigRem for the considered pentanol percentages

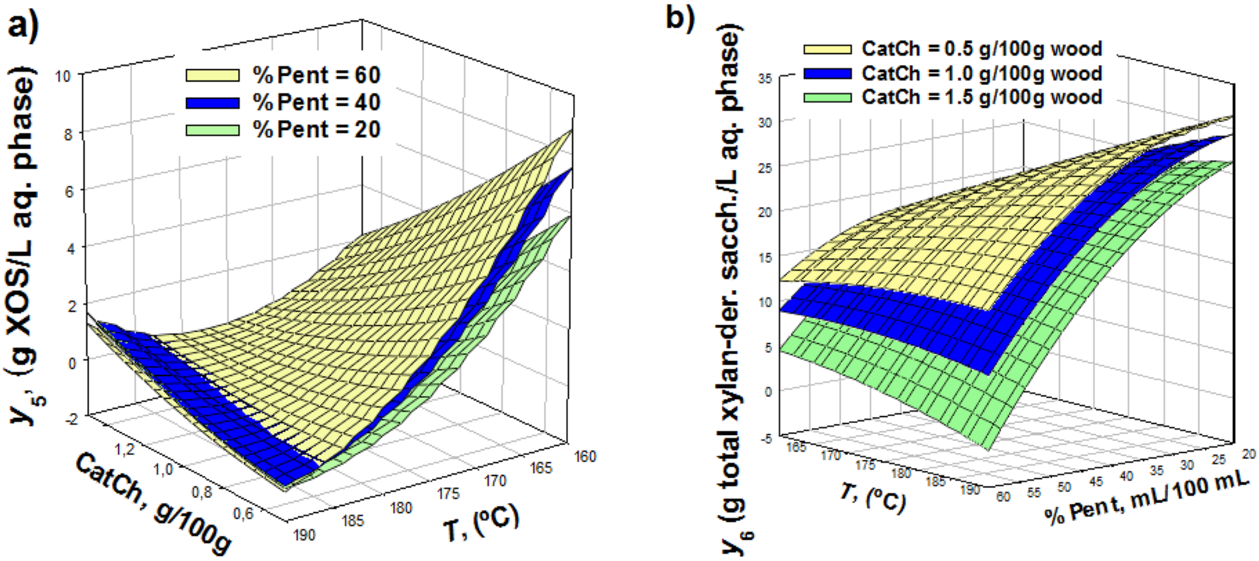

Fig. 3 Response surfaces calculated for variables y_5 and y_6 measuring the volumetric concentrations of XOS and total xylan-derived saccharides, respectively

saccharides" and "Optimization" is not obvious, owing to the mixed effects of the operational variables on cellulose recovery, extent of delignification and generation of xylan-derived saccharides. Because of this, an objective function (OF) was defined to measure the joint contribution of the following parameters: (a) relative amount of cellulose kept in the processed solids; (b) relative amount of lignin removed from the solid phase; and (c) relative amount of xylan-derived saccharides in the aqueous phase respect to the amount of acetylated xylan in wood.

Based on the definitions of the dependent variables, it can be seen that the values of OF can be calculated as follows:

$$\begin{aligned} \mathrm{OF} &= y_1 \cdot y_2 / y_{2w} + (100 - y_1 \cdot y_4 / y_{4w}) \\ &\quad + 100 \cdot y_6 \cdot V_{\mathrm{aq}} \cdot \mathrm{F}/20.21, \end{aligned} \tag{4}$$

where y_1, y_2, y_4, y_{2w}, and y_{4w} are as before, V_{aq} (in L) is the theoretical volume of aqueous phase obtained after the processing of 100 g wood (which depends on x_2), *HF* is the hydration factor (defined as the ratio between the molar masses of anhydroxylose and xylose), and 20.21 is the wood content of acetylated xylan (in wt%). With this definition, a good fractionation (leading to a solid phase with little contents of lignin and hemicelluloses, high cellulose retention, and near stoichiometric conversion of xylan into saccharides) would result in increased OF values, whereas poor fractionation conditions would correspond to decreased OF values.

