Hydrothermal and Acid Pretreatments Improve Ethanol Production from Lignocellulosic Biomasses

Danila Morais de Carvalho,[a,b,*] José Humberto de Queiroz,[c] and Jorge Luiz Colodette [a]

Hydrothermal and acid pretreatments using different acid charges (1.5%, 3.0%, and 4.5% H_2SO_4) were proposed for eucalyptus, sugarcane bagasse, and sugarcane straw prior to their bioconversion into ethanol using the semi-simultaneous saccharification and fermentation (SSSF) process. The hydrothermal and acid pretreatments were efficient for hemicelluloses removal from eucalyptus (63 to 96%), bagasse (25 to 98%), and straw (23 to 95%) and to remove a substantial amount of lignin from eucalyptus (10 to 34%) and bagasse (10 to 27%). During pretreatments, pseudo-extractives and pseudo-lignin were generated from biomasses. The SSSF was performed in pretreated biomasses using 24 h presaccharification followed by an additional 10 h of simultaneous saccharification and fermentation (SSF). With hydrothermal pretreatment, the eucalyptus presented the highest ethanol production, but only low values for SSSF parameters were obtained, as follows: ethanol yield (0.017 $g_{ethanol}/g_{biomass}$), volumetric productivity of ethanol (0.16 g L^{-1} h^{-1}), and ethanol concentration (1.6 g L^{-1}). On the other hand, using acid pretreatment, the straw (pretreated using 4.5% H_2SO_4) presented the highest ethanol production among the biomasses, assessed based on ethanol yield (0.056 $g_{ethanol}/g_{biomass}$), volumetric productivity of ethanol (0.51 g L^{-1} h^{-1}), and ethanol concentration (5.1 g L^{-1}).

Keywords: Acid pretreatment; Eucalyptus wood; Hydrothermal pretreatment; Semi-simultaneous saccharification and fermentation (SSSF); Sugarcane bagasse; Sugarcane straw

Contact information: a: Pulp and Paper Laboratory, Department of Forestry Engineering, Federal University of Viçosa, Av. P. H. Rolfs, s/n, Campus, 36570-900 Viçosa, Minas Gerais, Brazil; b: Department of Fibre and Polymer Technology, KTH, Royal Institute of Technology, Teknikringen 56-58, SE-100 44 Stockholm, Sweden; c: Department of Biochemistry, Federal University of Viçosa, Av. P. H. Rolfs, s/n, Campus, 36570-900 Viçosa, Minas Gerais, Brazil;
** Corresponding author: carvalho.danila@gmail.com*

INTRODUCTION

Bioethanol is the most promising biofuel to replace fossil-based fuels. The high octane number (108) of ethanol, which means the compression that this fuel can withstand before detonation, combined with its low cetane number (8) makes the bioethanol suitable to be used in neat form or blended with gasoline for a gasoline engine. In addition, the low cetane number (8) and the high heat of vaporization (0.91 MJ/kg) of bioethanol may prevent its self-ignition in a diesel engine (Demirbaş 2005; Balat *et al*. 2008; Balat and Balat 2009; Demirbas 2009). Bioethanol is a primary alcohol (C_2H_5OH). The oxygen present in its chemical structure improves the combustion process, reducing hydrocarbon, carbon monoxide, and particulate emissions during the burning, but possibly increases nitrogen oxide emission (Balat *et al*. 2008).

Lignocellulosic biomasses are strategic feedstocks for the sustainable production of bioethanol. Several lignocellulosic feedstocks have been investigated for bioethanol

production, including sugarcane bagasse (Laser *et al.* 2002; Dawson and Boopathy 2008; Souza *et al*. 2012; Asakawa *et al*. 2015), sugarcane straw (Oliveira *et al.* 2013), and eucalyptus wood (Romaní *et al.* 2010).

In Brazil, eucalyptus and sugarcane residues stand out as potential feedstocks for bioethanol production due their high production volume. Eucalyptus is a fast-growing genus and largely cultivated in Brazil for many forest-based industries. Sugarcane is one of the main Brazilian agricultural crops; the production of sugarcane bagasse (stalks) and sugarcane straw (leaves and tips) for 2015-16 harvest may be greater than 92 million tons for each residue (Oliveira *et al.* 2013; Conab 2015).

Bioethanol production through biological conversion includes pretreatments, enzymatic hydrolysis, and fermentation (Rubin 2008). Pretreatments and enzymatic hydrolysis disrupt the complex network of cellulose, hemicellulose, and lignin from which lignocellulosic biomasses are formed, which increases the cellulose accessibility for a more efficient enzymatic hydrolysis (Singh *et al.* 2015). Pretreatment conditions have been studied by various authors (Asakawa *et al.* 2015; Silveira *et al.* 2015; Singh *et al.* 2015; Carvalho *et al.* 2016). Acidic conditions, such as hydrothermal and dilute acid, are great alternatives for increasing cellulose accessibility and hemicelluloses removal, as well as inducing chemical changes in lignin and cellulose (Sun and Cheng 2005; Lee *et al*. 2010). Similar reactions occur in hydrothermal and acid pretreatment, but to a lesser extent during hydrothermal pretreatments (Carvalho *et al.* 2015).

Hydrothermal pretreatment requires no additional chemical besides water (Garrote *et al.* 2007; Vegas *et al.* 2008). This pretreatment is usually performed at higher temperature (150 to 250 °C), under pressure, during residence time from a minute to an hour (Laser *et al.* 2002; Lee *et al.* 2010; Romaní *et al.* 2010). During hydrothermal pretreatment, hydronium ions are released from water and acidic species from hemicelluloses, which results in the chemical transformation in biomasses (Parajó *et al.* 2004; Lee *et al.* 2009). Laser *et al*. (2002) and Romaní *et al*. (2010) highlighted hydrothermal pretreatment as a promising method for eucalyptus and sugarcane bagasse, respectively. From a chemical point of view, the hemicellulose from eucalyptus is a better source of acid groups than those from sugarcane bagasse or straw (Morais de Carvalho *et al.* 2017).

Similar reactions occur during hydrothermal and acid pretreatment, with the difference that in acid pretreatment an external acid source is required. Sulfuric acid is usually used as the source of acid. Acid pretreatments are performed at higher temperature (100 to 200 °C), under pressure during residence time from 2 to 300 min, and sulfuric acid concentration from 0.6% to 6.0% (w/w) (Esteghlalian *et al.* 1997; Aguilar *et al.* 2002; Lloyd and Wyman 2005; Saha *et al.* 2005; Sun and Cheng 2005).

Aguilar *et al*. (2002) observed about 90% of xylan removal from sugarcane bagasse, low cellulose degradation, and by-products formation using sulfuric acid concentration and reaction time of 2% and 24 min, respectively, in acid pretreatment at 122 °C. One disadvantage of acid pretreatment is the need for pH neutralization before enzymatic hydrolysis (Taherzadeh and Karimi 2007).

After pretreatments, biomasses are converted into bioethanol. Semi-simultaneous saccharification and fermentation (SSSF) has been studied as a promising arrangement of enzymatic hydrolysis and fermentation process for ethanol production (Gonçalves *et al.* 2014; Cotana *et al.* 2015). SSSF is divided in two steps: 1) pure enzymatic hydrolysis, also called presaccharification, in which a number of enzymes (cellulases) attack both amorphous and crystalline structure of cellulose, breaking it down into cellobiose and from

cellobiose to glucose; and 2) simultaneous saccharification and fermentation (SSF), in which enzymes (for saccharification) and yeast (for fermentation) are kept in the same reactor, and the reactions for enzymatic hydrolysis and fermentation occur at the same time, resulting in ethanol production, mainly from the glucose released. The presaccharification performed in SSSF is important to supply glucose in concentrations high enough to activate the yeast at the beginning of fermentation (Santos *et al.* 2010). Presaccharification improves ethanol yield and reduces enzyme inhibition (such as glucose and cellobiose in high concentration) and fermentation time (Gonçalves *et al.* 2014; Baeyens *et al.* 2015; Cotana *et al.* 2015).

The objectives of this study were: (i) to evaluate hydrothermal and acid pretreatments (1.5%, 3.0%, and 4.5% H_2SO_4) on the chemical composition of eucalyptus, sugarcane bagasse, and sugarcane straw and (ii) to evaluate the efficiency of bioethanol production from pretreated biomasses *via* SSSF.

