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Two-stage fractionation of sugarcane bagasse by autohydrolysis and glycerol organosolv delignification in a lignocellulosic biorefinery concept

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ABSTRACT

Bioethanol production from lignocellulose biomass offers a solution to current environmental challenges caused by fossil fuel energy resources, while satisfying the biorefinery concept. In this study, two-stage fractionation (autohydrolysis (AH) followed by glycerol organosolv delignification (GOD)) of sugarcane bagasse (SCB) was studied as a function of temperature, time, glycerol content and liquid-to-solid (*LSR*) ratio using experimental designs. The effect of three different AH pretreatment severities on delignification extents (*DE*) of the solid fractions were also evaluated. Scanning electron microscopy was used to examine the changes in the surface of SCB pretreated by AH. Energy balances of four fractionation conditions were estimated, and the use of pure and crude glycerol in GOD was evaluated based on *DE* and lignin contents. A *DE* of ~64% was obtained at 210.3 °C, 40 min, *LSR* of 6.5 (v/w) and 80% (v/v) pure glycerol for GOD of SCB pretreated by AH at 175.8 °C, 49 min and *LSR* of 5.3 (v/w), which resulted in an energetic profitability (*EP*) of 141.48 MJ/kg_{SCB}. The use of crude glycerol at 80% (v/v) under the same process conditions optimized for pure glycerol also proved to be feasible (*DE* of ~64% and *EP* of 142.67 MJ/kg_{SCB}), widening the possibilities for its direct use in GOD of SCB pretreated by AH in a 2G bioethanol integrated plant.

1. Introduction

The growing consciousness of climate change and noble efforts aimed at emission reduction has been a building topic for many years. Constantly challenged by depleting fossil fuel resources and the subsequent rise in fuel prices, it is necessary to evaluate the current reality.

Renewable energy serves as a viable alternative to traditional energy sources, providing clean and sustainable energy forms. Based on current renewable energy technologies involving lignocellulose biomass, projections for a global transition to renewable energy system by 2050 seems plausible (Cornelissen et al., 2012).

In regards to the use of renewable energy sources, Brazil is considered one of the pioneers in alternative fuel technologies and sustainable energy policies globally. Innovations in the production of sugarcane based bioethanol fuel, introduced in the 1970s, continue to produce new developments (Gurgel et al., 2012). Therefore, in view of this development scenario, the optimization of sugarcane bagasse (SCB) processing technologies in plants for the production of biofuels, bioenergy and bioelectricity is essential considering the power generating potential of this lignocellulose waste (Dias et al., 2012).

Among the main pretreatments commonly used for SCB processing and fractionating, autohydrolysis (AH) and autocatalyzed organosolv delignification are within the more environmentally advantageous processes for the removal of hemicelluloses and delignification (Baêta et al., 2016a; Novo et al., 2011; Sun and Chen, 2008; Vallejos et al., 2012; Yu et al., 2013a,b). Bearing in mind that organosolv pretreatment uses organic solvents, the type of solvent involved is still a matter of debate. The use of first generation (1G) ethanol, and glycerol derived from biodiesel production has been widely discussed. The use of ethanol, however, in comparison to glycerol, is a less favorable option

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due to its high demand for its use as a biofuel; while glycerol, on the other hand, is a byproduct of another biofuel production process, biodiesel production (Tan et al., 2013).

In order to meet the Brazil's domestic demand for biodiesel, a 5% blend of biodiesel with regular diesel is required. This caused biodiesel production and consumption to rise to 3.4 billion liters in 2014 (ANP, 2015). Thus, 340 million liters of crude glycerol were produced in 2014, which corresponds to 10% of all biodiesel production. Furthermore, a tendency of growth in biodiesel production is expected in the next years. Crude glycerol and its impurities generally maintain a low market value (Rivaldi et al., 2007). Therefore, the use of pure glycerol in the process of lignocellulose biomass delignification is viewed as unappealing from an economic standpoint. However, the use of crude glycerol (glycerol content of around 80–90%) in the delignification of SCB pretreated by AH remains unexplored (Raghavi et al., 2016; Sun et al., 2016).

Although the organosolv process is currently pointed as more costly (Sun et al., 2016) than some pretreatment processes discussed in literature in terms of energy, including heating reactors and solvent recovery, no energy balance resulting from the use of pure and crude glycerol, which could help increase process economic viability (Wan Isahak et al., 2015), was presented and discussed in the literature until the present moment. In addition, the effect of liquid-to-solid ratio (*LSR*) on the autocatalyzed glycerol (pure and crude) organosolv delignification of SCB pretreated by AH, which can significantly reduce the costs with chemicals and energy, still remains unexplored in the literature at the present moment. These data are of great scientific and industrial relevance to establish a viable technology for SCB processing, and therefore, need to be better evaluated.

In this context, the present study aimed to investigate the fractionation of SCB by AH pretreatment followed by organosolv delignification using a solvent mixture of glycerol (pure and crude)-water to achieve process conditions where the two-stage fractionation process can be economically feasible. For this, the effects of main process parameters influencing the hemicelluloses removal by AH pretreatment (temperature (*T*), time (*t*) and *LSR*) and the organosolv delignification of SCB pretreated by AH (*T*, *t*, *LSR* and glycerol content) were evaluated. The energy balances of four optimized strategies for second generation ethanol (2G ethanol) production were also performed. The energy balances considered the use of hemicelluloses for biomethane production, cellulose for 2G bioethanol production (by enzymatic hydrolysis with low content of enzymes and water followed by fermentation) and the black liquor containing glycerol (pure and crude) and lignin for energy generation.

2. Material and methods

2.1. Material

Raw SCB was provided by Ipiranga Ethanol and Sugar Company, Descalvado, SP, Brazil and air-dried at room temperature to approximately 10% humidity and stored under cool, dry conditions. Glycerol (99.5%), sulfuric acid (95–98%), cyclohexane and ethanol (99.5%) were purchased from Synth®, Brazil. Crude glycerol was collected at Darcy Ribeiro Biodiesel Plant (Petrobras Biocombustíveis, Montes Claros, MG, Brazil). This residue was obtained from biodiesel production by transesterification of vegetable and animal oils using sodium methoxide as a catalyst and methanol as a short-chain alcohol. Its composition is: 86.0% glycerol, 13.9% water, 0.29 g/L sulfate, 37.06 g/L chloride, 0.07 g/L phosphate and had a pH of 6.18, 1077.44 g/L total solids, 1499.4 g/L chemical oxygen demand (COD) and 1.27% lipid content. Chromatography standards used include cellobiose, D-glucose, D-xylose, L-arabinose, acetic acid, formic acid, 5-hydroxymethyl-2-furaldehyde (HMF), and 2-furaldehyde (furfural) and sulfuric acid (99.999%) were purchased from Sigma-Aldrich® (Brazil). Commercial cellulase (celluclast 1.5 L) and β-glucosidase (Novozyme 188) were purchased from Novozymes Latin America Ltd. Cellulase and β-glucosidase exhibited enzymatic activities of 83.53 FPU/mL (paper filter units by mL of enzymatic solution) and 544.37 IU/mL (international unit of enzymatic activity by mL of enzymatic solution), respectively.

2.2. Autohydrolysis (AH) pretreatment of sugarcane bagasse

The experiments for hemicelluloses extraction by AH pretreatment were conducted firstly in cylindrical reactors (autoclave type) made of 316-L stainless steel with a capacity of 195 mL (190 mm height × 58 mm internal diameter × 76 mm external diameter). The optimized pretreatments were then performed in a Regmed AU/E-27 reactor at the University of São Paulo (USP), São Carlos, Brazil. In a typical run, the reactor was loaded with 1000.00 g of SCB, on dry-weight basis. Distilled water was subsequently added, taking into consideration the selected liquid-to-solid ratio (*LSR*) and the moisture content of the SCB. The reactor was heated and kept at set temperature until the end of reaction time. After completion, the reactor was rapidly cooled by the slow opening of the pressure valve. The pretreated solid was transferred to a hydraulic press to extract the hemicelluloses rich hydrolysate and stored under cool, wet conditions.