OF maximization respect to the operational variables x_1, x_2 and x_3 with the restrictions $-1 \leq x_j \leq 1$ (*j*: 1 to 3) allowed the identification of optimal conditions (OF = 223.0) for $x_1 = -0.213$, $x_2 = 0.1713$, and $x_3 = -0.357$ (corresponding to CatCh = 0.894 g/100 g, %Pent = 43.4, and T = 169.7 °C). Under these conditions, the models predicted $y_1 = 53.4\%$, $y_2 = 85.9\%$, $y_3 = 2.40\%$, $y_4 = 11.0\%$, and $y_6 = 24.2$ g/L; corresponding to 99.5% cellulose recovery in solid phase and 72.5% lignin removal. The experimental validation of the optimal conditions resulted in average values of triplicate assays in accordance with the predicted ones: 51.8 ± 0.90%, 86.00 ± 0.21%,

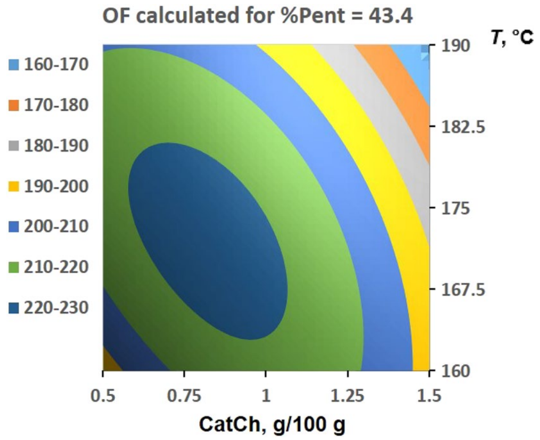

Fig. 4 Dependence of the objective function OF on the catalyst charge and temperature, calculated for media containing the pentanol concentration considered as optimal

1.89 ± 0.24%, 12.58 ± 0.93%, 22.7 ± 1.70 g/L, for y_1–y_4 and y_6, respectively.

Figure 4, which shows the OF dependence on CatCh and T predicted for media containing 43.4 1-pentanol, confirms the existence of a wide zone of the experimental domain where OF > 210, corresponding to an improved fractionation.

A comparative evaluation of the results reported here with literature can be done on the basis of the information summarized in Table 5, which includes (to our knowledge) all the data available for LB fractionation in media made up of 1-pentanol, water and sulfuric acid. In order to facilitate the interpretation of results, this Table list the type of substrate subjected to fractionation; the experimental variables fixed in the study and the ones employed for optimization. The set of experimental variables include %Pent, LSR, T, t, and CatCh. For the sake of simplicity, the data are summarized considering the most relevant measured effects (solid yield; cellulose content, lignin content, cellulose loss, lignin removal, and hemicellulose removal) obtained under the operational conditions identified as optimal by the authors.

The data in Table 5 have been obtained for both non-woody and woody substrates, which are expected to show diverse susceptibility to fractionation (for example, higher lignification degrees result in poorer component separation and selectivity).

The non-woody substrates include sorghum bagasse, wheat straw and moso bamboo. Teramura et al. [22] processed sorghum bagasse under fixed experimental conditions in a medium of low 1-pentanol content (12.5%) to achieve a limited fractionation, defined by limited cellulose enrichment and comparatively poor delignification degree, with no evaluation of hemicellulose conversion. Madadi et al. [5] considered the processing of wheat straw in a study using T and t as optimization variables, and reported optimal conditions leading to a processed solid of high cellulose content (96.4%) with high percentage of lignin removal, with limited cellulose loss (7.9%). Intermediate results were reported by Liu et al. [16], who employed moso bamboo as a fractionation substrate in a study using %Pent, CatCh, T and t as operational variables. Operating under selected conditions, the authors obtained a solid containing 85.4% cellulose and 7.7% lignin, with near complete hemicellulose removal.

