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Eucalyptus globulus wood fractionation by autohydrolysis and organosolv delignification

Aloia Romaní [a,b], Gil Garrote [a,b,*], Francisco López [c], Juan Carlos Parajó [a,b]

[a] Department of Chemical Engineering, Faculty of Science, University of Vigo (Campus Ourense), As Lagoas, 32004 Ourense, Spain
[b] CITI (Centro de Investigación, Transferencia e Innovación) – University of Vigo, Tecnopole, San Cibrao das Viñas, Ourense, Spain
[c] Department of Chemical Engineering, Faculty of Experimental Sciences, Carmen Campus, University of Huelva, Av. 3 de Marzo S/N, 21071 Huelva, Spain

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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 organosolv pulping 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 methods, which enabled the identification of the most favourable operational conditions.

1. Introduction

Lignocellulosic materials (LCM) are a basic resource for sustainable development. The biomass refinery approach is based on the selective separation of the major LCM components (cellulose, hemicelluloses and lignin), enabling their separate utilization for defined purposes. This conceptual framework enables an efficient utilization of LCM.

In biorefineries, feedstocks are subjected to successive processing stages, achieving an integral benefit (for example, in the production of biofuels and/or chemicals) with minimal or no waste generation (FitzPatrick et al., 2010; Michels and Wagemann, 2010; Zhang, 2008).

In biorefineries producing second-generation biofuels by enzymatic hydrolysis-fermentation, the fractionation stages enhance the susceptibility of the solid substrates to enzymatic hydrolysis, playing the same role of as 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 feedstocks; 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. This technology leads to a liquid phase rich in hemicellulose-derived sugars or oligomers without causing significant dissolution of cellulose and lignin (Garrote et al., 1999). Operating under optimized autohydrolysis conditions, most hemicelluloses can be recovered in liquors 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, limited cellulase susceptibility has been reported for *Eucalyptus* wood autohydrolyzed under the conditions leading to maximum oligosaccharide production (Romaní et al., 2010a).

Ethanol–water solutions have been employed for LCM pulping (Díaz et al., 2004), sometimes 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.

* Corresponding author. Tel.: +34 988397075; fax: +34 988387001.
E-mail address: gil@uvigo.es (G. Garrote).

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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 as a method for obtaining: i) valuable products derived from hemicelluloses (hemicellulosic saccharides); ii) solvent-soluble, sulphur-free lignin fragments; and iii) cellulase-susceptible solids suitable as substrates for the manufacture of second-generation bioethanol.

2. Methods

2.1. Raw Material

E. 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 of raw material and solid samples

E. globulus wood samples were milled to particle size less than 0.5 mm, and analyzed for extractives (TAPPI T-264-cm-97 method), moisture (TAPPI T-264-cm-97 method), and ashes (T 211 om-93 method). Additional samples were subjected to extractions and quantitative acid hydrolysis (QAH) (T-249-cm-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 min^{-1}. The solid residue from QAH was considered as Klason lignin (without correction for ash).

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 *E. globulus* wood (denoted AW) and solids resulting from autohydrolysis-delignification of *E. 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 *E. 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 °C, 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:

$$\mathrm{So} = \log \mathrm{Ro} = \log[\mathrm{Ro}_{\mathrm{HEATING}} + \mathrm{Ro}_{\mathrm{COOLING}}] = \log\left[\int_0^{t_{\mathrm{MAX}}} \frac{T(t) - T_{\mathrm{REF}}}{\omega} \times \mathrm{d}t + \int_{t_{\mathrm{MAX}}}^{t_{\mathrm{F}}} \frac{T'(t) - T_{\mathrm{REF}}}{\omega} \times dt\right] \quad (1)$$

where Ro is the severity factor, t_{MAX} (min) is the time needed to achieve T_A (°C), t_F (min) is the time needed for the whole heating–cooling period, and $T(t)$ and $T'(t)$ stand for the temperature profiles in heating and cooling, respectively. Calculations were made assuming reported data for ω and T_{REF} (14.75 °C and 100 °C, respectively). The So 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 121 °C for 30 min, filtered through 0.45 μm membranes, and analyzed by HPLC. The concentrations of oligomers were calculated on the basis of the increase in monosaccharide concentrations achieved upon posthydrolysis. The results obtained in autohydrolysis are shown in Table 1.

2.4. Organosolv delignification of autohydrolyzed E. globulus wood

AW samples were subjected to organosolv delignification with ethanol–water solutions at a LSR of 8 g liquor/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 hydrolyses of ADW were carried out at 48.5 °C 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 employed were LSR = 25 kg liquid/kg oven-dry ADW, enzyme to substrate ratio (denoted as ESR) = 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), data corresponding to individual experiments were fitted to the following equation (Holtzapple et al., 1984):