Three desirable AH conditions obtained from different temperatures, times and *LSR* were selected from the study of Baêta et al. (2016a) to obtain three distinct residual solid fractions for the evaluation of the effect of pretreatment severity (S_0) on the hemicelluloses extraction (Overend et al., 1987) and subsequently in the delignification of SCB pretreated by AH. The three conditions include: AH condition 1 (S_0 = 3.92: 175.8 °C, 49 min and *LSR* = 5.3 (v/w)), AH condition 2 (S_0 = 4.11: 180.4 °C, 55 min and *LSR* = 7.15 (v/w)) and AH condition 3 (S_0 = 4.04: 182.7 °C, 40 min and *LSR* = 4.38 (v/w)).

2.3. Glycerol organosolv delignification (GOD) of SCB pretreated by AH

GOD experiments of SCB pretreated by AH were also performed in 316-L stainless steel reactors with a capacity of 195 mL, each one containing a sealing O-ring made of polytetrafluoroethylene (PTFE) to ensure proper sealing to withstand high temperatures. In a typical run, the reactor was loaded with 10.000 g of SCB pretreated by AH (on-dry weight basis) followed by the addition of a solution of pure glycerol-water or crude glycerol-water. The composition of each solution in terms of glycerol content (v/v) and *LSR* were taken into account based on the set experimental condition. The reactor was heated in a thermostatic bath, containing glycerol as a heating fluid, for a determined time period, and then cooled in an ice bath at the end of reaction time (Novo et al., 2011).

The solid residues obtained after delignification were further subjected to a defibering process using a blunt bladed blender whereby the solid residue was simultaneously washed with 50 mL of 1% NaOH solution (w/v) for 30 s at 2800 rpm; this was repeated 3 times (Gurgel et al., 2016). Between each defibering, the solid residue was transferred to a Büchner funnel and washed with distilled water. This washing process aimed to remove lignin fragments from the fiber surface since its solubility in the glycerol solution is reduced at low temperatures after reactor cooling (Novo et al., 2011). After, the cellulose rich solid residue was washed with distilled water until the filtrate was neutral at pH ~7, and the wet solid fraction was stored in a freezer (−20 °C) to avoid microorganism growth.

2.4. Enzymatic hydrolysis

For the enzymatic hydrolysis experiments, 1.000 g of the solid fractions obtained from GOD of SCB pretreated by AH were hydrolyzed with 10.0 mL of an aqueous 0.05 mol/L citric acid-sodium citrate buffer solution (pH 4.8), 10 FPU of cellulase (Celuclast 1.5 L) and 20 IU of β-glucosidase (Novozym 188). Enzymatic hydrolysis experiments were performed in 50 mL Erlenmeyer flasks in an orbital shaker incubator

(New Brunswick 24 incubator series) at 50 ± 1 °C and 150 rpm for 72 h. After this time, aliquots were taken and the hydrolysates were placed in an ice bath to stop the enzymatic hydrolysis. The liquid and solid fractions were separated by centrifugation at 9,000 rpm for 15 min and the sugars present in the liquid fraction were separated and quantified by high performance liquid chromatography (HPLC) (Nascimento et al., 2016), as described in Section 2.5.

The enzymatic conversion (*EC*) was calculated using Eq. (1), as follows:

$$EC/\% = \left[\frac{w_{\text{glucose}} f_{\text{h}}}{w_{\text{sample}}\left(y_{\text{i}}/_{100}\right)}\right] \times 100 \quad (1)$$

where w_{glucose} (g) is the weight of glucose in the hydrolysate, w_{sample} (g) is the weight of sample, f_{h} is the cellulose hydrolysis conversion factor (0.9) and y_{i} (%) is the cellulose content in the sample.

2.5. Analytical methods for characterization of the liquid and solid fractions

Samples of SCB, SCB pretreated by AH and delignified SCB pretreated by AH were oven-dried at 105 °C for 2 h and left to cool in a desiccator. Then, they were ground to pass through a 0.40 mm (40 mesh) screen for the compositional tests. The ash (inorganic) content was determined according to the method "Ash in wood, pulp, paper and paperboard", TAPPI T211 om-02. The extractives content was determined (only for raw SCB) according to the method "Solvent extractives of wood and pulp", TAPPI T204 cm-07, using cyclohexane instead of benzene. The lignin content was determined according to the method "Determination of acid-insoluble lignin in biomass", NREL LAP-004. The carbohydrate content was determined according to the method "Determination of carbohydrates in biomass by high performance liquid chromatography", NREL LAP-002. Acid-soluble lignin (ASL) was measured on a UV–vis spectrophotometer (Hewlett-Packard®, model 8453) and was calculated by Eq. (2) (Lin and Dence, 1992).

$$ASL/(\%) = \left\{\frac{\left[\frac{(4.53A_{205}) - A_{280}}{300}\right]V}{w}\right\} \times 100 \quad (2)$$

where A_{205} and A_{280} are the absorbances at 205 and 280 nm, respectively, V (L) is the volume of hydrolysate solution and w (g) is the weight of SCB. All compositional tests were made in triplicate.

The separation and quantification of sugars, sugar degradation products and organic acids in the hydrolysate from acid-insoluble lignin (AIL) test was performed in a Shimadzu® high performance liquid chromatograph equipped with a binary pump system (LC-30AD); refractive index detector (RID-6); UV–vis detector (SPD-10AV); a micro-guard cartridge (Cation H^+, Aminex HPX-87H, Bio-Rad) and an Aminex HPX 87H column (300 × 7.8 mm, Bio-Rad); an oven temperature (CTO-10A) of 55 °C; an autosampler (SIL 30AC) and 0.5 mmol/L H_2SO_4 as eluent at a flow rate of 0.6 mL/min. Sugar detection was made using refractive index, whereas the detection of sugar degradation products and organic acids was made in a UV–vis detector at the wavelengths 210 nm (organic acids) and 274 nm (sugar degradation products).

For mass balances, the percentages of cellulose and hemicelluloses in the samples were calculated according to Eqs. (3) and (4) (Canilha et al., 2011; Gurgel et al., 2014).

$$\text{Cellulose} = 0.95\text{Cel}_{\%} + 0.90\text{Glu}_{\%} + 1.286\text{HMF}_{\%} + 3.522\text{FA}_{\%} \quad (3)$$

$$\text{Hemicelluloses} = 0.88\text{Xyl}_{\%} + 0.88\text{Ara}_{\%} + 1.375\text{FF}_{\%} + 0.717\text{AA}_{\%} \quad (4)$$

where $\text{Cel}_{\%}$, $\text{Glu}_{\%}$, $\text{HMF}_{\%}$, $\text{FA}_{\%}$, $\text{Xyl}_{\%}$, $\text{Ara}_{\%}$, $\text{FF}_{\%}$ and $\text{AA}_{\%}$ are the percentages (w/w) of cellobiose, glucose, 5-hydroxymethyl-2-furfuraldehyde, formic acid, xylose, arabinose, 2-furfuraldehyde and acetic acid in Eqs. (3) and (4), respectively.

The percentage weight loss (*WL*) was calculated from the ratio between the weight of the pretreated delignified SCB and SCB pretreated by AH. The delignification extent (*DE*) of solid residues was calculated according to Eq. (5) (Novo et al., 2011).

$$DE/(\%) = \left\{\frac{L_{\text{pretreated SCB}} - [L_{\text{delignified residue}}(Y_{\text{delignified residue}}/100)]}{L_{\text{pretreated SCB}}}\right\} \times 100 \quad (5)$$

where $L_{\text{pretreatedSCB}}$ is the amount of lignin contained in the SCB pretreated by AH condition 1, 2 or 3 (%), $L_{\text{delignified residue}}$ is the amount of lignin in the solid residue rich in cellulose after delignification (%), and $Y_{\text{delignified residue}}$ is the yield of the delignification reaction ($Y_{\text{delignified residue}}$ = 100–*WL*) (%).

2.6. Scanning electron microscopy (SEM)

The morphology of SCB pretreated by AH in conditions 1 and 3 were examined in a scanning electron microscope (Tescan/Oxford Instruments, model Vega3 SB SEM) operating with a filament voltage of 20 keV and secondary electrons detector (SE). All samples were previously dried at 90 °C for 1 h, and then sputter coated with a thin layer of gold in a modular high vacuum coating (Quorum Technologies, model Q150R ES) prior to analysis.