Concerning the 1-pentanol fractionation of woods, results have been reported for *Acacia confusa*, aspen and Monterey pine. Islam et al. [14] considered the fractionation of *Acacia confusa* wood in a study evaluating the effects obtained at diverse values of variables %Pent, CatCh, T and LSR. The conditions considered as optimal by the authors led to good cellulose retention in solid phase and extensive hemicellulose removal, but with poor results concerning both cellulose content (72.1%) and lignin removal (70.3%). Madadi et al. [15] reported

Table 5 Results reported for 1-pentanol fractionation of lignocellulosic substrates using H_2SO_4 as a catalyst

Feedstock	Fixed variables	Optimal conditions	Solid yield	Cellulose content, %	Lignin content, %	Cellulose loss, %	Lignin removal, %	%Hemicellulose removal	Ref.
Sorghum bagasse	%Pent, 12.5% CatCh, 1% T, 180 ºC LSR, 13.3 mL/g t, 45 min	All the operational variables were fixed	38	62.2	29	28.1	74.3	Not reported	22
Wheat straw	%Pent, 60% CatCh, 1% LSR, 10 mL/g	T, 170 ºC t, 45 min	Not reported	96.4	2.3	7.9	94.5	93.3	5
Moso bamboo	LSR = 6 mL/g	%Pent, 80% CatCh, 4% T, 130 ºC t, 20 min	40.9	85.4	7.7	9.0	87.8	96.2	16
Acacia confusa wood	t, 60 min	%Pent, 65% CatCh, 45 mM T, 170 °C LSR, 7 mL/g	Not reported	72.1	Not reported	11.5	70.3	92	14
Aspen wood	CatCh, 1% t, 60 min LSR, 10 mL/g	%Pent, 60% T, 160 ºC	49	78.3	3.2	8.9	85	91	15
Monterey pine	%Pent, 60% CatCh, 1% LSR, 10 mL/g	T, 170 ºC t, 45 min	Not reported	94.3	3.1	7.9	89.7	89.2	5

on the fractionation of aspen wood, using %Pent and T as operational variables. The best treatment led to a solid with limited Klason lignin content (3.2%), but with a cellulose content (78.3%) lower than expected for the delignification degree achieved. Considering the proportions of other components in the processed solid material (2.1% xylan, 0.28% acid-soluble lignin), the closure of material balances was poorer than in other studies. Fractionation in 1-pentanol media was also suitable for fractionation of softwoods, which are known to be less favorable substrates than hardwoods. Using Monterey pine as a substrate, a study performed at diverse temperatures and reaction times [5] reported remarkable results in terms of both lignin and cellulose removal (> 89%), while causing little cellulose solubilization (7.9%).

Enzymatic hydrolysis

Native lignocellulosic materials are recalcitrant to the action of cellulolytic enzymes, owing to compositional and structural factors (heterogeneous nature, limited cellulose content, barrier effects caused by lignin and hemicelluloses, irreversible cellulase adsorption, cellulose crystallinity, etc.). In general, the lignin contents of woody biomasses makes them more recalcitrant to enzymatic hydrolysis than other feedstocks (e.g., herbaceous materials), while the relative amounts of G, S, and H units in lignin is also influential [2]. The drawbacks cited above can be overcome, at least in part, by chemical fractionation. In particular, solids susceptible to enzymatic hydrolysis have been manufactured by LB fractionation in biphasic media made up of 1-pentanol [5, 14–16, 27].

The experimental plan employed to assess the susceptibility of solids treated under optimal conditions toward enzymatic hydrolysis was made up of 12 experiments (see Table 6), and considered as independent variables the reaction time (up to 75 h), CSR (5 or 15 FPU/g), and LSR (8 or 15 g/g).

Besides glucose, the reaction media contained xylose, coming from the hydrolysis of residual xylan in the substrate. The low xylan content of the treated substrate limited the xylose concentrations (which fell in the range 0.40–1.64 g/L, see Table 6). Because of this, the xylose present in the enzymatic hydrolysis media was considered as not relevant for the purposes of this study.