EXPERIMENTAL

Materials

Eucalyptus, sugarcane bagasse, and sugarcane straw were used for ethanol production. Wood chips from a seven-year-old clonal hybrid of eucalyptus (*Eucalyptus urophylla* × *Eucalyptus grandis*) were supplied by a Brazilian pulp company. Chips were sieved, and those with dimensions lesser than 0.5 cm by 3 cm by 3 cm were collected for chemical analyses and pretreatments. Five-month-old bagasse and straw (cultivar RB867515) were supplied by Center Sugarcane Experimentation (Oratórios, Minas Gerais State, Brazil) at the Federal University of Viçosa after chipping (bagasse and straw) and juice removal (bagasse), generating particles of 10 mm in diameter. Biomasses were dried at room temperature until reaching about 85% dryness and stored in polyethylene bags at room temperature prior to use. Moisture content was determined according to TAPPI T 264 cm-07 (2007). The chemicals used were sulfuric acid 95 to 97% (Merck Milipore, Germany), commercial cellulase Celluclast 1.5 L (from *Trichoderma reesei* ATCC 26921) (Sigma-Aldrich, Brazil), and *Saccharomyces cerevisiae* LBM-1 isolated from fermentation vats in Brazil.

Hydrothermal and Acid Pretreatments

One-hundred grams of eucalyptus wood, sugarcane bagasse, and sugarcane straw were subjected to hydrothermal (using only water) and acid pretreatments using three acid charges: (i) 1.5% H_2SO_4 (w/w H_2SO_4/biomass); (ii) 3.0% H_2SO_4 (w/w H_2SO_4/biomass); and (iii) 4.5% H_2SO_4 (w/w H_2SO_4/biomass). Pretreatments were performed in duplicate in a Regmed reactor, with a rotating pressure vessel (2 L capacity) using the following parameters: liquor:biomass ratio of 2:1 L kg^{-1} for eucalyptus and 7:1 L kg^{-1} for bagasse and straw (these last biomasses are more porous than eucalyptus and absorb more liquor, requiring a higher liquor:biomass ratio during pretreatments); maximum temperature of 175 °C; heating time of 90 min; and time at maximum temperature of 15 min. Severity factor for hydrothermal pretreatments and combined severity factor for acid pretreatments were calculated according to Eqs. 1 and 2 (Lloyd and Wyman, 2005) and the values are summarized in Table 1.

$$SF = \log\left[t \times exp\left(\frac{T - 100}{14.75}\right)\right] \quad (1)$$

where *SF* is severity factor, *t* is reaction time of pretreatment (min), and *T* is temperature of pretreatment (°C).

$$CSF = SF - pH \quad (2)$$

In Eq. 2, C*SF* is combined severity factor, *SF* is severity factor, and *pH* is the initial pH of liquor of acid pretreatments in terms of sulfuric acid concentration (the additional acidity from biomass was not taken into consideration for CSF calculation).

Table 1. Severity Factor and Combined Severity Factor of Pretreatments

Biomasses	Hydrothermal*	Acid 1.5%**	Acid 3.0%**	Acid 4.5%**
Eucalyptus	3.38	2.57	2.87	3.05
Bagasse	3.38	2.03	2.33	2.50
Straw	3.38	2.03	2.33	2.50
*Severity factor (SF) **Combined severity factor (CSF)				

After the pretreatment, the reactor was cooled, and a sample of the liquor was collected for pH measurement. The pretreated biomasses were washed with an excess of water until complete liquor removal and then they were dewatered. The pretreated biomasses were conditioned for 24 h at 23 ± 1 °C and 50 ± 2% relative humidity to a constant weight and then stored at room temperature in polyethylene bags.

Presaccharification Tests

Pretreated eucalyptus wood had a low enzymatic hydrolysis rate, probably associated to the particle size of wood chips. A way to improve enzymatic hydrolysis was by converting pretreated eucalyptus into 20/80-mesh sawdust by using a Wiley mill bench model. Bagasse and straw were not subjected to additional grinding in order to generate results that were more realistic relative to the industrial application. The commercial cellulase preparation Celluclast 1.5 L was used for enzymatic hydrolysis. In a 125 mL Erlenmeyer flask, 4 g of pretreated biomass were suspended in 50.0 mL of citrate buffer (50 mM, pH 4.8) and supplemented with 15 Filter Paper Units (FPU) of enzyme per gram of substrate (1.4 mL) (Souza *et al.* 2012). The flask was capped and incubated in a shaker at 50 °C for 24 h and 180 rpm agitation according to Carvalho *et al*. (2016).

After presaccharification, samples of enzymatic hydrolysis were centrifuged for 10 min at 10,000 × g, and the supernatants were used to evaluate glucose concentration. The glucose concentration in hydrolysates and presaccharification yield were used to select the best acid concentrations for pretreatments. Untreated biomasses were used as controls.

Semi-simultaneous Saccharification and Fermentation (SSSF)

Biomasses pretreated by hydrothermal pretreatment and by the optimal acid pretreatment defined during the presaccharification tests were used for presaccharification followed by SSSF. SSSF was performed in two steps, presaccharification and SSF. In a 125-mL Erlenmeyer flask, 4 g of pretreated biomass were suspended in 50.0 mL of fermentation medium (2.5 g L^{-1} yeast extract; 2.5 g L^{-1} peptone; 2 g L^{-1} NH_4Cl; 1 g L^{-1}

KH_2PO_4; and 0.3 g L^{-1} $MgSO_4.7H_2O$) in citrate buffer (50 mM, pH 4.8) and supplemented with 15 FPU of enzyme per gram of substrate (1.4 mL) (Souza *et al.* 2012). The flask was capped and incubated in a shaker at 50 °C and 180 rpm for 24 h.

During SSF, the yeast cultures (*Saccharomyces cerevisiae* LBM-1) were inoculated after 24 h presaccharification in sterile conditions in the flask from presaccharification, which was capped and incubated in a shaker at 37 °C and 180 rpm for 10 h. After SSSF, samples were centrifuged for 10 min at 10,000 × g, and the supernatants were analyzed for glucose and ethanol concentrations. SSSF was performed in duplicate for untreated and treated biomasses. Untreated biomasses were used as controls.

Determination of Chemical Composition of Biomasses and Pretreatment Parameters

Chemical characterization was performed using 40/60-mesh sawdust, produced by using a Wiley mill bench model. Sawdust was dried at room temperature (23 ± 1 °C and 50 ± 2% relative humidity) to constant a weight and saved in airtight containers. The moisture content was determined according to TAPPI T 264 cm-07 (2007). Chemical analyses for raw material were conducted in triplicate and for pretreated biomasses in duplicate.

The following chemical analyses were performed for determination of chemical composition: ash content (TAPPI 211 om-02 standard 2002), silica content (insoluble part of ash remained after acid hydrolysis with HCl) (TAPPI 244 cm-11 standard 2011), total extractives content (1:2 ethanol-toluene for 5 h → 95% ethanol for 4 h → hot water for 1 h) (TAPPI T 264 cm-07 standard 2007). Klason lignin was determined according to Gomide and Demuner (1986) and corrected by the silica content according to Carvalho *et al*. (2015). Soluble lignin (Goldschimid 1971), anhydrosugars content (glucose, xylose, galactose, mannose, and arabinose) (Wallis *et al.* 1996), uronic acids (Scott 1979), and acetyl groups (Solar *et al.* 1987) were determined as previously described. The complete mass balance for raw materials and pretreated biomasses was calculated according to Carvalho *et al*. (2015).

The pH was determined in the liquor after pretreatments, and the yield was determined gravimetrically on the solid fraction after pretreatments.

Assessment of Ethanol Production and SSSF Parameters

The glucose concentration was determined in samples collected after presaccharification by a high-performance liquid chromatography (HPLC) instrument (Thermo, ACCELLA IR, Analítica, São Paulo, Brazil) with refractive index detector and HPX-87 H / BIORAD column (300 mm by 8.7 mm; BIORAD, São Paulo, Brazil). The mobile phase was water with 0.05 mM sulphuric acid, with a flow rate of 0.5 mL min^{-1}; the column pressure and injected volume were 1200 psi and 20 µL, respectively.

Presaccharification yield was calculated according to Souza *et al.* (2012) (Eq. 3),

$$Y_{G/B} = \frac{Glu_f - Glu_i}{Biomass} \quad (3)$$

where $Y_{G/B}$ is presaccharification yield ($g_{glucose}/g_{biomass}$), Glu_f and Glu_i are the final and initial glucose mass (g) released from glucans compounds during presaccharification, respectively, (measured in samples collected from presaccharification medium), and *Biomass* is total mass of biomass (g) used for presaccharification test.