2.7. Design of experiments and statistical analysis

A Doehlert experimental design (DED) was created with 22 trials and duplicate at the center point. The matrix of DED experiments is shown in Table 2. This design aimed at maximizing lignin removal and cellulose preservation while maintain a low *LSR* as a way to save glycerol and water in the GOD. Four independent variables were evaluated: temperature (*T*, °C), time (*t*, min), liquid-to-solid ratio (*LSR*, mL/g) and glycerol content (*G*, v/v), whereby *LSR* and *G* were varied in 7 levels, *T* in 5 levels and *t* in 3 levels. The dependent variables (*DV*) evaluated were yield, cellulose and lignin content and *DE*.

In order to improve the modeling and prediction of the dependent variables (cellulose content and *DE*), a 2^2 experimental design was also devised. In this experimental design, the independent variables *LSR* and *t* were kept fixed according to the results obtained from DED. The matrix of experiments for the 2^2 experimental design is also shown in Table 2.

Experimental results were evaluated with Statistica 10.0 (StatSoft, Inc.) routines for analysis of variance (ANOVA), modeling the responses, regression coefficients and graphical analysis. Sum of squares of residuals (*SSR*) was chosen for statistical significance and computation of standard errors and the most complex model was used for DED and 2^2 design. Statistical analyses were performed with a 95% significance level. The second (DED) and first (2^2 design) degree polynomial equations generated by the model that best fitted the experimental data are shown in Eqs. (6) and (7).

$$\begin{aligned} DV = {} & a_0 + a_1T + a_2T^2 + a_3G + a_4G^2 + a_5LSR + a_6LSR^2 + a_7t + a_8t^2 \\ & + a_9T \times G + a_{10}T \times LSR + a_{11}T \times t + a_{12}G \times LSR + a_{13}G \times t \\ & + a_{14}LSR \times t \end{aligned} \quad (6)$$

$$DV = a_0 + a_1T + a_2G + a_3T \times G \quad (7)$$

where *DV* is the dependent variable, a_0, a_1, …, a_{14} are regression coefficients estimated by fitting the model to the experimental data.

2.8. Energetic balance of the process

In order to perform an energetic balance, some experimental conditions of DED for GOD of SCB pretreated by AH were chosen. The experiment 13 (203.5 °C for 40 min with 68.32% glycerol and a *LSR* of

Table 1
Autohydrolysis (AH) pretreatment parameters, chemical composition of raw sugarcane bagasse and sugarcane bagasse pretreated by AH 1–3 (on dry weight basis), lignin characterization and hemicellulose hydrolysate composition.

Autohydrolysis pretreatment parameters	Raw SCB (%)	SCB pretreated by AH 1 (%)	SCB pretreated by AH 2 (%)	SCB pretreated by AH 3 (%)
Severity (S_0)	–	3.92	4.11	4.04
Temperature (°C)	–	175.8	180.4	182.7
Time (min)	–	49	55	40
Liquid-to-solid ratio (*LSR*, mL/g)	–	5.30	7.15	4.38
Chemical composition (%)	–	–	–	–
Lignin	23.93 ± 0.74	28.91 ± 0.49	29.12 ± 0.85	30.92 ± 0.14
Cellulose	46.36 ± 2.20	63.56 ± 4.64	60.26 ± 5.64	56.52 ± 0.37
Hemicelluloses	20.61 ± 0.19	9.29 ± 1.53	12.65 ± 0.42	13.04 ± 1.43
Extractives	2.26 ± 0.05	–	–	–
Ash	2.19 ± 1.31	0.75 ± 0.14	0.53 ± 1.79	1.72 ± 0.65
Yield (*Y*, %)	–	70.0	70.3	71.1
Weight loss (*WL*, %)	–	30.0	29.7	28.9
Acetyl groups (%)	3.63 ± 0.34	0.52 ± 0.00	1.21 ± 0.06	1.15 ± 0.07
Deacetylation extent (%)	–	90.0	76.6	77.5
Residual cellulose (%)	–	90.15 ± 6.59	85.46 ± 8.00	80.15 ± 1.28
Hemicelluloses removal (%)	–	68.44 ± 5.28	57.05 ± 1.42	55.73 ± 4.86
Delignification extent (*DE*, %)	–	15.43 ± 1.42	14.81 ± 2.49	9.54 ± 0.40
Lignin characterization	–	–	–	–
AIL-to-ASL ratio	10.5 ± 1.5	20.9 ± 0.0	30.7 ± 2.7	33.7 ± 0.8
Hemicellulose hydrolysate composition (g/L)	–	–	–	–
Glucose	–	0.000	0.029	0.000
Xylose	–	1.577	4.566	2.885
Arabinose	–	1.381	0.724	1.405
Formic acid	–	2.551	0.741	0.998
Acetic acid	–	1.282	3.041	3.035
HMF	–	0.241	2.092	1.845
2-furfuraldehyde	–	0.442	1.331	0.863

3 mL/g) was chosen due to its lower *LSR*; whereas the experiment 12 (196.8 °C for 40 min with 80% glycerol and a *LSR* of 6.5 mL/g) due to its lower reaction temperature in comparison with experiment 13. The experiment 3 (210.3 °C for 40 min with 80% glycerol and a *LSR* of 6.5 mL/g) from the 2^2 experimental design was also chosen due to its higher *DE* (63.74%), and so was the experiment CG-80 for GOD of SCB (210.3 °C for 40 min and a *RLS* of 6.5 mL/g) employing crude glycerol at 80% (v/v), which was chosen due to the use of a solvent mixture, a residue from the biodiesel process. These data were used to determine the energy input of each fractionation strategy with the aim of evaluating the feasibility of the process.

An analysis for the production of methane by single-stage anaerobic digestion (1S-DA) of the hemicellulose hydrolysate obtained in the first fractionation stage (autohydrolysis) was integrated into the energetic balance based on condition DC2 (S_0 = 3.95; 178.6 °C for 43.6 min with a *LSR* of 4.2 (mL/g)) described by Baêta et al. (2016a), which generated 2.9 MJ/kg $SCB_{dry\text{-}weight}$, the most similar condition to the AH condition 1 (S_0 = 3.92; 175.8 °C for 49 min with a *LSR* of 5.3 (mL/g)) used in the present study.

Table 4 shows the parameters adopted in the simulations of the fractionation scenarios. The equations used for calculation of energetic balance are presented as follows:

$$\Delta E = E(+) - E(-) \tag{8}$$

$$E(+) = E_{AD} + E_{CBL} + E_{CE} + E_{CSR} \tag{9}$$

$$E_{CBL} = w_{hemi,BL} \times \Delta H^{o}_{comb,hemi} + w_{cellu,BL} \times \Delta H^{o}_{comb,cellu} + w_{lig,BL} \times \Delta H^{o}_{comb,lig} + w_{glyc,BL} \times \Delta H^{o}_{comb,glyc} \tag{10}$$

$$E_{AD} = BMP \times \Delta H^{o}_{comb,CH_4} \times \left(\frac{TOC_{Soluble}}{SCB_{dry\text{-}weight}} \right) \times 0.9 \tag{11}$$

$$E_{CE} = w_{ethanol} \times \Delta H^{o}_{comb,ethanol} \tag{12}$$

$$E_{CSR} = w_{cellu,SR} \times \Delta H^{o}_{comb,cellu} + w_{hemi,SR} \times \Delta H^{o}_{comb,hemi} + w_{lig,SR} \times \Delta H^{o}_{comb,lig} \tag{13}$$

$$E(-) = E_{AH} + E_{GOD} \tag{14}$$

$$E_{GOD} = C_{p,SCB} \times w_{SCB,AH} \times (T_{GOD} - T_{SCB,AH}) + C_{p,H_2O} \times w_{H_2O} \times (T_{GOD} - T_{H_2O,100°C}) + C_{p,glyc} \times w_{glyc} \times (T_{GOD} - T_{glyc,25°C}) \tag{15}$$

$$E_{AH} = C_{p,SCB} \times w_{SCB} \times (T_{AH} - T_{SCB,25°C}) + C_{p,H_2O} \times w_{H_2O} \times (T_{AH} - T_{H_2O,100°C}) \tag{16}$$

