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Eucalyptus globulus wood fractionation by autohydrolysis and organosolv delignification

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Abstract: This work provides an assessment on the fractionation of *Eucalyptus globulus*, wood by sequential stages of autohydrolysis (to cause the solubilization of hemicelluloses) and organosolv pulping (to dissolve lignin, leaving solids enriched in cellulose). With this approach, valuable products (hemicellulose-derived saccharides, sulphur-free lignin fragments and cellulosic substrates with low contents of residual hemicelluloses) are obtained in separate streams, according to the biomass refinery approach. Autohydrolysis was carried out under optimized operational conditions, and the organosolv pulping step was performed using uncatalyzed ethanol-water solutions. The effects of the most influential operational variables (autohydrolysis severity, delignification temperature and ethanol concentration in the organosolv stage) on solid yield, solid composition, cellulose susceptibility and recovery of the various fractions was assessed using statistical method, which enabled the identification of the most favourable operational conditions.

Keywords: autohydrolysis, biorefinery, delignification, enzymatic hydrolysis, *Eucalyptus globulus*.

1. Introduction

Lignocellulosic Materials (LCM) are a basic resource for sustainable development. The biomass refinery approach, based on the selective separation of the major LCM components and their separate utilization for defined purposes, provides a valuable conceptual framework for an efficient utilization of LCM, whose fractionation can be achieved on the basis of the different properties of their structural components (cellulose, hemicelluloses and lignin), which are interpenetrated making part of a three-dimensional network.

In biorefineries, feedsotcks are subjected to successive processing stages to extract value from each fraction, achieving an integral benefit with minimal or no waste generation, allowing the production of biofuels and/or chemicals (FitzPatrick et al., 2010; Michels and Wagemann , 2010; Zhang, 2008).

When biorefineries are designed to produce second-generation biofuels by enzymatic hydrolysis-fermentation, the fractionation stages have to enhance the susceptibility of the solid substrates to enzymatic hydrolysis, playing a role related to the ones of conventional pretreatments. The importance of pretreatments and their contribution to the operational costs of biorefineries have been pointed out (Eggeman and Elander, 2005; Mosier et al, 2005; Sun and Cheng, 2002). A convenient pretreatment should fulfill as many as possible of the following conditions: simple and economical structure; scalable to industrial size; limited requirements of energy, water and process chemicals; ability for breaking the structure of the feesdstock; reduced polysaccharide losses; maximal production of valuable hemicellulose-derived products with limited generation of undesired degradation compounds; maximal production of valuable by-products from lignin; generation of processed, cellulose-containing solids with high susceptibility towards enzymatic hydrolysis; and minimal generation of processing wastes (Cara et al., 2006; Jørgensen et al., 2007; Mosier et al., 2005; Petersen et al., 2009; Pienkos and Zhang, 2009; Sun and Cheng, 2002).

Hemicellulose solubilization by autohydrolysis (or hydrothermal) processing with hot, compressed water has been proposed as the first step of biorefineries, owing to the

possibility of obtaining a liquid phase rich in hemicellulose-derived sugars or oligomers without causing significative dissolution of cellulose and lignin (Garrote et al., 1999). Operating under optimized autohydrolysis conditions, most hemicelluloses can be recovered in liquid phase as xylooligosaccharides and xylose (Garrote et al., 2001), whereas cellulose and lignin remain in solid phase, and could show enhanced susceptibility to further fractionation (Kim et al., 2009; Laser et al., 2002). However, the cellulase susceptibility of *Eucalyptus* wood autohydrolyzed under conditions leading to maximum oligosaccharide production is limited (Romaní et al., 2010a).

Ethanol-water solutions have been employed for LCM pulping (Díaz et al., 2004), eventually in the presence of sulphuric acid (Zhu and Pan, 2010). This approach leads to lignin fragments suitable for a variety of purposes (Pan et al., 2006), and to delignified solids with improved susceptibility towards enzymatic hydrolysis. When this method is applied to raw LCM, hemicelluloses can be just partially dissolved (Hallac et al., 2010), or converted into sugar-dehydration products such as furfural or hydroxymethylfurfural (Pan et al., 2007).

Based on the above ideas, this work provides an experimental assessment on the sequential processing of *Eucalyptus globulus* by autohydrolysis - organosolv delignification for obtaining valuable products derived from hemicelluloses (hemicellulosic saccharides) and lignin (solvent-soluble, sulphur-free lignin fragments), as well as cellulase-susceptible solids suitable as substrates for the manufacture of second-generation bioethanol.

2. Materials and Methods

2.1. Raw Material

Eucalyptus globulus wood samples were obtained from a local pulp factory (ENCE, Galicia, NW Spain), milled to pass an 8 mm screen, air-dried, homogenized in a single lot to avoid differences in composition among aliquots, and stored in a dark and dry place until use.

2.2 Analysis and Processing of the Raw Material

Eucalyptus globulus wood samples were milled to particle size less than 0.5 mm, and analyzed for extractives (TAPPI T-264-om-88m method), moisture (TAPPI T-264-om-88m method), and ashes (T-244-om-93 method). Additional samples were subjected to extractions and quantitative acid hydrolysis (QAH) (T-249-em-85 method). The liquid phase from QAH was assayed by HPLC for sugars (glucose, xylose, arabinose) and acetic acid, using a Refractive Index detector and a BioRad Aminex HPX-87H column, eluted with 0.01 M H_2SO_4 at a flow rate of 0.6 $mL \cdot min^{-1}$. The solid residue from QAH was considered as Klason lignin.

The composition of the raw material (expressed in g/100 g wood in oven-dry basis ± standard deviation, based on four replicate determinations) was as follows: extractives 2.4 ± 0.15; ashes 0.23 ± 0.03; cellulose (as glucan), 44.39 ± 0.44; xylan 17.49 ± 0.65; arabinan 1.08 ± 0.05; acetyl groups 3.27 ± 0.23; and Klason lignin 27.67 ± 0.37.

Samples of autohydrolyzed *Eucalyptus globulus* wood (denoted AW) and solids resulting from autohydrolysis-delignification of *Eucalyptus globulus* (denoted ADW) were assayed for moisture and QAH using the same methods employed for the raw material.

2.3. Autohydrolysis and analysis of autohydrolysis liquors

Water and *Eucalyptus globulus* wood samples were mixed at a liquid to solid ratio (LSR) of 8 kg liquid/kg raw material (oven-dry basis). The mixture was reacted in a stainless steel reactor (Parr Instruments Company, Moline, IL) following the standard heating and cooling temperature profiles (Romaní et al., 2010b). Once the desired maximum autohydrolysis temperature (T_A) was reached, the media were immediately cooled, and aliquots of liquors were obtained by filtration. Based on reported data (Garrote and Parajó, 2002), operation was carried out at T_A of 185, 190, 195, 200 and 205 ºC, in order to cover the range of severity conditions leading to significant generation of valuable hemicellulose-derived soluble compounds.