Glucose yield was calculated according to Eq. 4,

$$G_Y = \frac{Glu_f - Glu_i}{Glu_B} \times 100 \quad (4)$$

where G_Y is glucose yield (%), Glu_f and Glu_i are the final and initial glucose mass (g) released from glucans compounds during presaccharification, respectively (measured in samples collected from presaccharification medium), and Glu_B is the glucan content (measured as glucose) present in the pretreated biomass used for presaccharification test obtained by the complete mass balance (g).

Glucose and ethanol concentrations were determined in samples collected after the SSSF by using a refractive index HPLC detector. Glucose was quantified according to the procedure previous described, and ethanol was measured following conditions: column, HPX-87 H / BIORAD, 300 mm by 8.7 mm diameter; mobile phase, water with 0.05 mM sulphuric acid; flow rate, 0.7 mL min^{-1}; column pressure, 1920 psi; and injected volume, 10 µL.

Bioethanol production was evaluated based on ethanol yield and volumetric productivity of ethanol, according to Eqs. 5 and 6, respectively (Souza *et al*. 2012),

$$Y_{E/B} = \frac{EtOH_f - EtOH_i}{Biomass} \quad (5)$$

where $Y_{E/B}$ is the ethanol yield ($g_{ethanol}/g_{biomass}$), $EtOH_f$ and $EtOH_i$ are the final and initial ethanol mass (g), respectively, (measured in samples collected from fermentation medium), and *Biomass* is total mass of biomass (g) used for SSSF.

$$Q_P = \frac{EtOH_f}{t} \quad (6)$$

In Eq. 6, the quantity Q_P is the volumetric productivity of ethanol (g L^{-1} h^{-1}), $EtOH_f$ is the maximum ethanol concentration achieved (measured in samples collected from fermentation medium), and *t* is the time of fermentation (h).

Means from the same parameters were compared using standard deviation or error bars (tables and figures, respectively). Means from different parameters were compared using coefficient correlation.

RESULTS AND DISCUSSION

Effects of Acid Charge on the Performance of Pretreatments and Chemical Composition of Biomasses

The final pH was preferred to the acid charge itself as a way to evaluate the chemical transformation of the biomass during the pretreatments. The acid charge was the amount of acid added for pretreatments. However, organic acids from polymeric biomass components were also released during pretreatments. As a consequence, the reactions in pretreatments were the result of the combination of external source of acid and the organic acids from the biomass itself. Unlike the acid charge, the pH was more comprehensive and accounted for both acid sources.

Hydrothermal pretreatment was a milder acidic condition for biomass pretreatment, as the only source of acid were the chemical components of biomass itself, especially from hemicelluloses. Eucalyptus wood naturally contains more acid groups in the xylan structure (39 acetyl groups: 100 xylose units) than bagasse (33 acetyl groups: 100 xylose units) and straw (10 acetyl groups: 100 xylose units) (Carvalho 2015; Morais de Carvalho *et al*. 2017). The acetyl groups released from hemicelluloses during hydrothermal pretreatment generated hydronium ions, which catalyzed the hydrolysis of hemicelluloses and other oligosaccharides (Garrote *et al*. 2007; Vegas *et al*. 2008). Considering the conditions used for hydrothermal pretreatments, organic acids released from eucalyptus generated more acid liquor than those generated from bagasse and straw (Table 2).

The decrease in the final pH reduced the total solid yield of pretreatment, irrespective of the biomass. Eucalyptus wood presented higher pretreatment yields than bagasse and straw, regardless of the pretreatment condition. The increased acid charge had a great effect on the pretreatment yield and final pH of pretreatments for bagasse and straw. Surprisingly, in the case of eucalyptus, the acid charge over 1.5% had little impact on final pH of pretreatments and, consequently, on pretreatment yield. One hypothesis was that the organic acids (*e.g*. acetyl groups from xylan) released from eucalyptus reacted with pretreatment liquor, forming a buffer solution. Three types of evidence supported this hypothesis. The first evidence was that, although in the various acid conditions the same acid charge (based on % w/w H_2SO_4/biomass) was used for eucalyptus, bagasse, and straw, the liquor concentration varied between biomasses. The liquor concentration in the various acid conditions was more acid for eucalyptus (liquor:biomass ratio of 2:1) than for bagasse and straw (liquor:biomass ratio of 7:1). This led to the higher values of CSF for acid pretreatments performed for eucalyptus than for bagasse and straw (Table 1). For CSF determination, acidity of biomasses (respect to organic acids) was not being taken into consideration, but definitely it contributed to the kinetics of reactions during pretreatment. Thus, the second evidence was the remarkable effect of organic acids released from eucalyptus on the liquor pH in comparison to bagasse and straw (see values for pH in hydrothermal pretreatments in Table 2). The hypothesis was that in acid conditions, the organic acids from biomasses reacted with the external source of acid (H_2SO_4) to form buffer in the pretreatment liquor. This hypothesis was supported by the third source of evidence, which consisted of the results for bagasse. According to the results, the organic acids released from bagasse generated a hydrothermal liquor with a lower acid concentration (higher pH) than that generated for eucalyptus (considering the pretreatments conditions used in the present study). Therefore, acid pretreatments performed for bagasse required a greater charge of external acid to reach the buffer capacity (apparently occurred between 3.0% and 4.5% acid) (Table 2). A kinetic study dealing with reaction mechanisms between organic acid (from biomasses) and external acids used in pretreatments would provide a better understanding about the formation of buffer solution. However, the elucidation of such mechanisms were not the purpose of the present study. Using the maximum acid charge (4.5% H_2SO_4), the values for final pH of pretreatments were similar for all biomasses as well as the CSF (Tables 1 and 2).

Table 2. Correlation Coefficient between Final pH and Total Solid Yield of Pretreatments

Biomasses	Parameters	Hydrothermal	Acid 1.5%	Acid 3.0%	Acid 4.5%	CC*
Eucalyptus	pH	3.4 ± 0.1	1.3 ± 0.1	1.5 ± 0.1	1.5 ± 0.3	0.97
	Yield (%)	91.5 ± 0.6	80.8 ± 1.0	80.1 ± 2.6	78.5 ± 3.3	
Bagasse	pH	4.5 ± 0.0	2.3 ± 0.0	1.4 ± 0.0	1.2 ± 0.0	0.99
	Yield (%)	84.5 ± 0.5	66.7 ± 2.9	57.5 ± 2.3	50.8 ± 0.1	
Straw	pH	5.3 ± 0.0	4.0 ± 0.0	3.2 ± 0.1	1.5 ± 0.0	0.93
	Yield (%)	80.2 ± 0.8	70.5 ± 0.3	58.2 ± 0.8	55.8 ± 0.3	
*CC, Correlation coefficient between final pH and the total solid yield of pretreatments						

The results indicated that for the conditions of acid pretreatments used in the present study, the CSF should not exceed 2.5. Values for CSF below 2.5 prevented buffer formation in pretreatment liquor and, consequently, improved efficiency of pretreatments.

Hemicelluloses were the main chemical components removed during hydrothermal and acid pretreatments. Hemicellulose removal from biomasses was very important to bioethanol production because it increased the enzyme accessibility to cellulose, which favored the subsequent enzymatic hydrolysis (Alvira *et al*. 2010). Conditions performed at lower pH intensified the hemicellulose removal during pretreatments, irrespective of biomass type (Table 3). For eucalyptus wood, 63%, 95%, 96%, and 95% of hemicelluloses were removed from the raw material by the hydrothermal acid 1.5%, acid 3.0%, and acid 4.5% pretreatments, respectively. In bagasse, the percentages were 25%, 86%, 96%, and 98%, respectively, and in straw, the values were 23%, 56%, 88%, and 95%, respectively. As observed for yield, there was no noticeable impact of acid charge over 1.5% on hemicellulose removal from eucalyptus wood because the final pH was similar for all amounts of acid in the pretreatments.

Our results confirmed those from the literature, which indicated that about 90% hemicellulose was removed from bagasse using 2% sulfuric acid at 122 °C for 24 min (Aguilar *et al*. 2002). Esteghlalian *et al*. (1997) also observed about 90% hemicellulose removal from corn stover, switchgrass, and poplar wood within the first minute of reaction by using 0.9% sulfuric acid at 180 °C.