where ΔE is the energetic profitability, $E(+)$ the energy generated, $E(-)$ the energy consumed, E_{AD} the energy produced by anaerobic digestion (AD) using hemicellulose hydrolysate, *BMP* the biochemical methane production, $\Delta H^{\circ}_{comb,CH4}$ the heat of combustion of methane, E_{CBL} the energy produced by combustion of black liquor, $w_{hemi,BL}$ the hemicellulose weight in the black liquor, $w_{cellu,BL}$ the cellulose weight in the black liquor, $w_{lig,BL}$ the lignin weight in the black liquor, $w_{glyc,BL}$ the glycerol weight in the black liquor, $\Delta H^{\circ}_{comb,hemi}$ the heat of combustion of hemicelluloses, $\Delta H^{\circ}_{comb,cellu}$ the heat of combustion of cellulose, $\Delta H^{\circ}_{comb,lig}$ the heat of combustion of lignin, $\Delta H^{\circ}_{comb,glyc}$ the heat of combustion of glycerol, E_{CE} the energy produced by combustion of ethanol, $\Delta H^{\circ}_{comb,ethanol}$ the heat of combustion of ethanol, $w_{ethanol}$ the ethanol weight obtained by enzymatic hydrolysis of pretreated SCB, E_{CSR} the energy produced by combustion of the solid residue after enzymatic hydrolysis, $w_{cellu,SR}$ the cellulose weight in the residual solid after enzymatic hydrolysis, $w_{hemi,SR}$ the hemicelluloses weight in the residual solid after enzymatic hydrolysis, $w_{lig,SR}$ the lignin weight in the residual solid after enzymatic hydrolysis, E_{AH} the energy used by AH pretreatment, E_{GOD} the energy used by GOD pretreatment, $C_{p,SCB}$ the heat capacity of SCB, $C_{p,H2O}$ the heat capacity of water, $C_{p,glyc}$ the heat capacity of glycerol, w_{SCB} the SCB weight for AH pretreatment, $w_{SCB,AH}$ the SCB weight for GOD pretreatment, w_{H2O} the water weight for pretreatment, w_{glyc} the glycerol weight for GOD pretreatment, T_{AH} the AH

pretreatment temperature, T_{GOD} the GOD pretreatment temperature, T_{SCB} the SCB temperature, T_{Glyc} the glycerol temperature and T_{H2O} the water temperature.

3. Results and discussion

3.1. Characterization of sugarcane bagasse (SCB)

Compositional characterization data for SCB is presented in Table 1. According to the data presented in Table 1, SCB chemical composition is in good agreement with those reported by Rocha et al. (2015) and Novo et al. (2011).

3.2. Autohydrolysis (AH) pretreatment of SCB

AH pretreatment of SCB has recently been studied by several authors such as Baêta et al. (2016a), Carvalho et al. (2015), Santucci et al. (2015), Vallejos et al. (2015), Yu et al. (2013a) and Yu et al. (2013b). One of the major advantages of this pretreatment is the fact that the addition of chemical reagents is not necessary, reducing investment in reactors since the use of only water in the process does not pose corrosion risks. Furthermore, the use of high temperatures (150–220 °C) allows the increase of the acid dissociation constant of water (K_w), substantially increasing the concentration of hydronium ions (H_3O^+) due to water autoionization. The hydronium ions generated in the reaction medium are responsible for the hydrolysis of acetyl groups in hemicelluloses from SCB, allowing the release of acetic acid in the medium, helping to sufficiently lower the pH of the reaction medium to 3.5–4.5. In this pH range, hemicelluloses hydrolysis reactions can occur without extensive degradation of five-carbon sugars (C-5) (e.g. xylose and arabinose) and six-carbon sugars (C-6) (e.g. glucose, galactose and mannose) to furans such as furfural and HMF, since the reaction time is relatively short (e.g. < 60 min and temperatures between 160 and 180 °C) (Santucci et al., 2015; Vallejos et al., 2012).

However, a major disadvantage of AH pretreatment is the fact that lignin can also be hydrolyzed/degraded during this process, undergoing chemical structural changes, causing the release of phenolic fragments in the reaction medium. These fragments have limited solubility in acidic aqueous medium, and are very toxic for microorganisms used in the recovery of the hemicellulose hydrolysate for the production of 2G ethanol (Gírio et al., 2010), methane (Baêta et al., 2016a, 2016b), and value-added bioproducts (Carvalho et al., 2015; Leschinsky et al., 2008a, 2008b; Vallejos et al., 2012).

Moreover, it has been reported in other studies that temperatures generally employed in the AH pretreatment (170–180 °C) of lignocellulose biomass (hardwood) are near to the glass transition temperature (T_g) of lignin (Ko et al., 2015; Zhuang et al., 2015). As a result, lignin can coalesce, and pass from a rigid physical state to a rubbery or viscous physical state.

It is hypothesized that near to T_g value, lignin coalesces into its viscous state and migrates from the middle lamella and cell wall, emerging out of the cell and onto the fiber surface (Ko et al., 2015). Because the lignin fragments with high molecular weights are sparingly soluble in the acidic aqueous medium, they precipitate on the surface of the fibers during reactor cooling, assuming a spherical shape, also known as lignin droplets (Zhuang et al., 2015). The lignin droplets can generally be observed in the corners of the cell walls that are ruptured during AH pretreatment, acting as a physical barrier and inhibiting the access of cellulolytic enzymes to the internal regions of the cell walls (Ko et al., 2015). As will be further discussed in detail, the formation of lignin droplets was also observed in this study.

Besides affecting the enzymatic digestibility of the solid residue after AH pretreatment, the structural changes of lignin can seriously affect the efficiency of the subsequent delignification stage. The value of acid-insoluble lignin to acid-soluble lignin (AIL-to-ASL) ratio can be used to evaluate the changes in the structure of lignin during the AH pretreatment according to Ko et al. (2015). The values of AIL-to-ASL ratio for SCB pretreated by AH condition 1–3 are shown in Table 1. As the severity of the AH pretreatment was increased the AIL-to-ASL ratio increased, suggesting that lignin structure may have changed to a more condensed structure (less reactive with respect to the strong acid medium used in the Klason lignin test). Similar results were reported by Ko et al. (2015).

The difficulty in lignin removal after AH pretreatment lies in the fact that lignin is repolymerized due to reactions that lead to the formation of carbonium ions intermediates during AH pretreatment, thereby promoting the formation of new carbon−carbon (C−C) bonds such as β-β, β-1 and β-5 (Leschinsky et al., 2008a, 2008b). According to Leschinsky et al. (2008a, 2008b), the main bonds broken during AH pretreatment include alkyl- and aryl-ether bonds, with α-O-4 and β-O-4 being most reactive. The disruption of ether bonds and the consequent generation of carbonium ions not only causes lignin condensation reactions with formation of C−C, but also leads to furan (furfural and HMF) condensation reactions with lignin. The formation of chemically modified lignin makes it more difficult to be removed in a subsequent delignification stage (Carvalho et al., 2015; Ko et al., 2015; Leschinsky et al., 2008a, 2008b). Lignin modified by condensation reactions of phenolic fragments and furans significantly decreases enzymatic digestibility of the pretreated solid by AH when compared to the lignin that remains from a delignification process (Carvalho et al., 2015; Ko et al., 2015).

Therefore, AH pretreatment of SCB as a single pretreatment stage before enzymatic hydrolysis faces the aforementioned disadvantages, and requires that AH pretreatment is mild enough to reduce furan condensation with lignin as well to minimize lignin repolymerization. However, a mild AH pretreatment of SCB (≤170 °C), although achieving hemicelluloses extraction in the form of xylooligomers (XOS), does not lead to monosaccharide formation in high concentrations as shown by Vallejos et al. (2012). In order to make use of hemicellulose hydrolysate for its conversion into ethanol and bioproducts, such as succinic acid, a subsequent stage would then be required to convert the XOS into monosaccharides. This could result in a considerable cost increase, and nonetheless lead to a hydrolysate with a high furan content, since this stage requires an aqueous acidic medium (dilute sulfuric acid), whereby hydrolysis is faster and more favorable (Vallejos et al., 2012).