The effects achieved in a given non-isothermal autohydrolysis experiment can be measured in terms of severity (So), which includes the combined effects of temperature and reaction time along heating and cooling. So was defined by Lavoie et al. (2010) as:

$$So = \log Ro = \log \left[Ro_{HEATING} + Ro_{COOLING} \right] = \log \left[\int_{0}^{t_{MAX}} \frac{T(t) - T_{REF}}{\omega} \cdot dt + \int_{t_{MAX}}^{t_F} \frac{T'(t) - T_{REF}}{\omega} \cdot dt \right] \quad \textbf{(1)}$$

where Ro is the severity factor, t_{MAX} (min) is the time needed to achieve T_A (ºC), t_F (min) is the time needed for the whole heating-cooling period, and T(t) and T'(t) represent the temperature profiles in heating and cooling, respectively. Calculations were made assuming the values reported in literature for ω and T_{REF} (14.75 ºC and 100 ºC, respectively). The So values calculated for the considered T_A were 3.35, 3.50, 3.64, 3.79 and 3.94.

AW samples were washed with distilled water and used to measure the solid yield of the autohydrolysis stage (Y_A, kg of AW/100 kg raw material, oven-dry basis). Other samples were employed for compositional analysis (see section 2.2). An aliquot of autohydrolysis liquors was filtered through 0.45 μm membranes and used for direct HPLC determination of glucose, xylose, arabinose, acetic acid, hydroxymethylfurfural (HMF) and furfural (F), using the same method specified above. A second aliquot was subjected to quantitative posthydrolysis (by triplicate) with 4% w/w sulphuric acid at 121ºC for 30 min, filtered through 0.45 μm membranes, and analyzed by HPLC for oligomer determination. The results obtained in autohydrolysis are shown in Table 1.

2.4. Organosolv delignification of autohydrolyzed *Eucalyptus globulus* wood

AW samples were subjected to organosolv delignification with ethanol-water mixtures at a LSR of 8 g solution/g oven-dry AW for 1 h at the selected temperature. Time zero was taken when the system reached the preset temperature. When desired, the mixture was cooled, and the autohydrolyzed-delignified solids (ADW) were recovered and washed (first with ethanol/water and then with distilled water). After washing, ADW were used for gravimetric determination of the solid yield corresponding to delignification (Y_D, kg of ADW /100 kg AW, on dry basis), and a ADW sample was used for analysis (see section 2.2).

2.5. Enzymatic hydrolysis of ADW

Enzymatic hydrolysis assays of ADW was carried out at 48.5ºC and pH= 4.85 (0.05 N citric acid-sodium citrate buffer) in 250 mL Erlenmeyer flaks with orbital agitation (150

rpm) using “Celluclast 1.5 L” cellulases and “Novozym” β-glucosidase, which were kindly provided by Novozymes (Madrid, Spain). The cellulase activity of “Celluclast 1.5 L” was measured by the Filter Paper assay, and the activity was expressed as Filter Paper Units (FPU) according to Ghose (1987). The β-glucosidase activity of “Novozym” was determined as International Units (IU) (Paquot and Thonart, 1982). The enzyme activities were 70.1 FPU/mL for “Celluclast 1.5 L” and 575 IU/mL for “Novozym”.

Enzymatic hydrolyses were carried out under the same conditions for ADW samples obtained under different conditions, to study the effects of processing on the cellulase digestibility. The conditions of enzymatic hydrolysis were LSR = 25 kg liquid/kg oven-dry ADW, cellulase/substrate ratio = 10.3 FPU/g oven-dry ADW, and β-glucosidase/cellulase ratio = 5 IU/FPU. The reaction time of enzymatic hydrolysis varied in the range 0-72 h. At the desired times, samples were withdrawn from the media, centrifuged, filtered and analyzed by HPLC for monosaccharides and acetic acid. Based on the typical variation pattern observed for the glucose concentration profiles (Garrote et al., 2008), the glucose concentration profiles determined for individual experiments were fitted to the following equation (Holtzapple et al., 1984):

$$CGC_t = CGC_{MAX}\frac{t}{t + t_{1/2}} \qquad \textbf{(2)}$$

where CGC_t is the cellulose-to-glucose conversion achieved at time t, calculated as:

$$CGC_t = 100 \cdot \frac{G_t - G_{t=0}}{G_{POT}} \qquad \textbf{(3)}$$

whereas CGC_{MAX} is the cellulose-to-glucose conversion predicted for an infinite reaction time, t is the enzymatic hydrolysis time (h), $t_{1/2}$ (h) is the time needed to achieve CGC= $CGC_{MAX}/2$, G_t is the glucose concentration (g/L) achieved at time t, $G_{t=0}$ is the glucose concentration at the beginning of the experiments, and G_{POT} represents the potential glucose concentration (corresponding to the stoichiometric conversion of the cellulose present in substrates into glucose). G_{POT} was calculated as:

$$G_{POT} = \frac{Gn}{100} \cdot \frac{180}{162} \cdot \frac{\rho}{LSR + 1 - \frac{KL}{100}} \qquad \textbf{(4)}$$

where Gn is the glucan content of ADW (kg glucan/100 kg ADW, on dry basis), 180/162 is the stoichometric factor, ρ is the density of the reaction medium (average value, 1005 g/L), LSR is the liquid-to-solid ratio (25 kg liquid/kg ADW) and KL is the Klason lignin content of ADW (kg Klason lignin/100 kg ADW).

2.6. Fitting of data

The experimental data were fitted to the proposed models using commercial software (Microsoft Excel by Microsoft, U.S.A.)

3. Results and Discussion

3.1 Structure of the experimental design

The selection of the operational parameters employed in this work and their variation ranges was based on the results obtained in preliminary experiments. Autohydrolysis was carried out at the severities indicated in Table 1, in order to achieve fractionation effects (mainly related to the selective removal of hemicelluloses). The results concerning the xylan contents of AW (from 10.9 to 2.73%) confirmed that the experimental range considered was suitable for covering all the situations of practical importance (from moderate up to extensive hemicellulose removal). As expected, due to the selectivity of the autohydrolyis stage, the results concerning solid yield (81.8-72.4%) and the contents of cellulose and lignin (in the ranges 54.3-61.4% and 26.3-32.0%) confirmed that both components were almost quantitatively retained in solid phases upon autohydrolysis. On the other hand, xylooligomers were the major hemicellulose-derived components present in liquid phase, with minor amounts of glucooligosaccharides, oligosaccharide substituents, monosaccharides, furans and acetic acid.

In order to obtain a preliminary insight on the susceptibility of AW samples to delignification, autohydrolyzed solids were processed with 60% ethanol under a variety of operational conditions (see Table 2), operating at selected combinations of delignification temperature (T_D in the range 175-200 ºC) and delignification times (t_D in the range 60-120 min); and the resulting solids (ADW) were assayed for yield (Y_D) and composition.

Additionally, ADW were assayed as substrates for enzymatic hydrolysis, and the cellulose conversions into glucose were measured after 72 h (CGG_{72}). The experimental results showed that significant fractionation could be achieved even in experiments performed under the mildest delignification conditions assayed (175 ºC, 60 min). In agreement with literature (Pan et al., 2007), the reaction time (in the range assayed) showed a moderate influence, whereas the results obtained under harsh conditions (200 ºC for 120 min) suggested the participation of lignin repolymerization reactions. Finally, the ADW susceptibility to hydrolysis increased steadily with the severity of the operational conditions.