Hydrothermal and acid pretreatments promoted transformation in lignin structure. It has been claimed that lignin can be removed and/or converted into condensed structures during hydrothermal and acid pretreatments (Lora and Wayman 1978; Alvira *et al*. 2010). During acidic pretreatments, lignin-like compounds, named pseudo-lignin, were generated from lignin and polysaccharide degradation products. The method for the quantification of lignin used in the present study was not able to distinguish lignin from pseudo-lignin, and both compounds were quantified together. Thus, the formation of pseudo-lignin was only demonstrated when the lignin content increased due to pretreatments, regarding to the original biomasses. Previous work showed that in hardwood, pseudo-lignin was preferentially generated when harsher acid conditions were used in pretreatments (Sannigrahi *et al*. 2011; Hu *et al*. 2012). In the present study, however, the formation of pseudo-lignin was also observed for grass plants (bagasse and straw), even in milder acid conditions (hydrothermal pretreatment and acid 1.5%). In the same pretreatment conditions, lignin from eucalyptus was hydrolyzed and removed from biomass. Although the formation of pseudo-lignin may also occur for eucalyptus, in the present study the lignin content decreased due to the pretreatments. The accumulation of pseudo-lignin in bagasse and straw resulted from the combination of low rate of lignin hydrolysis and intense

polysaccharide hydrolysis into degradation products. The explanation for such difference was in the chemical composition of eucalyptus, sugarcane bagasse, and sugarcane straw. Native lignin from bagasse and straw presented a remarkable amount of guaiacyl (G), syringyl (S), and p-hydroxyphenyl (H) units of lignin. In eucalyptus wood, only guaiacyl (G) and syringyl (S) units were present (Brandt *et al.* 2013). Because of methoxy groups in position C3 (for S and G lignin) and C5 (for S lignin), S lignin generated less condensed structures and, consequently, structures more easily removed from lignocellulosic biomasses than G lignin. Similarly, S and G lignins were more reactive and easily removed from lignocellulosic biomasses than H lignin (absence of methoxy groups in the lignin structure) (Santos *et al.* 2011). The higher lignin removal from eucalyptus during pretreatments was probably a result of the most favorable S/G ratio in eucalyptus lignin (2.7) than in bagasse lignin (1.1) or straw lignin (0.5) (Carvalho *et al*. 2015). The presence of pseudo-lignin in biomass used for ethanol production should be minimized, since this compound may impair enzyme activity during saccharification and/or reduce cellulose accessibility (Sannigrahi *et al*. 2011; Kumar *et al*. 2013).

Table 3. Correlation Coefficients between Final pH of Pretreatments and the Amount of Hemicelluloses, Lignin/Pseudo-Lignin, Glucan, and Extractives/Pseudo-Extractives Remaining in the Pretreated Biomasses

Biomasses	Parameters	Raw materials	Hydrothermal	Acid 1.5%	Acid 3.0%	Acid 4.5%	CC**
Eucalyptus	Final pH	-	3.4	1.3	1.5	1.5	-
	Hemicellulose*, g	20.3	7.5	1.0	0.8	1.0	0.99
	Lignin*, g	27.4	23.1	19.0	18.9	18.0	0.96
	Glucan*, g	49.9	49.4	46.1	46.1	43.8	0.86
	Extractives*, g	2.3	11.3	14.5	13.8	14.8	-0.96
Bagasse	Final pH	-	4.5	2.3	1.4	1.2	-
	Hemicellulose*, g	28.7	21.5	4.1	1.1	0.5	0.99
	Lignin*, g	18.0	21.5	16.1	14.1	13.1	1.00
	Glucan*, g	36.0	36.0	35.7	33.6	29.8	0.73
	Extractives*, g	15.0	4.7	9.9	7.9	6.7	-0.57
Straw	Final pH	-	5.3	4.0	3.2	1.5	-
	Hemicellulose*, g	29.8	22.8	13.1	3.5	1.6	0.93
	Lignin*, g	13.8	17.7	15.9	14.7	15.5	0.72
	Glucan*, g	36.3	32.4	32.4	30.4	29.2	0.93
	Extractives*, g	12.2	3.1	5.5	6.4	6.4	-0.86

* Amount of hemicelluloses, lignin/pseudo-lignin, glucan (various polysaccharides formed by glucose monomers), and extractives/pseudo-extractives present in biomasses after pretreatments expressed in grams (obtained by the combination of complete mass balance and actual yield of each biomass). Hemicelluloses represented by the sum of xylose, galactose, mannose, and arabinose. Only in raw materials hemicelluloses amount also includes uronic acid and acetyl groups. In pretreated biomasses, the amount of lignin refers to lignin + pseudo-lignin and the amount of extractives refers to extractives + pseudo-extractives, which were generated during pretreatments.
** CC, Correlation coefficient between final pH and the different parameters.

The lignin removal from eucalyptus wood was 16% (hydrothermal), 31% (acid 1.5% and 3.0%), and 34% (acid 4.5%) (Table 3). In bagasse, the lignin removal was 10% (acid 1.5%), 22% (acid 3.0%), and 27% (acid 4.5%). In bagasse pretreated by hydrothermal pretreatment and straw pretreated by hydrothermal and various acid pretreatments, pseudo-

lignin was generated. A positive correlation between final pH and amount of lignin remained in biomasses was observed for all biomasses. Only slight variations in lignin removal were observed from acid 1.5% to acid 4.5% for eucalyptus.

During pretreatments, the glucan content in biomasses decreased more slowly than the hemicelluloses or the lignin. Glucan removal increased with decreasing final pH, and the maximum glucan removal for eucalyptus, bagasse, and straw was 12.2%, 17.2%, and 19.5%, respectively (Table 3).

For eucalyptus, the formation of pseudo-extractives was observed in both hydrothermal and acid pretreatments. Pseudo-extractives were structures formed during pretreatments from fragments of lignin and polysaccharides that re-precipitated on fibers. Pseudo-extractives presented similar solubility to extractives from original raw material, so they were quantified together (Carvalho *et al*. 2015). The formation of pseudo-extractives was demonstrated only for eucalyptus because the extractives content increased due to pretreatments, regarding to the original biomass.

A negative correlation between final pH of pretreatments and the quantifiable amount of extractives in biomasses was observed for eucalyptus, bagasse, and straw, irrespective of the pretreatment condition (Table 3). The negative correlation between final pH and extractives/pseudo-extractives content indicated increasing pseudo-extractives formation when harsher acidic conditions were performed in biomasses. As observed for eucalyptus, the amount of extractives increased along with the decreased pH in pretreatments for bagasse and straw. Moreover, the extractives measured in these biomasses after acid pretreatments were higher than that in hydrothermal pretreated materials. These results suggested the formation of pseudo-extractives in bagasse and straw. The acid charge had little effect on the amount of eucalyptus pseudo-extractives above acid 1.5%. This trend was probably due to the formation of buffer in pretreatment liquors from 1.5% to 4.5% acid.

Fragments of polysaccharides and lignin were responsible for the formation of both pseudo-lignin and pseudo-extractives. In bagasse and straw, these fragments were preferentially incorporated in pseudo-lignin structures, whereas in the eucalyptus the fragments formed pseudo-extractives. In both cases, the chemical properties of lignin and polysaccharides (especially xylans) was probably the determinant factor for understanding the formation mechanisms of pseudo-lignin and/or pseudo-extractives. However, this finding needs further investigation.

During pretreatments performed in acid conditions, degradation products such as hydroxymethyl furfural (HMF), vanillin, p-hydroxy benzaldehyde, vanillic acid, ferulic acid, and syringyl alcohols could be generated in biomasses. Analytically, such degradation products would be determined in pretreatment liquor. An effective strategy for removing degradation products from biomasses and, consequently, improve SSF performance was by washing the biomasses after pretreatments (Liu and Chen 2017). In the present study the pretreatment liquor was not investigated regarding to the presence of degradation products because it was not used for SSF.

Semi-simultaneous Saccharification and Fermentation

Presaccharification tests

Unsurprisingly, a positive effect of pretreatment on enzymatic hydrolysis was observed for all tested biomasses (Fig. 1). Glucose release by untreated biomasses during presaccharification was extremely low, at 0.9, 0.7, and 1.4 g L^{-1} for eucalyptus, bagasse, and straw, respectively. The glucose concentration for eucalyptus, bagasse, and straw

hydrothermally pretreated after 24 h presaccharification was 12.9, 13.2, and 11.5 g L^{-1}, respectively.

The maximum glucose release in acid pretreated biomasses occurred at the acid charge of 1.5% for eucalyptus and 4.5% for bagasse and straw. Coincidently, these pretreatments conditions were performed using similar CSF (around 2.5). At these conditions, the glucose concentration for pretreated eucalyptus, bagasse, and straw were 15.7, 20.5, and 22.0 g L^{-1}, respectively. Souza *et al*. (2012) obtained about 20.0 g L^{-1} glucose at 24 h presaccharification of delignified bagasse. They noted a trend of decreasing glucose release rate with increasing time of enzymatic hydrolysis over 24 h.