Raw SCB underwent three different experimental conditions of AH pretreatment based on the study by Baêta et al. (2016a). These conditions were aimed at high hemicelluloses removal with minimal production of sugar degradation (furans) and phenolic lignin fragments, which are known as microbial inhibitors that might affect the subsequent anaerobic digestion of hemicellulose hydrolysate for methane production or its fermentation into bioethanol. The chemical compositions of hemicellulose hydrolysates obtained by AH pretreatment of SCB in the condition 1–3 are shown in Table 1.

The solid fractions resulting from the AH pretreatment were collected after hydraulic pressing and used as the starting materials for the GOD experiments (second fractionation stage). It is possible to observe (Table 1) that the AH pretreatment conditions 1–3 were effective and resulted in hemicelluloses removal of about 56–69%. The content of acetyl groups in hemicelluloses of SCB has a direct influence on AH pretreatment as acetic acid is produced upon their cleavage, promoting the hydrolysis of polysaccharides along with the release of hydronium ions during the water autoionization (Garrote et al., 2002). The deacetylation extents of SCB after AH pretreatment in conditions 1–3 are shown in Table 1. All AH pretreatment conditions resulted in great deacetylation efficiencies and, therefore, a lower pH was obtained, which contributed for greater hemicelluloses depolymerization.

Additionally, AH pretreatment condition 1 (AH 1) led to the best cellulose preservation despite removing most hemicelluloses with lower generation of sugar degradation products. Furthermore, among the three AH pretreatment conditions, condition 1 had undergone the

mildest reaction conditions (S_0 = 3.92: T = 175.8 °C, t = 49 min and LSR = 5.3 (v/w)). As a result, AH 1 adopted in the present study was similar to the pretreatment condition DC2 (S_0 = 3.95: T = 178.6 °C, t = 43.6 min and LSR = 4.2 (v/w)) that led to the highest methane production (2.896 MJ/kg_{SCB}), as reported by Baêta et al. (2016a).

Yu et al. (2013a) also investigated the liquid hot water (LHW) pretreatment of SCB in a batch reactor with magnetic stirring at 500 rpm. The best condition of LHW pretreatment of SCB was obtained at 180 °C for 20 min (LSR = 20 (v/w) and P = 1.0 MPa), which resulted in a hemicelluloses removal of 87.7% with a xylose recovery of 85% (8 g/L). This hemicelluloses removal was higher than those obtained in the present study for conditions AH 1, AH 2 and AH 3. In addition, Yu et al. (2013a) also reported a delignification extent of 10%, which was lower than those reported in the present study for conditions AH 1 and AH 2 and similar to AH 3. These authors also reported an energy consumption for LHW pretreatment of 13.02 MJ/kg_{SCB}, which was ~6.7 times higher than that reported in the present study (Table 5).

In an another study, Yu et al. (2013b) also studied the fractionation of SCB by liquid hot water (LHW) pretreatment (180 °C, t = 30 min (at set reaction temperature), LSR = 5 (v/w) and P = 2 MPa (N_2 was injected into the reactor)) followed by delignification with aqueous ammonia (LHWAA). For the single LHW pretreatment, these authors reported a total xylose concentration (xylose plus XOS) of 10.9 g/L with a total xylose recovered of ~25%, and furfural, HMF, acetic and formic acid concentrations of ~3, ~0.5, ~7 and ~0.7 g/L, respectively. Compared to the results obtained by Yu et al. (2013b), the condition AH 1 presented a lower xylose concentration, but the concentration of sugar degradation products and organic acids in the hydrolysate was lower.

3.3. Morphological changes of pretreated solids

The morphological changes of SCB pretreated by AH under conditions 1 and 3 (S_0 = 3.92 and 4.04, respectively), for example, were analyzed by SEM and are presented in Fig. 1a–f. Fig. 1a–f shows micrographs at magnitudes of 100 × , 10,000 × and 30,000 × for SCB pretreated by AH under conditions 1 and 3, respectively. Fig. 1a and b shows how the pressing stage affected fiber morphology, since the crushed and flattened features can be seen as a result of the pressing employed (9 tons). Moreover, it is also possible to observe how AH pretreatment led to the partial breakdown of lignocellulose fibers of SCB. In Fig. 1c–d, the formation of spherical droplets can be observed, being more prominently noticeable. It is also notable that the formation of lignin droplets became more apparent with increasing severity of AH pretreatment from condition 1 (S_0 = 3.92: T = 175.8 °C, t = 49 min and LSR = 5.3 (v/w)) to AH condition 3 (S_0 = 4.04: T = 182.7 °C, t = 40 min and LSR = 4.38 (v/w)). In Fig. 1e and f, with a magnitude increase of 30,000 × , the presence of lignin droplets are more pronounced; and, in Fig. 1f, under a more severe condition, it is possible to observe several lignin droplets emerging from the fiber surface as described by Zhuang et al. (2015) and Ko et al. (2015).

3.4. Organosolv delignification of SCB pretreated by AH using pure glycerol

The Pareto charts for responses yield and residual cellulose for DED are shown in Supplementary Fig. S1a and b. The variable G (2.839) and interaction between $T \times RLS$ (2.358) as well as T^2 (2.535) had a significant positive effect on yield, whilst the variables T (−6.507) and t (−3.722) and the interaction between $T \times t$ (−3.127) had a significant negative effect on yield (Supplementary Fig. S1a). The variables T^2 (5.062) and G^2 (2.384) and interaction between variables $T \times RLS$ (3.551) had a significant positive effect on cellulose content, while the variables t (-3.517) e G (−2.703) had a significant negative effect on cellulose content (Supplementary Fig. S1b). DED matrix and the experimental results for responses yield, residual cellulose, lignin content and DE are shown in Table 2. As can be seen in Table 2, DE values were in the range from 55% to 64% and the higher DE values were observed in experiments 2 (DE = 63.74%: 210.3 °C, 40 min, 80% glycerol and LSR = 6.5 (v/w)), 16 (DE = 62.61%: 203.5 °C, 40 min, 21.68% glycerol and LSR = 10 (v/w)), and 20 (DE = 61.79%: 203.5 °C, 60 min, 45% glycerol and LSR = 3.87 (v/w)).

Response surfaces for yield and residual cellulose are shown in Supplementary Fig. S2a and b. ANOVA, regression and determination coefficients (R_{adj}^2) for responses yield and residual cellulose for DED are shown in the Supplementary Tables S1 and S2, respectively. It was not possible to model the responses lignin content and DE due to the lack of model fit. Thus, to improve the modeling of the GOD of SCB pretreated by AH and prediction of the model for responses yield (R_{adj}^2 = 0.8013) and residual cellulose (R_{adj}^2 = 0.7909), lignin content and DE, a 2^2 experimental design was constructed with two independent variables, T and G. The variables t and RLS were fixed at 40 min and 6.5 mL/g as the use of shorter reaction times and lower LSR can improve the economic feasibility of the GOD process.

Based on the main conclusions from the DED, the responses evaluated in the 2^2 experimental design were lignin content and DE. The 2^2 experimental design matrix and experimental results for responses lignin content and DE are shown in Table 2. As can be seen in Table 2, experiment 3 (210.3 °C, 40 min, 80% glycerol and LSR = 6.5 (v/w)) led to the highest DE value (63.74%) and the lowest lignin content (13.69%). Evaluating the results of residual cellulose from the 2^2 experimental design, it is possible to observe that an increase in the variable glycerol content caused a decrease in the residual cellulose at low and high temperatures, as shown in Table 2. This confirms the Pareto charts data besides suggesting the interaction between glycerol and cellulose during the process.

Response surfaces for lignin content and DE are shown in the Supplementary Fig. S3a and b. ANOVA, regression and determination coefficients (R_{adj}^2) for responses lignin content and DE for 2^2 experimental design are shown in the Supplementary Tables S3 and S4, respectively. As shown in Table 2, the use of 2^2 experimental design improved the modeling of the GOD of SCB pretreated by AH and, consequently, the prediction of the model for lignin content (R_{adj}^2 = 0.9539) and DE (R_{adj}^2 = 0.9802). The Pareto charts for the responses lignin content and DE are shown in Supplementary Fig. S4a and b. The variable T (−8.206) and the interaction between variables $T \times G$ (−7.736) had a significant negative effect on lignin content, i.e. the higher the temperature the lower the lignin content in the solid fraction after the GOD, while the interaction between variables $T \times G$ (−7.736) indicated that the higher the temperatures and glycerol contents, the lower the lignin content (Supplementary Fig. S4a). The variable T (14.588) and the interaction between variables $T \times G$ (9.235) also showed a significant positive effect on DE, confirming that the delignification process is favored at higher temperature and glycerol content. In experimental condition 3 (210.3 °C, 40 min, 80% glycerol and LSR = 6.5 (v/w)), the value observed for DE was 63.74%.