Concerning glucose, the maximum experimental concentrations and conversions are listed in Table 6, while Fig. 5 illustrates the concentration profiles of the experiments. Since they presented a typical hyperbolic variation pattern, the experimental data were fitted to the equation proposed by Holtzapple and Humphrey [33]:

$$X = X_{\max} \cdot t/\left(t + t_{1/2}\right) \tag{5}$$

where X is the cellulose conversion into glucose achieved at time t, $X_{\max}$ is the maximum conversion achievable under the considered conditions (corresponding to an infinite reaction time), and $t_{1/2}$ is the time needed to reach 50% of $X_{\max}$. The values determined for the fitting parameters are listed in Table 6.

The results obtained in the various experiments indicated that the media containing mixtures of Celluclast

Table 6 Operational conditions and results achieved in enzymatic hydrolysis experiments

Exp	Enzyme	Operational Conditions		Experimental Results			Fitting parameters	
		CSR	LSR	C_{Xylmax}	C_{Gmax}	X_{maxexp}	X_{max}	t ½
R1	Cellic® Ctec 3	5	15	0.61	52.2	90.3	100	14.1
R2		15	15	0.79	55.8	96.6	100	2.75
R3		5	8	1.39	81.9	79.1	81.6	7.90
R4		15	8	1.08	102.4	99.0	100	5.53
R5	Cellic® Ctec 2	5	15	0.49	44.1	76.2	87.9	12.0
R6		15	15	0.82	55.5	94.7	100	3.51
R7		5	8	1.00	65.8	63.5	72.2	13.64
R8		15	8	1.64	98.7	95.3	100	6.59
R9	Celluclast® & Novozym® 188	5	15	0.40	42.3	73.1	88.9	15.9
R10		15	15	0.89	50.1	86.6	89.2	3.36
R11		5	8	0.48	65.1	62.9	75.4	13.5
R12		15	8	0.54	87.4	84.4	91.4	6.38

C_{Gmax}: maximum experimental glucose concentration, g/L; C_{Xylmax}: maximum experimental xylose concentration, g/L; X_{maxexp}, maximum experimental cellulose conversion into glucose, %; X_{max}: maximum glucose concentration, %, calculated from the Holtzapple and Humphrey (1984); $t_{1/2}$, kinetic parameter calculated from the Holtzapple and Humphrey (1984) model, h