Based on the results achieved in these preliminary experiments, an experimental plan (following a Doehlert design) was performed to assess the autohydrolysis-delignification of *Eucalyptus* wood (see Figure 1). The structure of the experimental plan is summarized in Table 3. The independent variables considered were the severity of the autohydrolysis stage (denoted So, also measured by the normalized variable x_1), the delignification temperature (T_D or x_2), and the ethanol concentration in the delignification stage (C or x_3). The liquor to solid ratio in both autohydrolysis and delignification stages was fixed in 8 g/g, and the delignification time in 1 h. The dependent variables include the combined yield (denoted Y_C or y_1, measuring the recovery of solids after autohydrolysis and delignification, defined as kg ADW/100 kg raw material, oven dry basis), variables measuring the composition of ADW samples (contents of glucan, xylan, acetyl groups and Klason lignin, denoted variables y_2 to y_5, respectively), and the susceptibility of ADW towards enzymatic hydrolysis, which was measured by the two kinetic parameters of eq. 2 (CGC_{MAX} and t½, or y_6 and y_7 in generalized nomenclature).

For calculation purposes, the operational variables were expressed in terms of dimensionless, normalized variables (x_1, x_2, and x_3) with variation ranges (-1,1), which are linearly related to the corresponding independent variables (So, T_D or C) as follows:

$$x_1 = 2 \cdot \frac{So_i - So_{me}}{So_{max} - So_{min}} \quad \textbf{(5)}$$

$$x_2 = 2 \cdot \frac{T_{D_i} - T_{D\,me}}{T_{Dmax} - T_{Dmin}} \quad \textbf{(6)}$$

$$x_3 = 2 \cdot \frac{C_i - C_{me}}{C_{max} - C_{min}} \quad \textbf{(7)}$$

where the subscripts i, me, min and max correspond to the experiment considered, and to the mean, minimum and maximum values of the variation ranges, respectively.

The interrelationship between dependent and independent variables was established by empirical models following the generalized expression:

$$y_j = b_{0j} + \sum_i b_{ij} x_j + \sum_i \sum_k b_{ikj} x_j x_k \quad \textbf{(8)}$$

where y_j is the dependent variable considered (j: 1 to 12), x_i or x_k (i or k: 1 to 3, k≥i) are the dimensionless, independent variables defined by equation (2), and b_{0j}....b_{ikj} are the regression coefficients, calculated from the experimental data by multiple regression using the least-squares method. Table 4 shows the experimental values achieved for the experimental variables, whereas Table 5 lists the sets of regression coefficients b_{0j}....b_{ikj} and their significance (based on the Student's t-test), as well as the statistical parameters measuring the correlation (R^2) and significance of models (based on the Fisher's F-test).

3.2. Organosolv delignification of AW and enzymatic hydrolysis of the resulting samples (ADW)

According with the data in Table 4, combined yield of autohydrolysis-delignification (Y_C or y_1) varied in the range 47.9 - 66.6 kg ADW/100 kg wood, the extreme values corresponding to experiments 10 and 12. Figure 2 shows the surface response calculated for Y_C as a function of the most influential operational variables. As a general trend, Y_C decreases with T_D, particularly in media containing low ethanol concentrations. So showed a related influence, but its effects were less marked.

The variation range determined for the ADW glucan content (variable Gn or y_2) was 70.3 – 86.9 kg glucan/100 kg oven-dry (o. d.) ADW (corresponding to experiments 4 and 6, respectively). Figure 3 shows the calculated dependence of y_2 on the most influential independent variables. The highest glucan content predicted by the model (for operation in media containing 50.2 kg ethanol/100 kg liquor) was 87.6 kg glucan/100 kg ADW,

operating at So = 3.50 and T_D = 202.7 ºC and. As a general pattern, the higest glucan contents corresponded to samples treated at medium or high severities and at high T_D, with a limited influence of the ethanol concentration.

Considering ADW samples as potential substrates for biofuel production by enzymatic hydrolysis, their high glucan contents are an advantage, as they increase the potential glucose concentration for a given substrate loading. For example, the ADW sample with highest cellulose content obtained in this work corresponds to a potential glucose concentration 40% higher than the one reported in a related study (Romaní et al., 2010a).

As expected, ADW samples presented limited xylan contents (3.0 – 8.3 kg/100 kg), which decreased with So. The rest of independent variables did not play a significant role on this parameter. The lowest xylan content predicted by the models (for media containing 50.2 kg ethanol/100 kg) was 1.96 kg /100 kg ADW (operational conditions: So = 3.89, T_D = 203 ºC). This content corresponds to 93.4% xylan removal.

The experimental trends found for variable y_4 (ADW content of acetyl groups) were similar to the ones observed for the xylan content. The experimental range was 0.39 – 1.24 kg/100 kg, suggesting that this variable plays a role of limited importance in the whole process.

The Klason lignin content of samples (variable KL or y_5) varied in the range 10.2 – 22.7 kg/100 kg o. d. ADW. Figure 4 presents the surface responses calculated for KL as a function of the most influential operational variables. In general terms, comparatively high lignin contents are predicted for low values of T_D and So. For example, ADW with as much as 31.5 kg KL/100 kg were predicted operating at So = 3.35 and T_D = 172 ºC in media containing 69.8 kg ethanol/100 kg liquor. Starting from the mildest operational conditions, delignification was first improved by higher So and/or T_D, up to reach the minimum KL (10.2 kg KL/100 kg ADW) under operational conditions defined by So = 3.54, T_D = 199 ºC (values calculated for C = 64.0 kg ethanol/100 kg mixture). Beyond these values, further increases in So and/or T_D led to increased KL, a fact ascribed to lignin repolymerization (Parajó et al., 1995). Considering the zone of the experimental domain defined by high So and T_D, the models predicted the highest KL (29.6 kg/100 kg ADW) for samples treated at

So = 3.94 and T_D = 203 ºC (data calculated for media containing 50.2 kg ethanol/100 kg liquor).

All the ADW samples obtained in this work were susceptible towards enzymatic hydrolysis. The data in Table 4 concerning variables y_6 (or CGC_{MAX}) and y_7 (or $t_{½}$) confirmed that 10 in 15 samples subjected to autohydrolysis-delignification reached CGC_{MAX} close to 100%, whereas the lowest CGC_{MAX} (65.7%) corresponded to the sample treated under the mildest conditions. The second kinetic parameter ($t_{½}$) varied in the range 6.1 - 29.2 h. Comparatively, the enzymatic hydrolysis of autohydrolyzed *Eucalyptus globulus* wood (not delignified) carried out under similar conditions (LSR = 20 kg/kg and ESR = 10.3 FPU/g, So in the range 3.64 – 3.94) led to $t_{½}$ in the range 26 – 28 h and to CGC_{MAX} in the range 54% - 98% (Romaní et al., 2010a). Obtaining autohydrolyzed solids (not subjected to delignification) with an enzymatic digestibility similar to the ADW samples considered in this work would entail aqueous processing under harsh conditions (So $\geq$ 4.67), which would lead to degradation of hemicellulosic sugars and partial cellulose solubilization, worsening the integrated benefit of the whole raw material.