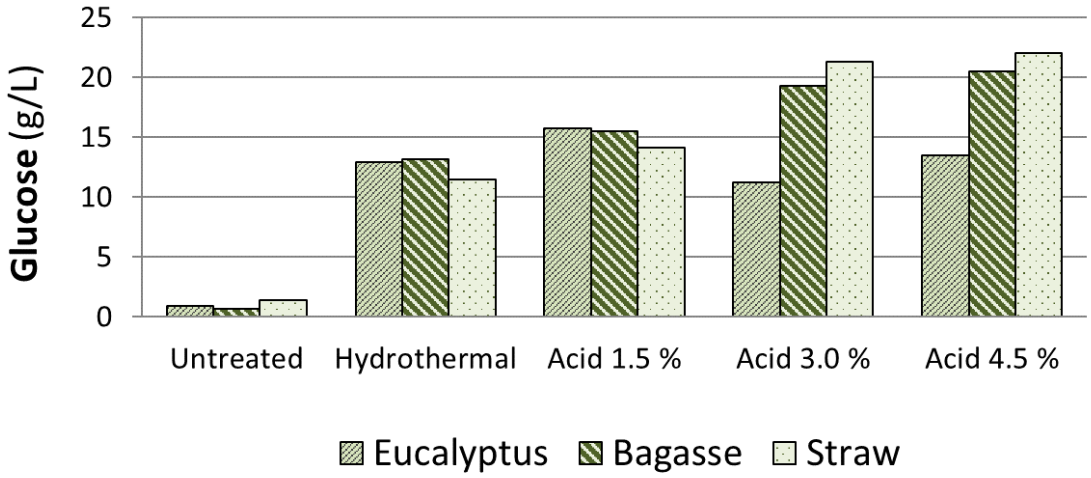

Fig. 1. Glucose concentration (g L^{-1}) released after 24 h presaccharification of pretreated and untreated biomasses

The lower glucose release in untreated biomasses compared to their pretreated counterparts were most likely due to the natural low porosity and, as a consequence, low enzyme accessibility of untreated biomasses. In addition, in untreated biomasses the natural barrier made of lignin and hemicelluloses the cellulose reduced the enzyme activity by limiting the cellulose accessibility. This barrier justified the need for pretreatments to improve sugar release during the process for bioethanol production from lignocellulosic materials (Öhgren *et al*. 2007; Taherzadeh and Karimi 2007).

The presaccharification yield was calculated from glucose concentration after 24 h of presaccharification; thus, similar trends were observed between the aforementioned parameters (Table 4). Presaccharification yields of untreated biomasses were only 0.012, 0.008, and 0.018 $g_{glucose}/g_{biomass}$ for eucalyptus, bagasse, and straw, respectively. The acid charge in acid pretreatment that maximized the glucose release maximized also the presaccharification yield (acid 1.5% for eucalyptus and acid 4.5% for bagasse and straw). An increase in presaccharification yields was observed from hydrothermal to the various acid pretreatment, irrespective of biomass type.

A narrow correlation between the final pH in pretreatments and presaccharification yield was observed for bagasse and straw (Table 4), which suggested that for these biomasses, pH control was an important tool to improve the presaccharification process.

The results implied that chemical transformation of eucalyptus, bagasse, and straw during pretreatments, such as removal of hemicelluloses and/or lignin removal, contributed to the improved cellulose accessibility during presaccharification. One evidence was that

the lower residual hemicellulose/lignin content improved enzymatic saccharification. Untreated straw had lower hemicellulose/lignin content (44%) than bagasse (47%) and eucalyptus (48%) and the higher glucose release during presaccharification. For hydrothermally biomasses, the higher presaccharification yield was observed for eucalyptus, which had only 33% hemicellulose/lignin content, compared to the 51% and 50% presented in bagasse and straw, respectively. In acid pretreatments, the highest presaccharification yields were achieved using eucalyptus (acid 1.5%), bagasse (acid 4.5%), and straw (acid 4.5%) containing 25%, 27%, and 31% hemicellulose/lignin content, respectively.

Table 4. Correlation Coefficient between the Final pH in Pretreatments and Presaccharification Yield

Biomasses	Parameters	Hydrothermal	Acid 1.5%	Acid 3.0%	Acid 4.5%	CC***
Eucalyptus	pH*	3.4	1.3	1.5	1.5	-0.24
	$Y_{G/B}$**, ($g_{glucose}/g_{biomass}$)	0.162	0.198	0.141	0.170	
Bagasse	pH*	4.5	2.3	1.4	1.2	-0.93
	$Y_{G/B}$**, ($g_{glucose}/g_{biomass}$)	0.166	0.195	0.243	0.258	
Straw	pH*	5.3	4.0	3.2	1.5	-0.91
	$Y_{G/B}$**, ($g_{glucose}/g_{biomass}$)	0.145	0.177	0.268	0.277	

Note: All presaccharification tests were performed using buffer at pH 4.8
* Final pH in pretreatments
** Presaccharification yield
*** CC, Correlation coefficient. Negative values indicate negative correlation, *i.e.*, by decreasing pH in pretreatments the presaccharification yield of biomasses increased

Glucose yield represented the amount of glucose, from the total glucans compounds present in biomass, that was released during presaccharification. After presaccharification, the highest glucose yields were observed for biomasses pretreated by acid processes compared to those pretreated by hydrothermal processes (Fig. 2). Straw pretreated by 4.5% acid achieved 52.8% glucose release during pure presaccharification. It is worth mentioning that values for glucose yields were probably higher during the whole process of SSSF, since the glucose was released continuously.

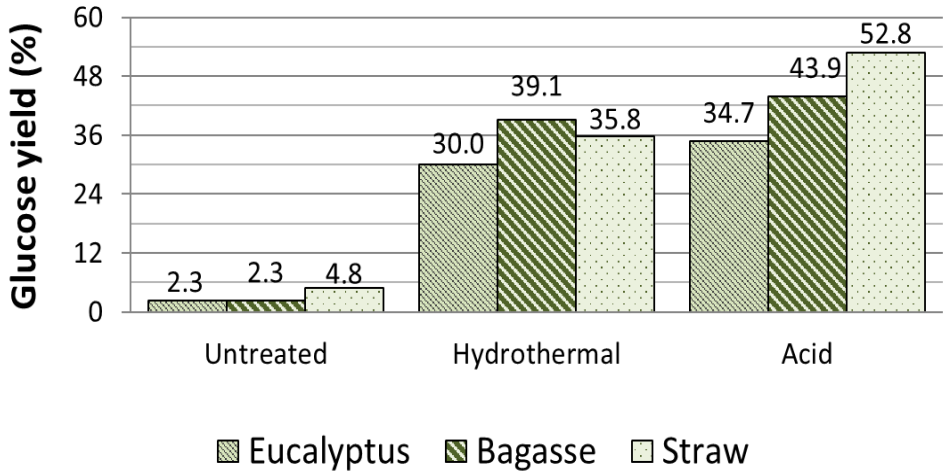

Fig. 2. Glucose yield (G_Y) measured at 24 hours presaccharification for untreated biomasses and biomasses pretreated by hydrothermal and acid pretreatments at optimal acid condition (1.5% acid for eucalyptus and 4.5% acid for bagasse and straw)

Assessment of bioethanol production

As expected, besides improving enzymatic hydrolysis of biomasses, the pretreatments enhanced bioethanol production (Fig. 3). The ethanol yields for bagasse and straw after acid pretreatment (acid 4.5%) were respectively 7.7 and 8.1 times higher than the values after hydrothermal pretreatment. One possible reason was that acid pretreated bagasse and straw had a lower hemicellulose/lignin content than their hydrothermally pretreated counterparts. Another reason was that the pseudo-lignin, a potential yeast inhibitor, was generated in lower amount during acid pretreatments than during hydrothermal pretreatment.

The eucalyptus pretreated by the hydrothermal process generated an ethanol yield 1.2 times higher than the eucalyptus pretreated with 1.5% acid. Unlike what was observed for bagasse and straw, the lower hemicellulose/lignin content in acid-pretreated eucalyptus did not improve the ethanol production. On the other hand, a greater pseudo-extractives content was generated during acid pretreatments. The lower ethanol yield for acid-pretreated eucalyptus together with the greater pseudo-extractives generation suggested their inhibition of yeast activity. These results implied that, although the hemicellulose/lignin content affected the SSSF process, this factor was not a first-order affect. Probably, the first order effect would be better explained by the entire chemical structure of biomasses. Thus, the first order condition would be reflective of the polymer array in the biomass, including specific characteristics such as which polymers would be present and in what amount, how the polymers would be entangled or bound to each other, what type of bond would be present, among others. New structures generated from degradation products during pretreatments should be also included in the first-order affect.