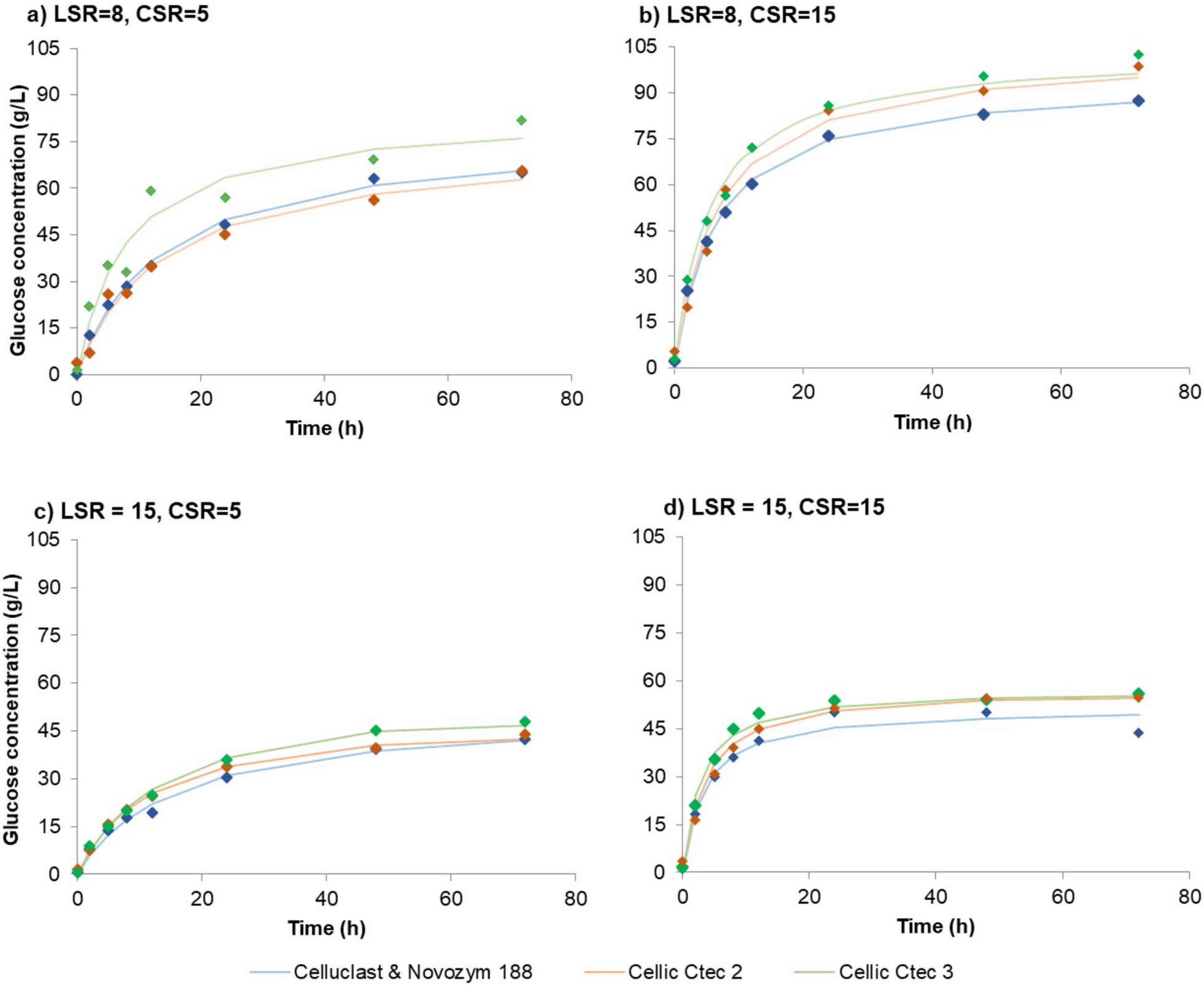

Fig. 5 Experimental and calculated results obtained in enzymatic hydrolysis experiments performed with the diverse types of enzymes at the enzymatic and solid charges considered

1.5L and Novozyme 188 led to poorer results (X_{max} in the range 62.9–86.6%) than the ones achieved with Cellic Ctec 2 or Cellic Ctec 3. For example, Cellic Ctec 2 provided X_{max} approximately 95% in experiments R6 and R8, with were achieved at LSR of 15 and 8, with CSR = 15 FPU/g. In comparison, the conditions R8 seem more favorable, since the lower LSR resulted in a higher glucose concentration (98.7 g/L in comparison with 55.5 g/L), although the kinetics was considerably slower ($t_{1/2}$ of 6.59 h in comparison with 3.51 h). Cellic Ctec 3 led to the best results, both in terms of conversion and kinetics: for example, near quantitative cellulose conversion was achieved in experiments R2 and R4, which provided high glucose concentrations (55.8 and 102.4 g/L, respectively), with a fast kinetics in both cases ($t_{1/2}$ of 2.79 and 5.53 h, respectively). These results confirmed the excellent behavior of the treated solids as substrates for glucose production by enzymatic hydrolysis.

Lignin characterization

The recovered lignin obtained in the selected optimal conditions presented 89.1 ± 1.0% purity (measured as insoluble material upon processing with 72% sulfuric acid, following the Klason lignin analysis conditions), and was characterized by very low contents of contaminating polysaccharides (0.08 g xylan/100 g lignin and 0.34 g glucan/100 g lignin). The lignin also contained 0.40 g acetyl groups/100 g lignin, and an acid-soluble fraction. The FTIR spectrum showed a general pattern typical for hardwoods, mainly made up of guayacyl and syringyl units in which the S groups are predominant. The dominant peak of the spectrum, appearing at 1112 cm^{-1}, corresponded to