Table 3 shows the results of GOD experiments for SCB pretreated by AH under conditions 1–3 under the best delignification conditions (210.3 °C, 40 min, 80% glycerol and LSR = 6.5 (v/w)) obtained by the 2^2 experimental design, as well as comparative GOD experiments with Novo et al., 2011Novo et al. (2011). As can be seen in Table 3, as the DE of pretreated SCB by AH decreased the severity of AH pretreatment also increased, thereby indicating that changes in the lignin structure during AH pretreatment made the delignification of SCB pretreated by AH more difficult. Thus, it is clear that the delignification of SCB pretreated by AH under conditions 2 and 3 was limited by the AH pretreatment conditions. A possible explanation for this can be the higher formation of low molecular weight lignin fragments with increasing severity during AH pretreatment. The lignin fragments formed during the process when exposed to glycerol at high temperatures, and time may participate in polymerization reactions with the glycerol on the fiber surface, thereby limiting the mass transfer between the solvent and

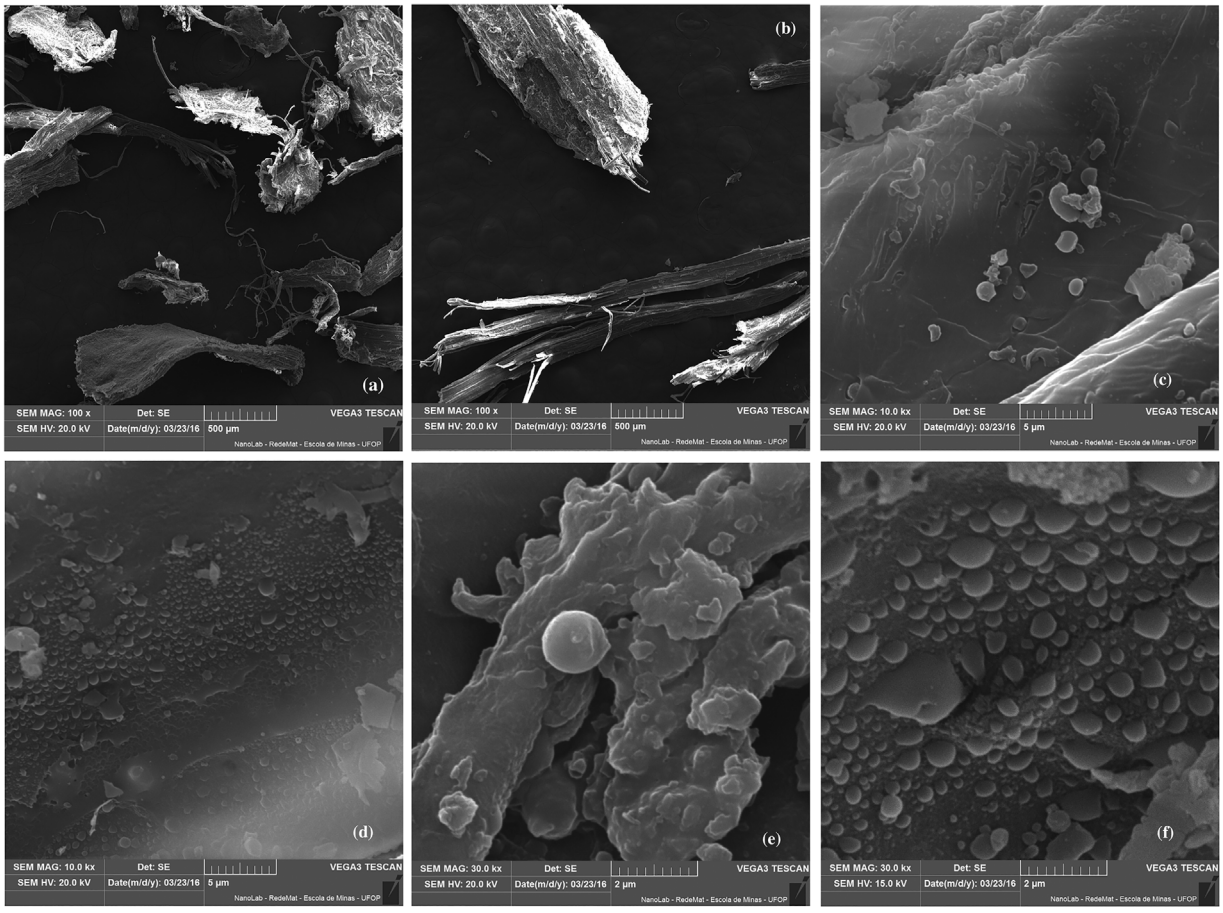

Fig. 1. a–f Scanning electron microscopy (SEM) micrographs of sugarcane bagasse pretreated by autohydrolysis conditions 1 (a, c and e) (S_0 = 3.92: T = 175.8 °C, t = 49 min and LSR = 5.3 mL/g) and 3 (b, d and f) (S_0 = 4.04: T = 182.7 °C, t = 40 min and LSR = 4.38 mL/g).

Table 2
Doehlert and 2^2 experimental designs and results obtained for glycerol organosolv delignification of sugarcane bagasse pretreated by autohydrolysis condition 1 (S_0 = 3.92: T = 175.8 °C, t = 49 min and LSR = 5.3 mL/g).

Design	Experiment	T (°C)	Glycerol (%)	LSR (mL/g)	t (min)	Yield (%)	Delignification extent (%)	Lignin (%)	Residual cellulose (%)
Doehlert Design	1	217.0	45.0	6.50	40	74.73	55.48 ± 0.09	17.22 ± 0.04	99.86 ± 11.67
	2	210.3	80.0	6.50	40	76.58	63.74 ± 0.96	13.69 ± 0.11	87.07 ± 1.79
	3	210.3	56.7	10.00	40	78.26	55.65 ± 1.18	16.38 ± 0.44	89.11 ± 6.46
	4	210.3	56.7	7.37	60	71.89	59.52 ± 0.52	16.28 ± 0.21	83.46 ± 3.86
	5	190.0	45.0	6.50	40	85.61	53.41 ± 0.54	15.73 ± 0.18	100.38 ± 0.47
	6	196.8	10.0	6.50	40	78.98	55.36 ± 0.35	16.34 ± 0.13	96.45 ± 1.11
	7	196.8	33.3	3.00	40	77.69	57.54 ± 0.03	15.80 ± 0.01	89.36 ± 2.86
	8	196.8	33.3	5.63	20	77.38	59.57 ± 0.00	15.10 ± 0.00	102.27 ± 2.57
	9	210.3	10.0	6.50	40	76.26	55.17 ± 0.55	16.99 ± 0.21	97.35 ± 5.63
	10	210.3	33.3	3.00	40	72.54	58.37 ± 1.88	16.59 ± 0.75	70.11 ± 1.36
	11	210.3	33.3	5.63	20	77.90	60.06 ± 0.45	14.82 ± 0.17	89.80 ± 2.85
	12	196.8	80.0	6.50	40	82.64	56.40 ± 0.21	15.25 ± 0.07	91.36 ± 3.52
	13	203.5	68.3	3.00	40	78.87	58.77 ± 0.12	15.11 ± 0.05	83.31 ± 0.86
	14	203.5	68.3	5.63	20	81.73	59.43 ± 1.10	14.35 ± 0.39	83.09 ± 8.75
	15	196.8	56.7	10.00	40	77.24	55.25 ± 0.08	16.75 ± 0.03	83.75 ± 0.84
	16	203.5	21.7	10.00	40	75.99	62.61 ± 0.50	14.22 ± 0.19	89.67 ± 6.44
	17	203.5	45.0	9.13	20	78.63	57.06 ± 0.71	15.79 ± 0.26	92.94 ± 4.34
	18	196.8	56.7	7.37	60	80.39	54.92 ± 0.40	16.21 ± 0.14	81.61 ± 12.47
	19	203.5	21.7	7.37	60	75.59	60.27 ± 1.41	15.19 ± 0.54	83.69 ± 0.67
	20	203.5	45.0	3.87	60	72.34	61.79 ± 0.26	15.27 ± 0.11	84.33 ± 2.95
	21	203.5	45.0	6.50	40	77.35	59.58 ± 0.75	15.10 ± 0.28	81.99 ± 10.90
	22	203.5	45.0	6.50	40	75.56	58.99 ± 0.00	15.69 ± 0.00	82.65 ± 5.21
2^2 Design	1	190.0	10.0	6.50	40	82.7	50.80 ± 1.28	17.19 ± 0.45	90.74 ± 4.90
	2	190.0	80.0	6.50	40	79.2	44.29 ± 9.05	20.33 ± 3.30	86.16 ± 3.77
	3	210.3	80.0	6.50	40	76.6	63.74 ± 0.96	13.69 ± 0.11	87.07 ± 1.79
	4	210.3	10.0	6.50	40	76.3	55.17 ± 0.55	16.99 ± 0.21	97.35 ± 5.63
	5	200.1	45.0	6.50	40	75.7	56.89 ± 3.91	16.46 ± 1.49	83.10 ± 3.40
	6	200.1	45.0	6.50	40	76.7	56.25 ± 2.96	16.54 ± 1.01	82.00 ± 3.14
	7	200.1	45.0	6.50	40	76.5	54.74 ± 2.54	17.09 ± 0.96	78.94 ± 4.30