3.3. Recovery of components in ADW samples and enzymatic hydrolysis yield

Based on knowledge of the composition of wood, and on the availability of empirical models describing the dependences of ADW composition of combined yield on the operational conditions, the recoveries of the various fractions in ADW can be directly calculated. For this purpose, the following variables were defined:

$$\text{Glucan recovery} = \text{Rec}_{Gn} = Y_C \cdot \frac{Gn}{Gn_{RM}} \quad \textbf{(9)}$$

$$\text{Klason lignin recovery} = \text{Rec}_{KL} = Y_C \cdot \frac{KL}{KL_{RM}} \quad \textbf{(10)}$$

where the subscripts RM refer to the contents of the considered component in the raw material, and the rest of variables have been defined before (see Table 3).

The availability of suitable parameters for equations 2 and 8 enables a generalized interpretation of the autohydrolysis-delignification-enzymatic hydrolysis process. Eq. 9 predicted glucan recoveries in the range 82.8% (operating under severe conditions, defined

by So = 3.94, T_D = 200 ºC and C = 61.9 kg ethanol/100 kg mixture) up to 100% (for samples treated under a variety of conditions). On the other hand, the recovery of Klason lignin in processed samples reached its minimum predicted value (19.1%) under conditions defined by So = 3.56, T_D = 198 ºC and C = 59.5 kg ethanol/100 kg liquor, and its maximum predicted value (65.6%) operating at So = 3.94, T_D = 203 ºC and C = 50.2 kg ethanol/100 kg liquor.

The high recovery of cellulose in processed solids, together with the extensive removal of hemicelluloses and lignin from solid phase, confirmed the suitability of the approach considered in this study for an efficient fractionation of *Eucalyptus globulus* wood.

In order to assess the fractionation effects achieved, Figure 5 shows the results calculated for variables Rec_{Gn}, Rec_{KL}, CGC_{24} (cellulose to glucose conversion after 24 h) and CGC_{72} (cellulose to glucose conversion after 72 h) along the most favourable zone of the experimental domain (defined by So in the range 3.65 - 3.94 and T_D in the range 180 - 200 ºC. For the sake of simplicity, the results have been calculated for concentration media containing the intermediate ethanol concentration (60 kg ethanol/100 kg liquor). Under these conditions, cellulose was almost or totally retained in ADW, whereas the degree of lignin removal reached values up to 81%, CGC_{24} was higher than 80%, and CGC_{72} was close to 100%.

In order to highlight the fractionation effects achieved under suitable conditions and the susceptibility of the resulting samples to hydrolysis, Figure 6 shows the ranges calculated for the mass flows of fractions (or compounds) for the range of operational conditions considered in Figure 5. Stream B (autohydrolysis liquors) accumulated 67 – 75% of the initial hemicelluloses in the form of saccharides (mainly of oligomeric nature). These latter are valuable, as xylooligosaccharides find direct application in pharmaceutical and food industries (Moure et al., 2006) or can be hydrolyzed to xylose and further converted by chemical methods (for example, hydrogenation to xylitol), or by fermentation (for example, to lactic acid or ethanol). Stream C (pulping liquors) contained up to 67% of the initial lignin. Stream E (enzymatic hydrolysis media after 72 h) contains glucose at a yield in the range 31.1 – 43.6 kg / 100 kg de raw material.

Comparative advantages can be found for operational conditions defined by So = 3.64, T_D = 198 ºC, C = 60 kg ethanol/100 kg liquor, and duration of enzymatic hydrolysis 72 h. These conditions led to the following yields: 18.1 kg of oligomers, as monomers, and sugars in stream B/100 kg o. d. wood, 17.9 kg of soluble lignin in stream C/100 kg o. d. wood, and 41.9 kg glucose/100 g o. d. wood. As a whole, the processing of 100 kg of o. d. wood would result in the recovery of 77.9 kg of valuable fractions or products in three separate streams, each fraction being suitable for the individual manufacture of fuels and/or chemicals.

4. Conclusions

The processing method enabled an efficient fractionation of *Eucalytpus globulus* wood. Hemicelluloses were converted into mono- and oligosaccharides, whereas autohydrolyzed wood was extensively delignified using uncatalyzed ethanol-water solutions. Autohydrolyzed - delignified wood samples (ADW) obtained under a variety of operational conditions were highly susceptible to enzymatic hydrolysis. The availability of empirical models giving the interrelationship of selected variables (measuring the combined yield and the composition of ADW, as well as the kinetic parameters involved in enzymatic hydrolysis) allowed a generalized interpretation of data, as well as the identification of conditions leading to favourable fractionation and enzymatic hydrolysis yield.

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Table 1. Data obtained in wood autohydrolysis: solid yield (Y_A), composition of autohydrolyzed wood (AW) and composition of autohydrolysis liquors.

So	3.35	3.50	3.64	3.79	3.94
Y_A (kg AW/100 kg raw material, on dry basis**)**	81.8	74.2	74.0	73.3	72.4
Autohydrolyzed *Eucalyptus globulus* wood composition (kg/100 kg AW, on dry basis**)**					
Gn (glucan)	54.3	57.5	58.7	61.4	60.6
Xn (xylan)	10.9	6.18	5.17	4.34	2.73
AcG (Acetyl groups)	1.73	0.98	0.86	0.36	0.37
KL (Klason lignin)	26.3	30.8	32.8	32.0	32.2
Liquid phase composition (g/L**)**					
GO (Gluco-oligomers [1])	0.53	0.36	0.86	0.92	0.98
XO (Xylo-oligomers [1])	8.60	12.5	14.6	14.0	10.2
ArO (Arabinosyl moities in oligomers [1])	0.41	0.42	0.00	0.00	0.00
AcGO (Acetyl groups in oligomers [1])	1.83	2.88	2.71	2.74	2.20
G (Glucose)	0.23	0.47	0.12	0.16	0.20
X (Xylose)	0.58	1.83	2.73	4.16	5.60
Ar (Arabinose)	0.29	0.28	0.46	0.56	0.44
AcH (Acetic acid)	0.20	0.20	0.62	0.85	1.23
HMF (Hydroxymethylfurfural)	0.01	0.02	0.03	0.07	0.09
F (Furfural)	0.07	0.14	0.22	0.56	0.78

[1]**:** expressed as monomer equivalents

Table 2. Results obtained in preliminary experiments of autohydrolyzed wood pulping (performed in pulping media containing 60 weight percent ethanol)

(Nomenclature: AW: autohydrolyzed wood; ADW: autohydrolyzed delignified wood; So: severity, dimensionless; T_D: delignification temperature, ºC; t_D: delignification time, min; Y_D: delignification yield, kg ADW/100 kg AW, on dry basis; Gn: glucan content, kg Gn/100 kg ADW, on dry basis; Xn: xylan content, kg Gn/100 kg ADW, on dry basis; KL: Klason lignin content, kg KL/100 kg ADW, on dry basis; CGC_{72}: Cellulose conversion into glucose achieved after 72 h, kg glucose/ 100 kg potential glucose)

Experimental conditions				**Experimental results**				
Exp	**So**	**T_D**	**t_D**	**Y_D**	**Gn**	**Xn**	**KL**	**CGC_{72}**
P01	3.64	175	60	75.4	74.5	2.10	20.2	39.4
P02			120	72.8	77.3	3.16	17.5	46.8
P03		200	60	68.6	81.3	4.24	14.0	54.1
P04			120	67.2	85.2	3.84	10.7	53.9
P05	3.79	175	60	75.8	74.8	3.92	17.2	47.1
P06			120	73.0	77.8	3.17	14.5	57.4
P07		200	60	70.0	83.7	2.70	13.1	58.2
P08			120	66.6	87.1	2.53	10.3	58.3
P09	3.94	175	60	76.7	77.3	2.80	16.2	47.5
P10			120	72.9	78.1	2.93	17.2	49.6
P11		200	60	70.1	83.0	2.46	13.0	54.9
P12			120	68.2	83.6	2.57	11.8	60.9

Table 3. Variables involved in the experimental design considered in this work (AW: autohydrolyzed wood, ADW: autohydrolyzed delignified wood, o.d.b.: on dry basis).