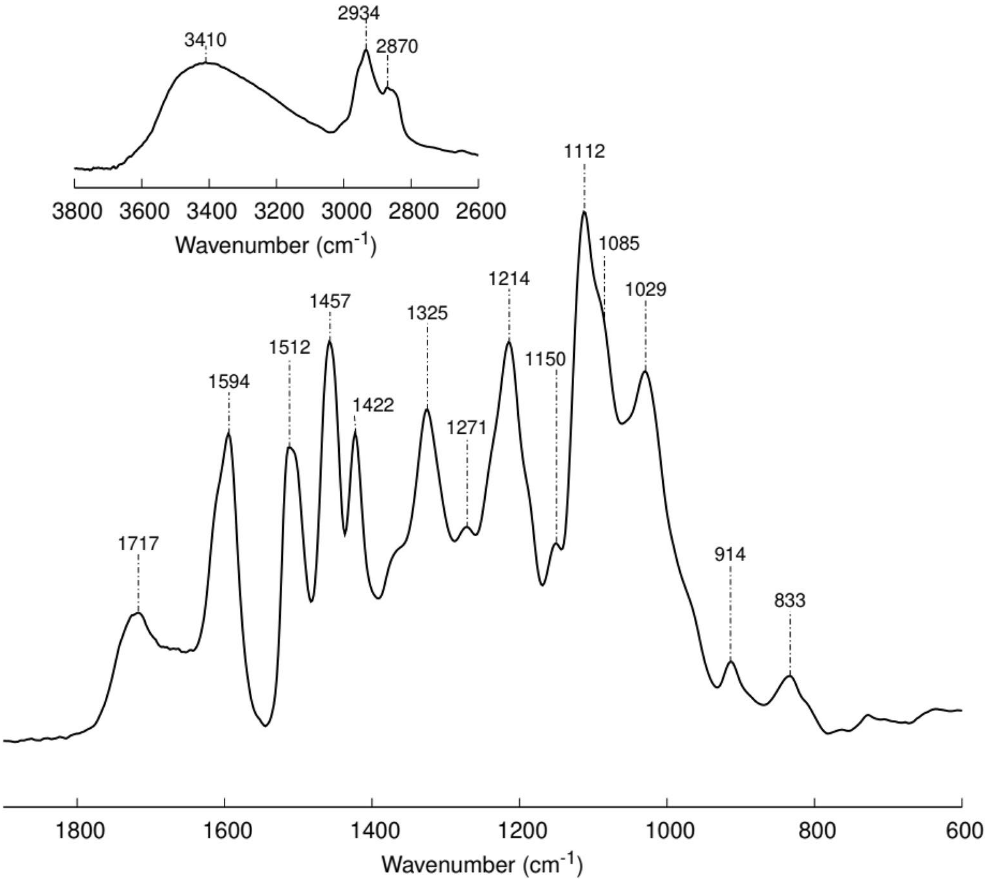

Fig. 6 FTIR spectrum of the recovered lignin

the deformation in the plane of the C–H bonds of the S units, which is distinguishable from the peak corresponding to the G units at 1150 cm^{-1}. Both the dominant peak at 1112 cm^{-1} and a relatively high peak at 1325 cm^{-1} (corresponding to the C–O stretching of the S-units) are typical of hardwood lignins. This peak can be used to calculate a ratio between S and G units by deconvoluting the peaks at 1325 and 1271 cm^{-1} typical of G units [34]. This calculation led to an S/G ratio of 2.6. Other typical hardwood bands are the well-defined signals appearing at 833 and 914 cm^{-1}. The peaks corresponding to the aromatic ring (1594, 1512 and 1422 cm^{-1}) show the same intensity. In this region, a higher intensity peak corresponding to the C–H deformation in CH_3 and CH_2 (1457 cm^{-1}), typical of lignins with a high S/G ratio, is also present; whereas the small peak at 1717 cm^{-1} corresponded to the vibration of the C=O group of esters and carboxylic acids (Fig. 6).