Table 3
Results of organosolv delignification of raw sugarcane bagasse, sugarcane bagasse pretreated by autohydrolysis conditions 1–3 using pure and crude glycerol as organic solvents.

SCB pretreated by AH condition	Severity (S_0)	Organic solvent	T (°C)	t (min)	LSR (mL/g)	Glycerol (%)	Yield (%)	Delignification extent (%)	Lignin content (%)
–	–	–	210.3	40	6.50	80	81.02	47.95 ± 2.53	15.37 ± 0.78
–	–	–		120	10.0		51.95	84.63 ± 2.24	7.08 ± 1.03
–	–	–		240	10.0		48.30	83.18 ± 0.22	8.33 ± 0.11
1	3.92	Pure glycerol		40	6.50		72.95	67.65 ± 1.39	12.89 ± 0.55
1	3.92	Pure glycerol		40	10.0		80.03	58.67 ± 0.55	14.93 ± 0.12
1	3.92	Pure glycerol		120	6.50		77.40	56.95 ± 1.55	16.07 ± 0.58
1	3.92	Pure glycerol		240	6.50		72.50	56.53 ± 4.23	17.32 ± 1.69
1	3.92	Pure glycerol		240	10.0		78.50	63.68 ± 0.02	13.38 ± 0.01
2	4.11	Pure glycerol		40	6.50		81.55	52.74 ± 0.59	16.88 ± 0.05
3	4.04	Pure glycerol		40	6.50		75.10	66.41 ± 0.64	13.83 ± 0.26
1	3.92	Crude glycerol		40	6.50	80	71.93	63.89 ± 1.58	14.59 ± 0.64
1	3.92	Crude glycerol		40	6.50	86	65.84	68.08 ± 0.43	14.11 ± 0.73

Table 4
Parameters adopted in the fractionation scenario simulations, whereby all proposed pretreatment processes, combustion and 2G bioethanol production are performed in the same plant considering 1.0 kg sugarcane bagasse on dry–weight basis.

Parameter	Value	Unit
$C_{p,H2O}$	4.19×10^{-3}	MJ/kg °C
$C_{p,SCB}$	1.76×10^{-3}	MJ/kg °C
m_{SCB}	1.0	kg
$T_{inlet,SCB}$	25	°C
$T_{inlet,H2O}$ [a]	100	°C
T_{AH}	175.8	°C
ρ_{H2O}	1.0	kg/L
$\Delta H^{\circ}_{comb,cellu}$	17	MJ/kg
$\Delta H^{\circ}_{comb,hemi}$	16.63	MJ/kg
$\Delta H^{\circ}_{comb,lig}$	26.7	MJ/kg
LSR used in AH pretreatment	5.3	L/kg
Time used in AH pretreatment	49	min
Yield of AH pretreatment	69.75	%
$C_{p,glycerol}$ [b]	248.3435	J/mol°C
$C_{p,glycerol}$	0.002697	MJ/kg°C
Molecular weight of glycerol	92.09	g/mol
$\rho_{glycerol}$	1.21	kg/L
$T_{inlet,glyc}$	25	°C
$\Delta H^{\circ}_{comb,glyc}$	21	MJ/kg
$\Delta H^{\circ}_{comb,ethanol}$	27.43	MJ/kg

[a] The energy required to heat the water from 25 °C from 100 °C is considered to be generated from the condensing turbines of the cogeneration system.
[b] Righetti et al. (1998).

lignin. Sun and Chen, 2008Sun and Chen (2008) observed similar behavior in their studies of pretreatment of wheat straw with crude glycerol at 220 °C and 3 h. In addition, when comparing the severity of AH pretreatment in the conditions 2 and 3, it is also clear that the LSR also had a great influence.

In order to compare the two-stage fractionation process suggested in the present study with the single-stage fractionation process suggested by Novo et al. (2011), GOD experiments were performed in some GOD conditions explored by these authors, e.g. increasing the reaction time from 40 to 240 min or increasing LSR from 6.5 to 10 mL/g. The results of these experiments are shown in Table 3. The increase in the reaction time from 40 to 240 min did not lead to any increase in the DE. Nevertheless, the increase in the lignin contents for longer reaction times suggests that lignin repolymerization may have occurred. In addition, the increase in the reaction time from 40 to 240 min for a LSR of 6.5 mL/g also led to an increase in the lignin content, but the same increase in the reaction time for a LSR of 10 mL/g did not lead to an increase in the lignin content. These results revealed that the use of low LSR in GOD experiments also led to an increase in the lignin content, thereby suggesting that low water content may have favored lignin repolymerization.

The application of the optimized GOD conditions (210.3 °C, 40 min, 80% glycerol and LSR = 6.5 (v/w)) obtained in the present study on raw SCB showed that it was not possible to attain greater DE for lower reaction times and LSR. However, the use of longer reaction times and higher LSR led to greater DE, indicating that it is only possible to attain lower lignin contents if the raw SCB is used. Therefore, these results corroborate the previous discussions and show how AH pretreatment limits the delignification of SCB pretreated by AH.

According to Schrems et al. (2011), it is well established that cleavage of alkyl- and aryl-ether bonds is a key factor in the delignification of lignocellulose biomass. Moreover, cleavage of alkyl- and aryl-ether linkages such as α-O-α, β-O-β, α-O-4 and β-O-4 are strongly affected by the pH of the reaction medium. However, the removal of hemicelluloses, and therefore acetyl groups, during AH pretreatment prevents the reaction medium from being sufficiently acidic without an external source of hydronium ions, therefore interfering with DE. This was observed by Novo et al. (2011), who studied the GOD of SCB and obtained a solid residue with a 7.75% lignin content and a 81.4% DE when the SCB had not been previously pretreated by AH.

Similarly, the ethanol organosolv delignification (EOD) of SCB pretreated by AH (S_0 = 3.84: 170 °C, 60 min and LSR = 6:1 (w/w)) was investigated by Vallejos et al. (2015). The pretreated SCB was subjected to EOD using 50% ethanol-water (v/v), 190 °C for 150 min and LSR of 6:1 (w/w). The delignification process yielded a DE of 86.7% and 90% cellulose preservation, indicating that a high DE for pretreated SCB can be attained. The use of ethanol as an organic solvent has been extensively studied and ethanol is considered an efficient solvent for organosolv process (Zhao et al., 2009). When comparing the results obtained by Vallejos et al. (2015) with those obtained in the present study, it is important to note that the AH pretreatment conditions studied by Vallejos et al. (2015) involved a lower severity or temperature, and may probably have led to minor lignin structural modifications, as well as the fact that ethanol is a more efficient solvent for organosolv process (Balogh et al., 1992).