Nomenclature		Variable	Value or range	Units
Fixed variables				
LSR_A		Liquid to solid ratio in autohydrolysis	8	kg liquid/ kg raw material, o.d.b.
LSR_D		Liquid to solid ratio in delignification	8	kg liquid/ g AE, o.d.b.
t_D		Isothermal delignification time	1	h
Independent variables				
So	x_1	Severity of hydrothermal treatment	3.35-3.94	Dimensionless (So = Log Ro, with Ro in min)
T_D	x_2	Delignification temperature	172-203	ºC
C	x_3	Ethanol concentration in pulping media	48-72	kg ethanol/100 kg liquor
Dependent variables				
Y_C	y_1	Combined yield		kg ADW/100 kg raw material, o.d.b.
Gn	y_2	Glucan content of ADW		kg glucan/100 kg ADW, o.d.b.
Xn	y_3	Xylan content of ADW		kg xylan/100 kg ADW, o.d.b.
AcG	y_4	Acetyl groups content of ADW		kg acetyl groups/100 kg ADW, o.d.b.
KL	y_5	Klason lignin content of ADW		kg Klason lignin/100 kg ADW, o.d.b.
CGC_{MAX}	y_6	Maximum cellulose-to-glucose conversion in enzymatic hydrolysis		kg glucose/ 100 kg potential glucose
$t_{½}$	y_7	Time to achieve CGC_{MAX} /2 in enzymatic hydrolysis		h

Table 4. Operational conditions considered (expressed in terms of dimensional and dimensionless independent variables) and experimental results obtained for dependent variables y_1 to y_7 (see Table 3 for definitions and units).

	Independent variables			**Dimensionless, normalized independent variables**			**Dependent variables**						
Exp	**So**	**T_D (°C)**	**C (% ethanol)**	**x_1**	**x_2**	**x_3**	**y_1**	**y_2**	**y_3**	**y_4**	**y_5**	**y_6**	**y_7**
1	3.94	188	60	1	0	0	53.5	77.7	3.48	0.42	20.6	100	10.5
2	3.35	188	60	-1	0	0	57.7	70.7	8.34	1.24	18.1	65.7	24.8
3	3.79	203	60	0.5	0.866	0	50.0	79.0	4.20	0.40	17.8	100	8.0
4	3.50	172	60	-0.5	-0.866	0	65.6	70.3	6.64	0.68	22.7	93.9	29.2
5	3.79	172	60	0.5	-0.866	0	56.2	77.8	4.60	0.75	17.9	71.5	22.0
6	3.50	203	60	-0.5	0.866	0	57.4	86.9	5.32	0.65	10.2	100	8.4
7	3.79	193	72	0.5	0.289	0.817	49.6	84.9	4.59	0.44	13.6	100	8.7
8	3.50	182	48	-0.5	-0.289	-0.817	61.2	78.1	5.90	0.82	17.6	100	17.6
9	3.79	182	48	0.5	-0.289	-0.817	63.3	81.0	3.88	0.55	16.8	100	7.1
10	3.64	198	48	0	0.577	-0.817	47.9	85.8	2.99	0.39	13.2	100	6.1
11	3.50	193	72	-0.5	0.289	0.817	62.7	81.0	6.03	0.84	12.0	98.6	23.3
12	3.64	177	72	0	-0.577	0.817	66.6	77.6	4.86	0.71	15.0	85.5	24.8
13	3.64	188	60	0	0	0	52.5	82.6	4.90	0.68	11.5	100	16.0
14	3.64	188	60	0	0	0	51.3	81.9	5.05	0.83	12.2	100	16.6
15	3.64	188	60	0	0	0	49.5	82.6	4.69	0.68	11.7	100	15.3

Table 5. Regression coefficients and statistical parameters measuring the correlation and significance of the models.

Parameter	y_1	y_2	y_3	y_4	y_5	y_6	y_7
b_0	51.09***	82.38***	4.878***	0.7302***	11.80***	1.000***	15.98***
b_1	-4.534*	2.523**	-2.044***	-0.3120***	1.073***	0.05947	-7.678***
b_2	-6.688**	5.542***	-0.5806**	-0.1354*	-3.635***	0.09483	-9.716***
b_3	1.327	-0.3119	0.5543**	0.04723	-1.421***	-0.03236	5.304**
b_{11}	4.514	-8.180***	1.033**	0.1031	7.518***	-0.1714	1.644
b_{22}	1.125	-8.918***	0.5290	-0.1860	7.166***	0.1291	3.912
b_{33}	-9.722	3.803	0.1657	-0.01332	-1.061	-0.03727	-3.840
b_{12}	6.750	-2.465	0.06686	-0.1817	4.630***	-0.05820	0.6747
b_{13}	3.524	1.307	1.325**	0.06334	0.2682	0.1376	-2.166
b_{23}	8.377*	1.196	-0.5329	-0.1379	1.292**	0.01778	-2.634
R^2	0.806	0.963	0.969	0.873	0.995	0.673	0.948
F	2.30	14.6	17.4	3.82	110	1.14	10.2
Significance level	92%	> 99%	> 99%	98%	> 99%	< 80%	> 99%

** Coefficients significant at the 99% confidence level; ** Coefficients significant at the 95% confidence level; * Coefficients significant at the 90% confidence level

FIGURE LEGENDS

Figure 1: Scheme of the autohydrolysis-delignification process considered in this work.

Figure 2: Calculated dependence of the combined yield (Y_C, kg ADW/100 kg raw material, on dry basis) on autohydrolysis severity (So, dimensionless) and delignification temperature (T_D, ºC). Results calculated from media containing 60 kg ethanol/100 kg liquor.

Figure 3: Calculated dependence of glucan content of ADW (Gn, kg glucan/100 kg ADW, on dry basis) on autohydrolysis severity (So, dimensionless) and delignification temperature (T_D, ºC). Results calculated from media containing 60 kg ethanol/100 kg liquor.

Figure 4: Calculated dependence of Klason lignin content of ADW (KL, kg Klason lignin /100 kg ADW, on dry basis) on autohydrolysis severity (So, dimensionless) and delignification temperature (T_D, ºC). Results calculated from media containing 60 kg ethanol/100 kg liquor.