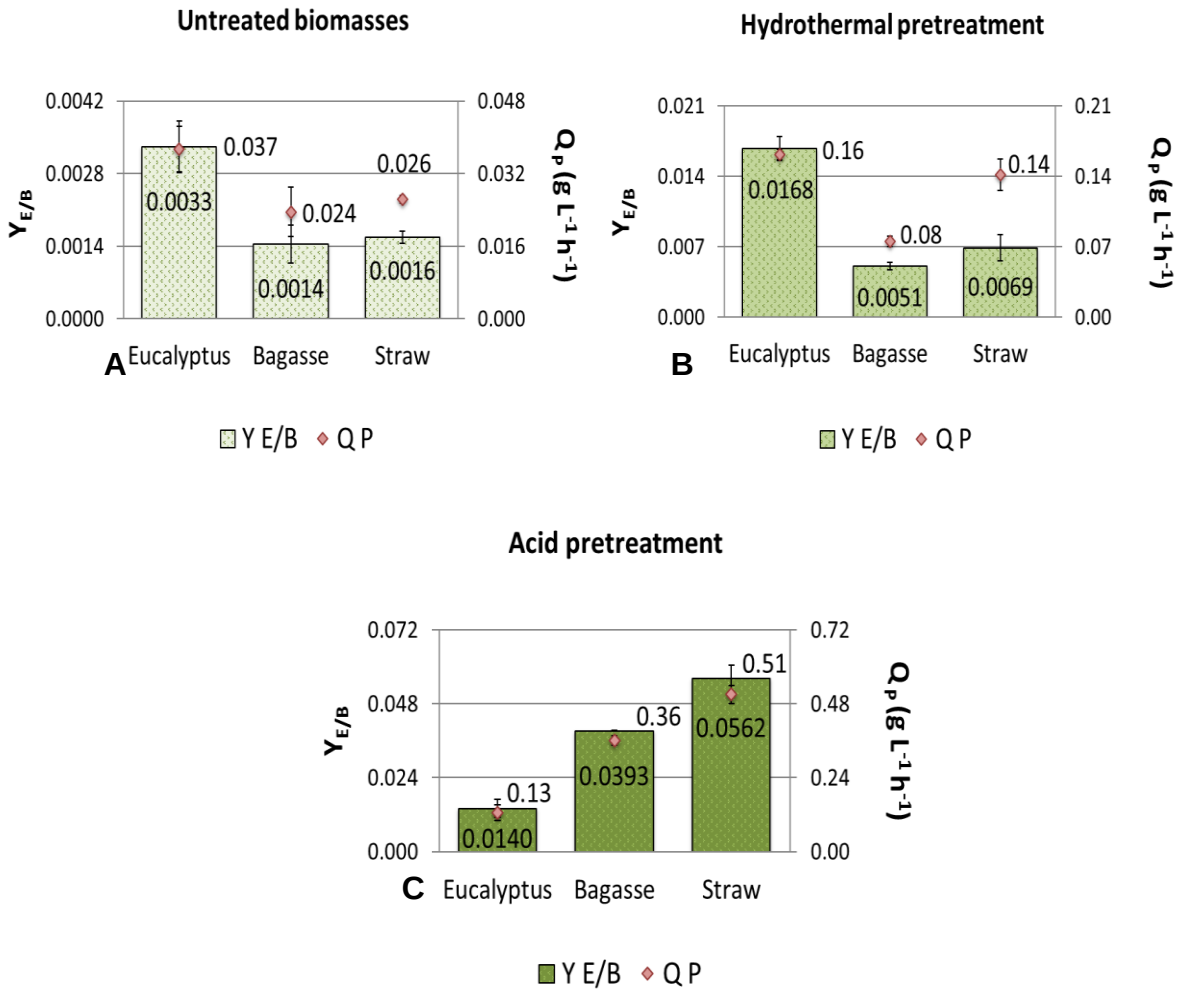

Fig. 3. The column graphic shows the ethanol yield ($Y_{E/B}$), and the scatter graphic shows the volumetric productivity of ethanol (Q_P) after 24 h of presaccharification and 10 h of SSSF for untreated biomasses (**A**) and biomasses pretreated by hydrothermal (**B**) and acid (**C**) pretreatment. Acid pretreatment was performed at 1.5% acid for eucalyptus and 4.5% acid for bagasse and straw.

Untreated eucalyptus and eucalyptus pretreated by hydrothermal process presented ethanol yield and volumetric productivity of ethanol noticeable higher than those from untreated and hydrothermally pretreated bagasse and straw. These results differed from those from presaccharification, in which similar presaccharification yields were observed for untreated biomasses and also between biomasses pretreated by hydrothermal process. On the other hand, results confirmed the effect of the entire chemical structure of pretreated biomasses and of new structures generated during pretreatments on the performance of SSSF and the inhibition of yeast. The lower ethanol production observed for bagasse and straw in comparison to eucalyptus was probably due to the inhibition caused by the substantial amount of pseudo-extractives generated during hydrothermal pretreatment. Eucalyptus, bagasse, and straw pretreated by hydrothermal process produced ethanol yields, respectively, 5.1, 3.6, and 4.3 times higher than their untreated counterparts.

The acid process was more efficient as pretreatment for bioethanol production from bagasse and straw. Ethanol yield was increased by 28.3 and 35.1 times for bagasse and straw, respectively, compared with untreated biomasses. For eucalyptus pretreated by acid,

ethanol yield increased only 4.2 times compared with untreated biomass and also decreased compared to eucalyptus pretreated by hydrothermal process. Straw presented the highest ethanol yield and volumetric productivity of ethanol among the biomasses. Straw pretreated with 4.5% acid produced an ethanol concentration of 5.1 g L^{-1}. Sugarcane bagasse and eucalyptus prepared using acid pretreatments produced only 3.6 g L^{-1} and 1.3 g L^{-1} ethanol, respectively.

Santos *et al*. (2010) achieved an ethanol concentration for sugarcane bagasse almost threefold higher than that obtained in the present study by performing 40 h SSSF (from which 16 h were pure presaccharification); however, these authors used alkaline pretreatment for bagasse delignification, which most likely favored the enzymatic hydrolysis by improving the cellulose accessibility. The volumetric productivity of ethanol achieved by these aforementioned authors for bagasse (0.30 g L^{-1} h-1) was higher than the observed in the present study after hydrothermal pretreatment (0.08 g L^{-1} h^{-1}), but lower than the observed after acid pretreatment (0.36 g L^{-1} h^{-1}). However, these authors considered the time for volumetric productivity of ethanol as the sum of presaccharification time (16 h) and SSSF (24 h). A remarkable value for volumetric productivity of ethanol for delignified bagasse was also observed by Souza *et al*. (2012) (~ 1.1 g L^{-1} h^{-1}) for 24 h presaccharification and 8 h SSSF. This information combined with the results in present study confirmed the observation of Cardoso *et al*. (2013) that residual lignin affected enzymatic hydrolysis more intensely than residual hemicelluloses. The ethanol yield achieved by Souza *et al*. (2012) was higher than that obtained in the present study, but these authors used alkaline pretreatment.

The effect of glucose yield (measured after presaccharification) on SSSF parameters differed between hydrothermal and acid pretreatments. For acid pretreatment, a positive correlation was observed between glucose yield and ethanol yield, as well as between glucose yield after presaccharification and volumetric productivity of ethanol (Table 5). The results indicated similar behavior of enzymatic hydrolysis during presaccharification and SSSF. Surprisingly, for hydrothermal pretreatment a negative correlation was observed between these aforementioned parameters. The differences between presaccharification and SSSF probably resulted from the combined effect of the different chemical composition of biomasses and the inhibition reactions. In addition to have different residual amount of hemicelluloses and lignin, the biomasses also contained a certain amount of potential inhibitors, which were generated during pretreatments (*i.e*., pseudo-lignin and pseudo-extractives). The inhibition effect of pseudo-lignin on enzymatic action was suggested previously (Sannigrahi *et al*. 2011). The effect of pseudo-extractives on presaccharification and SSSF processes for ethanol production was not addressed in the present study and still requires further investigation. However, the increasing pseudo-extractives content in eucalyptus from hydrothermal to acid processes along with the decreasing in ethanol production would be an evidence of a negative effect of the presence of pseudo-extractives on the biochemistry processes.