Conclusions

The statistical modeling of 1-pentanol-based fractionation of *Eucalyptus globulus* wood provided mathematical equations giving an exhaustive information on the effects caused by the most influential variables on key experimental variables, including the solid recovery yield and the composition of the aqueous, organic and solid phases from processing. Based on this information, the ranges of the experimental domain leading to a reasonable compromise among the desired effects (efficient removal of lignin and hemicelluloses, high cellulose recovery in solid phase) were identified (intermediate or high 1-pentanol contents, low or intermediate temperatures, and low or intermediate catalyst charges). Further optimization, based on an objective function measuring the relative amounts of cellulose kept in the processed solids, the solubilized lignin, and the generation of xylan-derived saccharides, was carried out to provide a deeper assessment on the overall fractionation. The values of the operational variables identified from the optimization calculations (0.894 g catalyst/100 g, 43.4% of 1-pentanol, 169.7 °C) led to solids with high cellulose content (which were almost stoichiometrically hydrolyzed by commercial cellulases under diverse conditions), and aqueous phases containing up to 22.87 g xylan-derived saccharides/L. The lignin isolated from the 1-pentanol-rich phase coming from fractionation showed structural features typical for hardwoods. These results confirmed the potential of 1-pentanol as a solvent for *Eucalyptus globulus* wood-based biorefineries.

List of symbols

Independent, dimensional variables

CatCh	Catalyst (H_2SO_4) charge, g/100 g
%Pent	Volume percent of 1-pentanol in the liquid phase
T	Isothermal reaction temperature, ºC

Independent, dimensionless variables

x_1	Dimensionless catalyst charge
x_2	Dimensionless 1-pentanol volume percent
x_3	Dimensionless reaction temperature)

Dependent variables

y_1	Solid yield, mass of treated solid/100 g oven-dry wood
y_2	Cellulose content of treated samples, wt% in oven-dry basis
y_3	Hemicellulose content of treated samples, wt% in oven-dry basis
y_4	Lignin content of treated samples, wt% in oven-dry basis
y_5	Concentration of xylooligosaccharides in the aqueous phase, g/L
y_6	Concentration of xylan-derived saccharides in the aqueous phase, g/L

Abbreviations employed for other variables or parameters (alphabetic order)

%CelRec	Percentage of cellulose recovery in solid phase
%LigRem	Percentage of lignin removal
$b_{0j}\ldots b_{ikj}$	Regression coefficients
CSR	Cellulase to substrate ratio
LB	Lignocellulosic biomass
LSR	Liquor to solid ratio (g/g or mL/g)
OF	Objective function
t	Time, min
$t_{1/2}$	Kinetic parameter (time needed to reach 50% of the maximum conversion)
y_{2w}	Cellulose content of native wood, wt% in oven-dry basis
y_{4w}	Lignin content of native wood, wt% in oven-dry basis
X	Cellulose conversion into glucose
X_{max}	Kinetic parameter (maximum conversion)

Acknowledgements
The authors thank the "Ministerio de Ciencia e Innovación" (MCIN/AEI) for the support provided to the BIOPLATFUN Project (Grant number PID2020-116717RB-I00). S. Rivas thanks the financial support of MCIN/AEI /https://doi.org/10.13039/501100011033 and European Union NextGeneration EU/PRTR for her research contract RYC2021-031964-I. Authors acknowledge Universidade de Vigo and CISUG for open access funding.

Author contributions
Conceptualization, S.R., J.L.A and J.C.P.; Methodology: M.F-B., S.R.; validation, M.F-B. S.R., R.Y, J.L.A and J.C.P; investigation, M.F-B. S.R., R.Y, J.L.A and J.C.P; resources, S.R., R.Y., J.L.A and J.C.P; writing-original draft preparation, J.C.P.; writing-review and editing, M.F-B, S.R., J.L.A and J.C.P.; supervision, S.R., J.L.A and J.C.P; funding, S.R., R.Y. and J.L.A. All authors have read and agreed to the published version of the manuscript.

Funding
"Ministerio de Ciencia e Innovación" of Spain (MCIN/AEI) in the framework of the BIOPLATFUN Project (Grant number PID2020-116717RB-I00) and the Research Contract RYC2021-031964-I (MCIN/AEI /https://doi.org/10.13039/501100011033 and European Union NextGeneration EU/PRTR).

Data availability
Not applicable.

Declarations

Competing interests
The authors declare no conflict of interest.

Received: 4 December 2023 Accepted: 16 March 2024

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