Figure 5: Contour lines calculated for variables Rec_{Gn} (kg de Gn in ADW/100 kg Gn in raw material, on dry basis), Rec_{KL} (kg KL en ADW/100 kg KL in raw material, on dry basis), CGC_{24} (cellulose to glucose conversion after 24 h) and CGC_{72} (cellulose to glucose conversion after 72 h). Results calculated from media containing 60 kg ethanol/100 kg liquor.

Figure 6: Summary of material balances for selected ranges of the most influential independent variables.

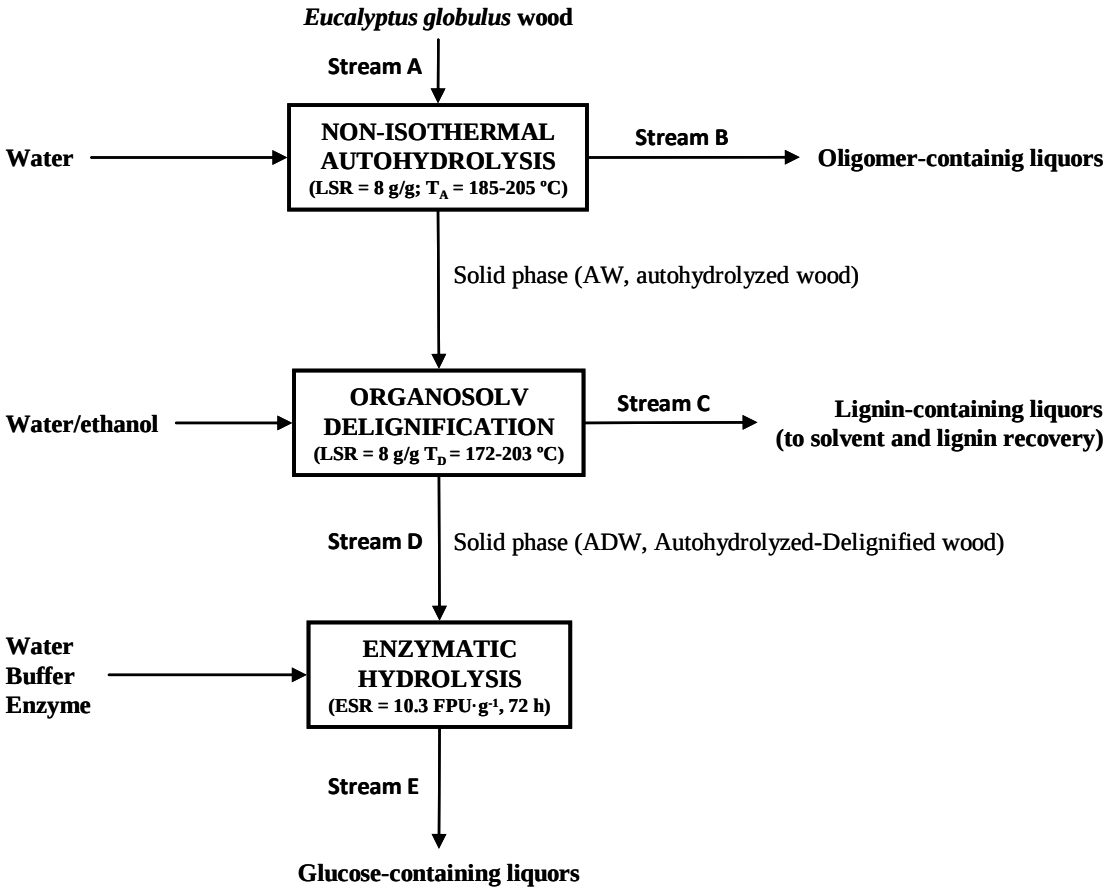
Eucalyptus globulus wood
Stream A
NON-ISOTHERMAL AUTOHYDROLYSIS
(LSR = 8 g/g; T_A = 185-205 ºC)
Water
Stream B
Oligomer-containig liquors
Solid phase (AW, autohydrolyzed wood)
ORGANOSOLV DELIGNIFICATION
(LSR = 8 g/g T_D = 172-203 ºC)
Water/ethanol
Stream C
Lignin-containing liquors
(to solvent and lignin recovery)
Stream D
Solid phase (ADW, Autohydrolyzed-Delignified wood)
ENZYMATIC HYDROLYSIS
(ESR = 10.3 FPU·g^{-1}, 72 h)
Water
Buffer
Enzyme
Stream E
Glucose-containing liquors

Figure 1

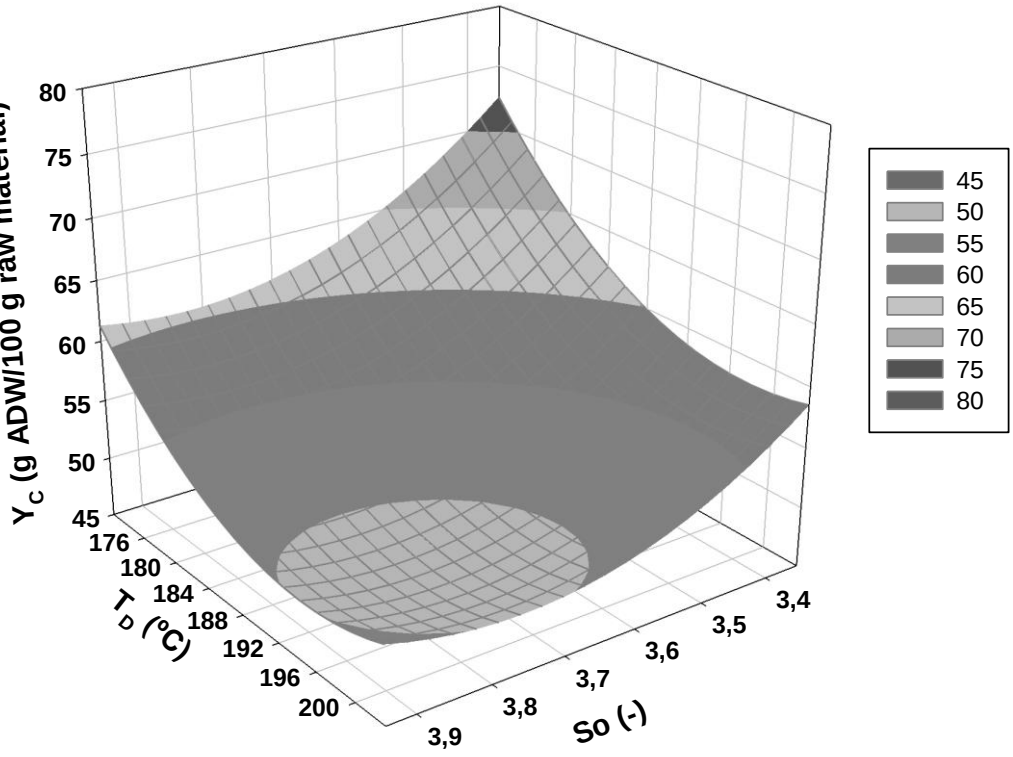
80
75
70
65
60
55
50
45
Y_C (g ADW/100 g raw material)
176
180
184
188
192
196
200
T_D (°C)
3,9
3,8
3,7
3,6
3,5
3,4
So (-)
45
50
55
60
65
70
75
80