Table 5. Correlation Coefficient between Glucose Yield (G_Y), Presaccharification and Ethanol Yield ($Y_{E/B}$) and Volumetric Productivity of Ethanol (Q_P) of all Biomasses Tested at Different Pretreatments Applied

Parameters	Eucalyptus	Bagasse	Straw	CC*
Hydrothermal pretreatment				
G_Y, %	30.0	39.1	35.8	-
$Y_{E/B}$, $g_{ethanol}/g_{biomass}$	0.0168	0.0051	0.0069	-0.98
Q_P, g L^{-1} h^{-1}	0.16	0.08	0.14	-0.91
Acid pretreatment				
G_Y, %	34.7	43.9	52.8	-
$Y_{E/B}$, $g_{ethanol}/g_{biomass}$	0.0140	0.0393	0.0562	0.99
Q_P, g L^{-1} h^{-1}	0.13	0.36	0.51	0.99
* CC, Correlation coefficient between G_Y and $Y_{E/B}$ and between G_Y and Q_P. Negative values indicate negative correlation.				

The residual glucose content was similar for all samples (1.9 to 2.7 g L^{-1}), irrespective of pretreatment type. The residual glucose concentrations of this study were lower than those observed by Santos *et al*. (2010) in a similar study considering 16 h presaccharification and 10 h SSSF for delignified bagasse. Souza *et al*. (2012) found no residual glucose after 10 h SSSF.

CONCLUSIONS

1. The increased acidity in pretreatments affected the chemical transformation of biomasses and the ethanol production.
2. Hydrothermal and acid pretreatments were efficient for hemicelluloses removal from eucalyptus (63 to 96%), bagasse (25 to 98%), and straw (23 to 95%). Pretreatments substantially removed lignin from eucalyptus (10% to 34%) and bagasse (10% to 27%), but promoted the formation of potential inhibitors in biomasses (pseudo-extractives and pseudo-lignin).
3. The increased acidity in pretreatments promoted the increased formation of pseudo-extractives.
4. The ethanol production from eucalyptus was higher for biomass pretreated by hydrothermal process than by acid process. Probably, the greater pseudo-extractives content in acid pretreated eucalyptus inhibited the yeast activity during SSSF.
5. Straw pretreated by acid process (4.5% H_2SO_4) presented the highest ethanol yield (0.056 $g_{ethanol}/g_{biomass}$), ethanol concentration (5.1 g L^{-1}), and volumetric productivity of ethanol (0.51 g L^{-1} h^{-1}) among biomasses.

ACKNOWLEDGMENTS

The authors are thankful for the financial support provided by the funding from the European Community's Seventh Framework Program FP7/2007-2013 under the grant agreement number KBBE-2009-3-244362 LignoDeco (EU/Brazil co-operation), the

Coordination for the Improvement of Higher Education Personnel (CAPES), the Brazilian National Council for Scientific and Technological Development (CNPq), and the Science without Borders program (CsF).

REFERENCES CITED

Aguilar, R., Ramírez, J. A., Garrote, G., and Vázquez, M. V. (2002). "Kinetic study of the acid hydrolysis of sugarcane bagasse," *Journal of Food Engineering* 55(4), 309-318. DOI: 10.1016/S0260-8774(02)00106-1

Alvira, P., Tomás-Pejó, E., Ballesteros, M., and Negro, M. J. (2010). "Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review," *Bioresource Technology* 101(13), 4851-4861. DOI: 10.1016/j.biortech.2009.11.093

Asakawa, A., Kohara, M., Sasaki, C., Asada, C., and Nakamura, Y. (2015). "Comparison of choline acetate ionic liquid pretreatment with various pretreatments for enhancing the enzymatic saccharification of sugarcane bagasse," *Industrial Crops and Products* 71, 147-152. DOI: 10.1016/j.indcrop.2015.03.073

Baeyens, J., Kang, Q., Appels, L., Dewil, R., Lv, Y., and Tan, T. (2015). "Challenges and opportunities in improving the production of bio-ethanol," *Progress in Energy and Combustion Science* 47, 60-88. DOI: 10.1016/j.pecs.2014.10.003

Balat, M., and Balat, H. (2009). "Recent trends in global production and utilization of bio-ethanol fuel," *Applied Energy* 86(11), 2273-2282. DOI: 10.1016/j.apenergy.2009.03.015

Balat, M., Balat, H., and Öz, C. (2008). "Progress in bioethanol processing," *Progress in Energy and Combustion Science* 34(5), 551-573. DOI: 10.1016/j.pecs.2007.11.001

Brandt, A., Gräsvik, J., Hallett, J. P., and Welton, T. (2013). "Deconstruction of lignocellulosic biomass with ionic liquids," *Green Chemistry* 15, 550-583. DOI: 10.1039/C2GC36364J

Cardoso, W. S., Tardin, F. D., Tavares, G. P., Queiroz, P. V., Mota, S. S., Kasuya, M. C. M., and Queiroz, J. H. de. (2013). "Use of sorghum straw (*Sorghum bicolor*) for second generation ethanol production: Pretreatment and enzymatic hydrolysis," *Química Nova* 36(5), 623-627. DOI: 10.1590/S0100-40422013000500002

Carvalho, D. M. de. (2015). *Study on the structure and properties of xylan extracted from eucalyptus, sugarcane bagasse and sugarcane straw*, Licentiate's Thesis, Royal Institute of Technology, Stockholm, ON, Sweden.

Carvalho, D. M. de., Queiroz, J. H. de., and Colodette, J. L. (2016). "Assessment of alkaline pretreatment for the production of bioethanol from eucalyptus, sugarcane bagasse and sugarcane straw," *Industrial Crops and Products* 94, 932-941. DOI: 10.1016/j.indcrop.2016.09.069

Carvalho, D. M. de., Sevastyanova, O., Penna, L. S., Silva, B. P. de., Lindström, M. E., and Colodette, J. L. (2015). "Assessment of chemical transformations in eucalyptus, sugarcane bagasse and straw during hydrothermal, dilute acid, and alkaline pretreatments," *Industrial Crops and Products* 73, 118-126. DOI: 10.1016/j.indcrop.2015.04.021

Conab. (2015). "Companhia nacional de abastecimento," (http://www.conab.gov.br/OlalaCMS/uploads/arquivos/16_02_23_17_34_53_boletim_cana_portugues_-_3o_lev_-_15-16.pdf), Accessed 25 January 2015.

Cotana, F., Cavalaglio, G., Gelosia, M., Coccia, V., Petrozzi, A., Ingles, D., and Pompili, E. (2015). "A comparison between SHF and SSSF processes from cardoon for ethanol production," *Industrial Crops and Products* 69, 424-432. DOI: 10.1016/j.indcrop.2015.02.064

Dawson, L., and Boopathy, R. (2008). "Cellulosic ethanol production from sugarcane bagasse without enzymatic saccharification," *BioResources* 3(2), 452-460. DOI: 10.15376/biores.3.2.452-460

Demirbaş, A. (2005). "Bioethanol from cellulosic materials: A renewable motor fuel from biomass," *Energy Sources* 27(4), 327-337. DOI: 10.1080/00908310390266643

Demirbaş, A. (2009). "Political, economic and environmental impacts of biofuels: A review," *Applied Energy* 86(1), S108-S117. DOI: 10.1016/j.apenergy.2009.04.036

Esteghlalian, A., Hashimoto, A. G., Fenske, J. J., and Penner, M. H. (1997). "Modeling and optimization of the diluted-sulfuric-acid pretreatment of corn stover, poplar, and switchgrass," *Bioresource. Technology* 59(2-3), 129-136. DOI: 10.1016/S0960-8524(97)81606-9

Garrote, G., Kabel, M. A., Schols, H. A., Falque, E., Dominguez, H., and Parajó, J. C. (2007). "Effects of *Eucalyptus globulus* wood autohydrolysis conditions on the reaction products," *Journal of Agricultural and Food Chemistry* 55(22), 9006-9013. DOI: 10.1021/jf0719510

Goldschimid, O. (1971). "Ultraviolet spectra," in: *Lignins: Ocurrence, Formation, Structure and Reaction*, K. V. Sarkanen, C. H. Ludwig (ed.), John Wiley & Sons, New York, NY, pp. 241-266.

Gomide, J. L., and Demuner, B. J. (1986). "Determinação do teor de lignina em madeira: método Klason modificado," *O Papel* 47(8), 36-38.