Figure 2

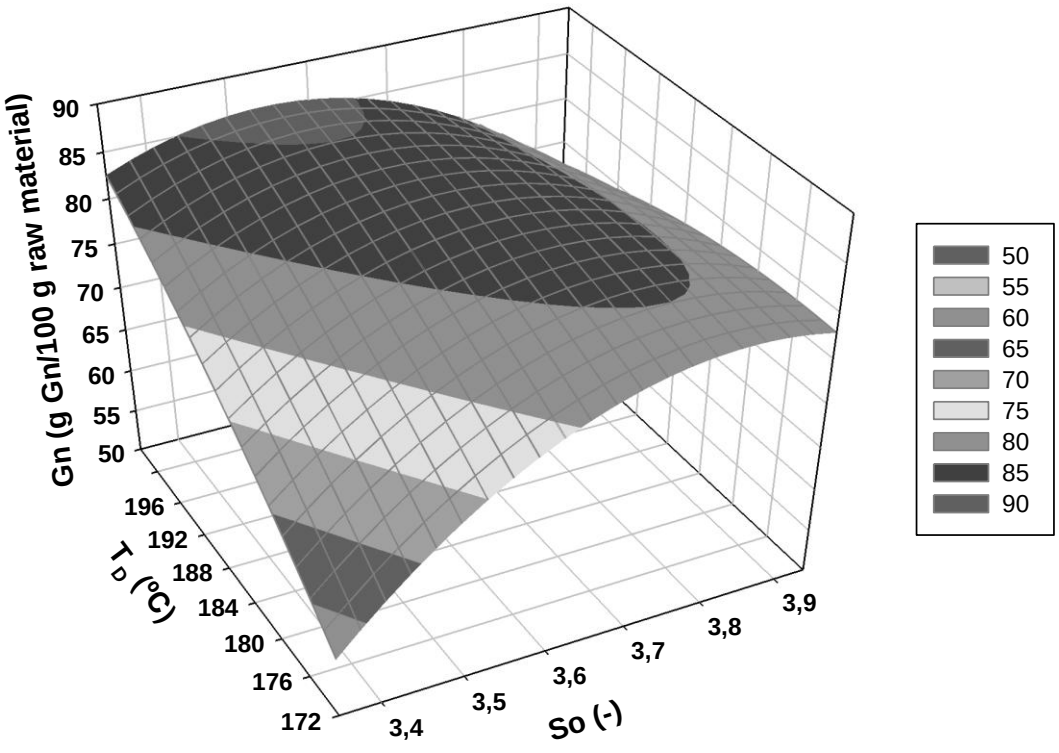
Gn (g Gn/100 g raw material)
90
85
80
75
70
65
60
55
50
196
192
188
184
180
176
172
Td (°C)
3,4
3,5
3,6
3,7
3,8
3,9
So (-)
50
55
60
65
70
75
80
85
90

Figure 3

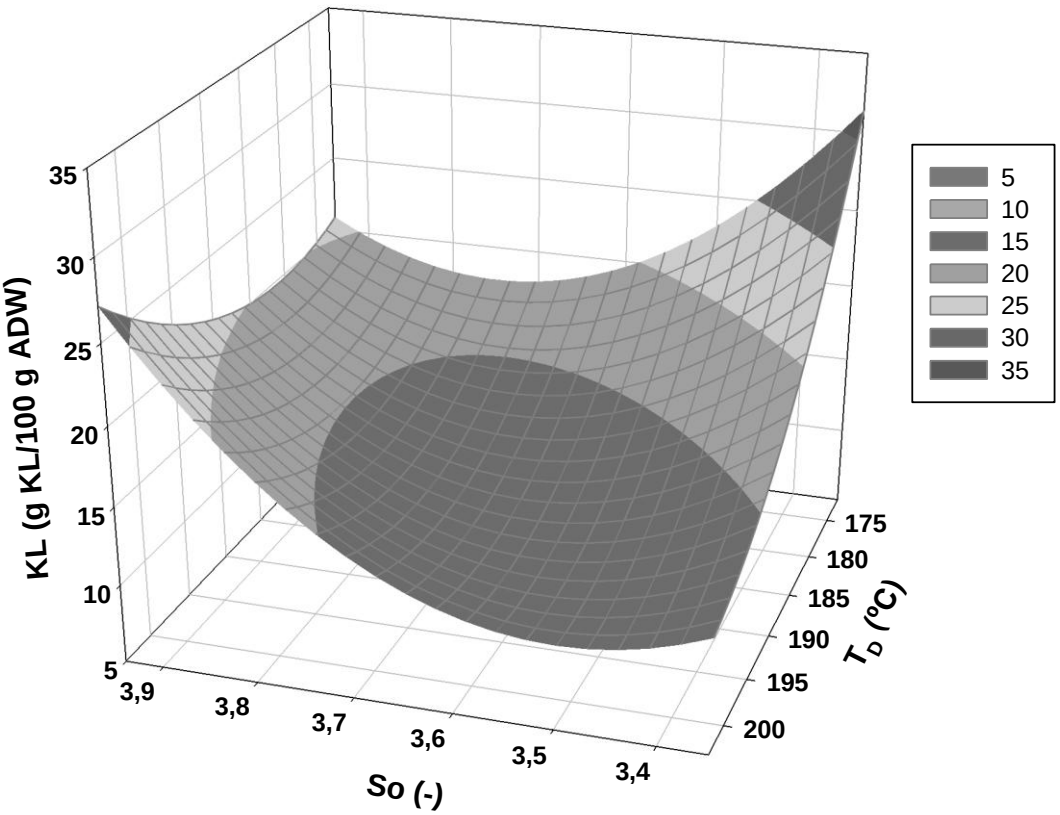
KL (g KL/100 g ADW)
35
30
25
20
15
10
5
3,9
3,8
3,7
3,6
3,5
3,4
So (-)
175
180
185
190
195
200
T_D (°C)
5
10
15
20
25
30
35