Gonçalves, F. A., Ruiz, H. A., Nogueira, C. C., Santos, E. S., Teixeira, J. A., and Macedo, G. R. (2014). "Comparison of delignified coconuts waste and cactus for fuel-ethanol production by the simultaneous and semi-simultaneous saccharification and fermentation strategies," *Fuel* 131, 66-76. DOI: 10.1016/j.fuel.2014.04.021

Hu, F., Jung, S., and Ragauskas, A. (2012). "Pseudo-lignin formation and its impact on enzymatic hydrolysis," *Bioresource Technology* 117, 7-12. DOI: 10.1016/j.biortech.2012.04.037

Kumar, R., Hu, F., Sannigrahi, P., Jung, S., Ragauskas, A. J., and Wyman, C. E. (2013). "Carbohydrate derived-pseudo-lignin can retard cellulose biological conversion," *Biotechnology Bioengineering* 110(3), 737-753. DOI: 10.1002/bit.24744

Laser, M., Schulman, D., Allen, S. G., Lichwa, J., Antal Jr., M. J., and Lynd, L. R. (2002). "A comparison of liquid hot water and steam pretreatments of sugar cane bagasse for bioconversion to ethanol," *Bioresource Technology* 81(1), 33-44. DOI: 10.1016/S0960-8524(01)00103-1

Lee, J. M., Jameel, H., and Venditti, R. A. (2010). "One and two stage autohydrolysis pretreatments for enzyme hydrolysis of Coastal Bermuda grass to produce fermentable sugars," *BioResources* 5(3), 1496-1508. DOI: 10.15376/biores.5.3.1496-1508

Lee, J. M., Jameel, H., and Venditti, R. A. (2009). "Autohydrolysis pretreatments of Coastal Bermuda grass for increased enzyme hydrolysis," *Bioresource Technology* 100, 6434-6441. DOI: 10.1016/j.biortech.2008.12.068

Liu, Z-H., and Chen H-Z. (2017). "Two-step size reduction and post-washing of steam exploded corn stover improving simultaneous saccharification and fermentation for

ethanol production," *Bioresource Technology* 223, 47-58. DOI: 10.1016/j.biortech.2016.10.049

Lloyd, T. A., and Wyman, C. E. (2005). "Combined sugar yields for dilute sulfuric acid pretreatment of corn stover followed by enzymatic hydrolysis of the remaining solids," *Bioresource Technology* 96(18), 1967-1977. DOI: 10.1016/j.biortech.2005.01.011

Lora, J. H., and Wayman, M. (1978). "Delignification of hardwoods by autohydrolysis and extraction," *TAPPI Journal* 61(6), 47-50.

Morais de Carvalho, D., Martínez-Abad, A., Evtuguin, D. V., Colodette, J. L., Lindström, M. E., Vilaplana, F., and Sevastyanova, O. (2017). "Isolation and characterization of acetylated glucuronoarabinoxylan from sugarcane bagasse and straw," *Carbohydrate Polymers* 156, 223-234. DOI: 10.1016/j.carbpol.2016.09.022

Öhgren, K., Bura, R., Saddler, J., and Zacchi, G. (2007). "Effect of hemicellulose and lignin removal on enzymatic hydrolysis of steam pretreated corn stover," *Bioresource Technology* 98(13), 2503-2510. DOI: 10.1016/j.biortech.2006.09.003

Oliveira, F. M. V., Pinheiro, I. O., Souto-Maior, A. M., Martin, C., Golçalves, A. R., and Rocha, G. J. M. (2013). "Industrial-scale steam explosion pretreatment of sugarcane straw for enzymatic hydrolysis of cellulose for production of second generation ethanol and value-added products," *Bioresource Technology* 130, 168-173. DOI: 10.1016/j.biortech.2012.12.030

Parajó, J. C., Garrote, G., Cruz, J. M., and Dominguez, H. (2004). "Production of xylooligosaccharides by autohydrolysis of lignocellulosic materials," *Trends in Food Science & Technology* 15(3-4), 115-120. DOI: 10.1016/j.tifs.2003.09.009

Romaní, A., Garrote, G., Alonso, J. L., and Parajó, J. C. (2010). "Bioethanol production from hydrothermally pretreated *Eucalyptus globulus* wood," *Bioresource Technology* 101(22), 8706-8712. DOI: 10.1016/j.biortech.2010.06.093

Rubin, E. M. (2008). "Genomics of cellulosic biofuels," *Nature* 454, 841-845. DOI: 10.1038/nature07190

Saha, B. C., Iten, L. B., Cotta, M. A., and Wu, Y. V. (2005). "Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol," *Process Biochemistry* 40(12), 3693-3700. DOI: 10.1016/j.procbio.2005.04.006

Sannigrahi, P., Kim, D. H., Jung, S., and Ragauskas, A. J. (2011). "Pseudo-lignin and pretreatment chemistry," *Energy Environmental Science* 4(4), 1306-1310. DOI: 10.1039/C0EE00378F

Santos, R. B., Capanema, E. A., Balakshin, M. Y., Chang, H.-M., and Jameel, H. (2011). "Effect of hardwoods characteristics on kraft pulping process: Emphasis on lignin structure," *BioResources* 6(4), 3623-3637. DOI: 10.15376/biores.6.4.3623-3637

Santos, J. R. A. dos, Souto-Maior, A. M., Gouveia, E. R., and Martín, C. (2010). "Comparison of SHF and SSF processes from sugar cane bagasse for ethanol production by *Saccharomyces cerevisiae*," *Química Nova* 33(4), 904-908. DOI: 10.1590/S0100-40422010000400027

Scott, R. W. (1979). "Colorimetric determination of hexenuronic acid in plant materials," *Analytical Chemistry* 51(7), 936-941.

Silveira, M. H. L., Morais, A. R. C., Lopes, A. M. da C., Olekszyszen, D. N., Lukasik, R. B., Andreaus, J., and Ramos, L. P. (2015). "Current pretreatment technologies for the development of cellulosic ethanol and biorefineries," *ChemSusChem* 8(20), 3366-3390. DOI: 10.1002/cssc.201500282

Singh, J., Suhag, M., and Dhaka, A. (2015). "Augmented digestion of lignocellulose by steam explosion, acid and alkaline pretreatment methods: A review," *Carbohydrate Polymers* 117, 624-631. DOI: 10.1016/j.carbpol.2014.10.012

Solar, R., Kačĺc, F., and Melcer, I. (1987). "Simple semimicro method for the determination of O-acetyl groups in wood and related materials," *Nordic Pulp & Paper Research Journal* 2(4), 139-141. DOI: 10.3183/NPPRJ-1987-02-04-p139-141

Souza, C. J. A. de, Costa, D. A., Rodrigues, M. Q. R. B., dos Santos, A. F., Lopes, M. R., Abrantes, A. B. P., Costa, P. dos S., Silveira, W. B., Passos, F. M. L., and Fietto, L. G. (2012). "The influence of presaccharification, fermentation temperature and yeast strain on ethanol production from sugarcane bagasse," *Bioresource Technology* 109, 63-69. DOI: 10.1016/j.biortech.2012.01.024

Sun, Y., and Cheng, J. J. (2005). "Dilute acid pretreatment of rye straw and Bermudagrass for ethanol production," *Bioresource Technology* 96(14), 1599-1606. DOI: 10.1016/j.biortech.2004.12.022

Taherzadeh, M. J., and Karimi, K. (2007). "Acid-based hydrolysis processes for ethanol from lignocellulosic materials: A review," *BioResources* 2(3), 472-499. DOI: 10.15376/biores.2.3.472-499

TAPPI T211 om-02 (2002). "Ash in wood, pulp, paper and paperboard: Combustion at 525°C," TAPPI Press, Atlanta, GA.

TAPPI T264 cm-07 (2007). "Preparation of wood for chemical analysis," TAPPI Press, Atlanta, GA.

TAPPI T244 cm-11 (2011). "Acid-insoluble ash in wood, pulp, paper, and paperboard," TAPPI Press, Atlanta, GA.

Vegas, R., Kabel, M., Schols, H. A., Alonso, J. L., and Parajó, J. C. (2008). "Hydrothermal processing of rice husks: effects of severity on product distribution," *Journal of Agricultural and Food Chemistry* 83(7), 965-972. DOI: 10.1002/jctb.1896

Wallis, A. F. A., Wearne, R. H., and Wright, P. J. (1996). "Chemical analysis of polysaccharides in plantation eucalyptus wood and pulps," *Appita Journal* 49(4), 258-262. ISSN: 1038-6807

Article submitted: December 7, 2016; Peer review completed: February 2, 2017; Revised version received and accepted: February 28, 2017; Published: March 7, 2017.
DOI: 10.15376/biores.12.2.3088-3107