Figure 4

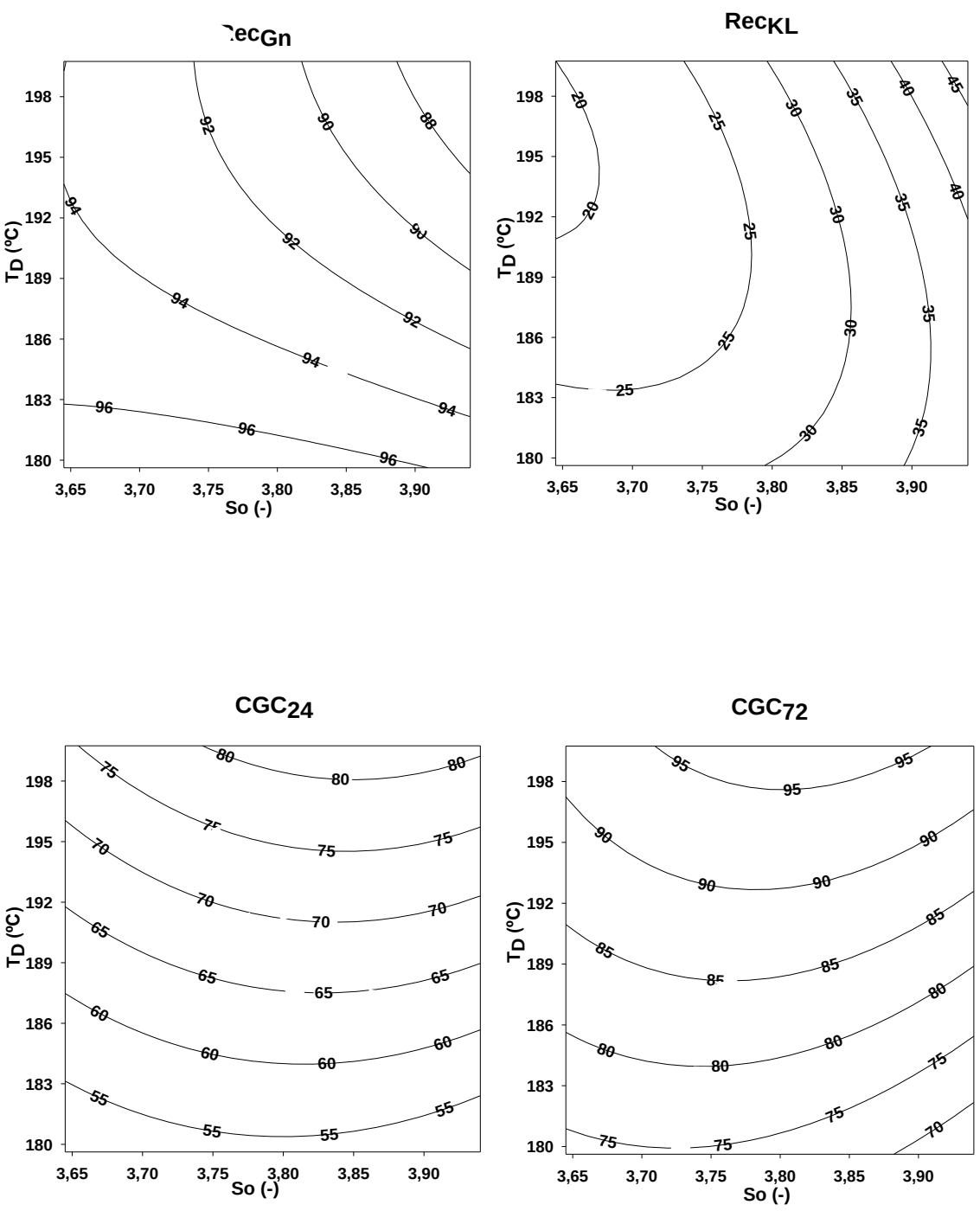
RecGn
RecKL
CGC24
CGC72
TD (ºC)
So (-)
198
195
192
189
186
183
180
3,65
3,70
3,75
3,80
3,85
3,90

Figure 5

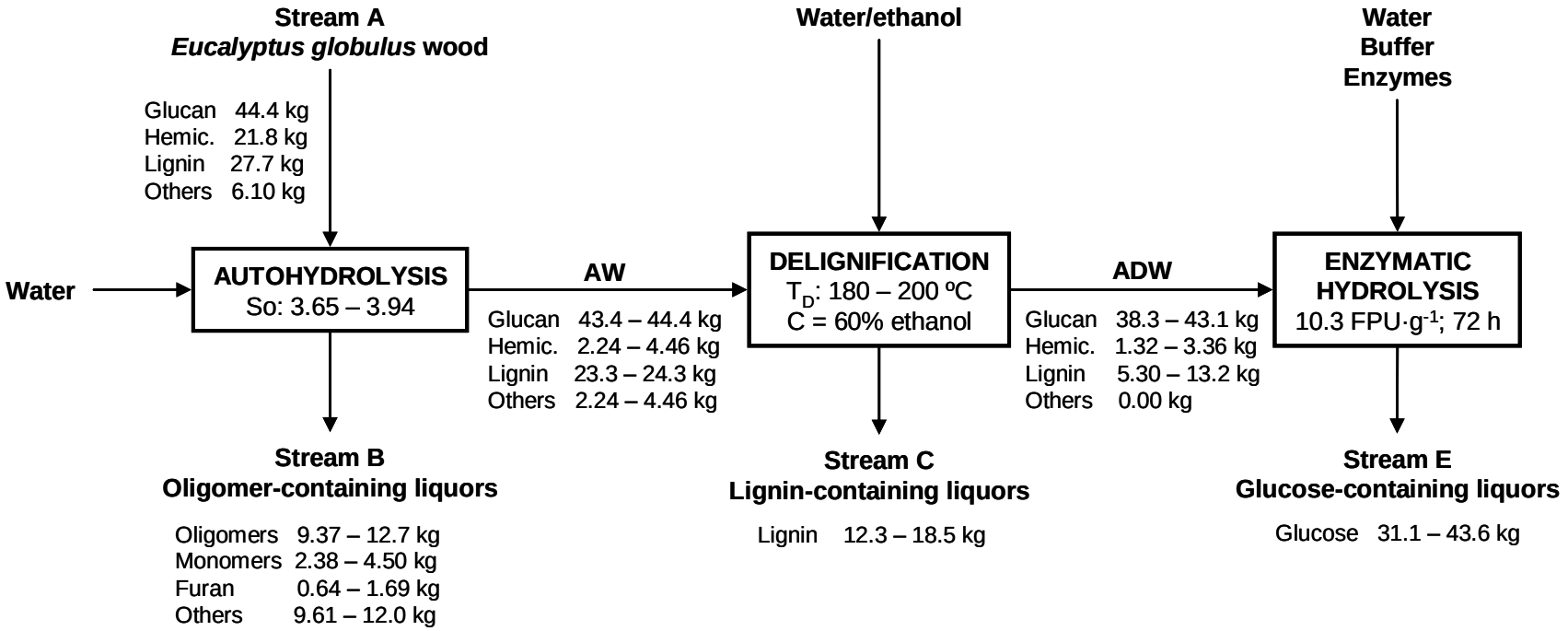
Stream A
Eucalyptus globulus wood
Glucan 44.4 kg
Hemic. 21.8 kg
Lignin 27.7 kg
Others 6.10 kg
Water/ethanol
Water
Buffer
Enzymes
Water
AUTOHYDROLYSIS
So: 3.65 – 3.94
AW
Glucan 43.4 – 44.4 kg
Hemic. 2.24 – 4.46 kg
Lignin 23.3 – 24.3 kg
Others 2.24 – 4.46 kg
DELIGNIFICATION
T_D: 180 – 200 ºC
C = 60% ethanol
ADW
Glucan 38.3 – 43.1 kg
Hemic. 1.32 – 3.36 kg
Lignin 5.30 – 13.2 kg
Others 0.00 kg
ENZYMATIC HYDROLYSIS
10.3 FPU·g^{-1}; 72 h
Stream B
Oligomer-containing liquors
Oligomers 9.37 – 12.7 kg
Monomers 2.38 – 4.50 kg
Furan 0.64 – 1.69 kg
Others 9.61 – 12.0 kg
Stream C
Lignin-containing liquors
Lignin 12.3 – 18.5 kg
Stream E
Glucose-containing liquors
Glucose 31.1 – 43.6 kg

Figure 6