Zhang et al. (2013) studied the single-stage fractionation of SCB using aqueous acidified GOD in laboratory and pilot scale. These authors reported a 33.5% delignification at 130 °C for 60 min with a LSR of 10:1 (w/w) and a 1.2% HCl (w/w) acidified glycerol solution at 80% (w/w) in pilot scale. On laboratory scale, a 37.4% delignification at 130 °C for 60 min with a LSR of 10:1 (w/w) and a 1.2% HCl (w/w) acidified glycerol solution at 97.5% (w/w) was reported. The experimental conditions for the acid-catalyzed GOD reported by Zhang et al. (2013) were milder in terms of temperature, but in general composed of more severe conditions due to the addition of hydrochloric acid (from 0.4 to 2.4% (w/w)) in comparison to the conditions used to obtain the greatest DE (~64%) in the present study (210.3 °C, 40 min, 80% glycerol and LSR = 6.5 (v/w)). Furthermore, the use of strong mineral acids such as HCl contributes to corrosion of the reactors and other parts of a 2G bioethanol plant. Another drawback included the high LSR used in the studies of Zhang et al. (2013) in comparison to that used in the present study.

In addition, in the study of Zhang et al. (2013), SCB that underwent

acid-catalyzed GOD had not been previously pretreated by AH, whereby the structural modifications of lignin during AH could have possibly changed the delignification behavior of the solid fractions in the present study. Furthermore, the absence of a basic or acid catalyst may have been another reason for low *DE* values (not exceeding 64%) in the present study.

Therefore, comparing the studies reported in the literature (Novo et al., 2011; Romaní et al., 2013; Vallejos et al., 2015; Zhang et al., 2013) with the present study, it can be observed that due to a lack of an endogenous source of hydronium ions such as acetyl groups, the severity of the operational conditions of GOD defined the *DE*. This, combined with possible lignin repolymerization reactions during GOD due to the high temperatures (190–210.3 °C) needed for delignification in the absence of an acid or base catalyst, was not enough to breakdown the lignin, thereby hampering its solubility in the reaction medium.

According to Li et al. (2007), who studied the steam explosion of aspen wood, the depolymerization and repolymerization of lignin was associated with increasing the pretreatment severity (increased temperature and/or time). Under endogenous or exogenous acidic conditions (addition of organic or mineral acids), lignin fragmentation by acidolysis of β-O-4 bonds occurs. This was followed by lignin repolymerization by acid-catalyzed condensation between C-6 aromatic units and carbonium ions, leading to the formation of C—C bonds and an increase in lignin molecular weight, and generally a more heterogeneous lignin structure, making it difficult to be removed.

Yu et al. (2013b) studied the fractionation of SCB by a combined pretreatment method involving the use of liquid hot water (LHW) (T = 180 °C, t = 30 min (at set reaction temperature), LSR = 5 (v/w) and P = 2 MPa (N_2 was injected into the reactor)) followed by aqueous ammonia (T = 180 °C, t = 30 min, LSR = 5 (v/w) and ammonia loading of 25% w/v). The enzymatic hydrolysis of the solid residue pretreated by LHWAA was performed with commercial enzymes (T = 50 °C, t = 72 h, pH = 4,8, LSR = 20 (v/w)), 21.5 mg of protein per gram of dry solid (cellulase with activity of 214 FPU/g and protein concentration of 306.9 mg/g, and β-glucosidase activity of 297 IU/g) and reported a glucan digestibility of 55%. However, the addition of xylanase (20 $IU/g_{dry\ solid}$ and xylanase activity of 163 × 10^3 IU/g) improved the glucan digestibility to 65%. Yu et al. (2013b) also reported an energy consumption for LHWAA pretreatment of 2.835 MJ/kg_{SCB}. This energy consumption was lower than those reported for GOD of SCB pretreated by AH (experiments 3-PG, 12-PG and CG-80) and higher than that reported for experiment 13-PG, as can be seen in Table 5.

3.5. Organosolv delignification of SCB pretreated by AH using crude glycerol

Based on the previous analyses of the 2^2 experimental design for the optimization of GOD experimental conditions to obtain the maximum *DE* (Tables 2 and 3), GOD experiments using crude glycerol were done under the following conditions: 210.3 °C, 40 min, 80% glycerol and *LSR* of 6.5 mL/g. As can be seen in Table 3, the *DE* value obtained when using pure glycerol (80% glycerol content) was very similar to those achieved using crude glycerol. Cellulosic preservation was high (86–91%), similar to those cellulose contents preserved in organosolv delignification experiments using pure glycerol (Table 2). The experiment with crude glycerol employing 86% of glycerol content (without dilution) attained an even higher *DE* than the experiment employing crude glycerol at 80% (residual cellulose of 91.04%), but with a smaller cellulose preservation (86.40%). Sun and Chen (2008) studied the GOD of raw wheat straw using residual glycerol from biodiesel production, attaining 17% delignification and 94% cellulose preservation under conditions of *LSR* of 20:1, 70% glycerol, 220 °C for 3 h. In the present study, crude glycerol also showed better properties for delignification of SCB pretreated by AH, attaining *DE* values of ~64–68% under milder conditions (*LSR* of 6.5 mL/g, 80% glycerol and 210.3 °C for 40 min). Sun and Chen (2008) attributed the low delignification to the lipophilic compounds present (0.3–4%) in crude glycerol, which deposited on the fiber surface, hindering delignification and lowering its efficiency. The source of crude glycerol used in their study was not informed, nor other information about the source of oil (vegetable, animal or combination) used in the transesterification reaction, which could possibly elucidate the properties of lipophilic compounds present. Contrastingly, the crude glycerol used in the present study had a higher lipid content that apparently did not affect delignification efficiency.

Crude glycerol as a biodiesel industry byproduct is an attractive option as an organosolv solvent. Its direct use in GOD of lignocellulose biomass is very desirable from an economic standpoint. Furthermore, the use of crude glycerol could essentially eliminate the need for glycerol purification. The *DE* values attained when using the crude glycerol represented a promising option for its viable application in a lignocellulose biorefinery especially considering the sugarcane bagasse biorefinery. Due to the good *DE* values obtained with the substitution of pure glycerol by crude glycerol, energetic analyses were made to model its incorporation into the two-stage fractionation process of SCB suggested in the present study in a lignocellulose biorefinery concept.

Table 5
Energetic balance of glycerol organosolv process using raw sugarcane bagasse and sugarcane bagasse pretreated by autohydrolysis condition 1 (S_0 = 3.92: T = 175.8 °C, t = 49 min and LSR = 5.3 mL/g) (experiments 3, 12, 13 and CG-80).

Description of the fractionation strategy	Energy Input (MJ/kg_{SCB})		Energy Output (MJ/kg_{SCB})				
	Autohydrolysis (AH) pretreatment	Glycerol organosolv delignification (GOD)	Single–Stage Anaerobic Digestion (DC2)	Combustion of black liquor	Combustion of 2G bioethanol	Combustion of non-converted cellulose rich solid residue	Energetic profitability
Experiment 3-PG[a,c]	1.95	3.78	2.90	138.95	3.09	2.27	141.48
Experiment 12-PG[a,d]	1.95	3.47	2.90	137.19	2.88	3.78	141.33
Experiment 13-PG[a,e]	1.95	1.63	2.90	58.38	2.85	3.40	63.93
Experiment CG − 80[b,f]	1.95	3.78	2.90	140.41	2.75	2.33	142.67

[a] PG = Pure Glycerol.
[b] CG = Crude Glycerol.
[c] *EC* of 53.8%.
[d] *EC* of 55.5%.
[e] *EC* of 60.3%.
[f] *EC* of 54.6%.

3.6. Energetic balance of the proposed fractionation process in a biorefinery concept

It is important to emphasize that the solid fraction obtained after two-stage fractionation has low lignin and hemicelluloses content, a great advantage for the subsequent enzymatic hydrolysis process for the production of fermentable sugars and consequently for the fermentation into 2G bioethanol. Table 5 shows the energy input and output of each simulated fractionation scenario, whereby energy input describes the energy required to power the pretreatments involved, and energy output the potential energy sources produced by utilization of pretreatment co-products. As seen in Table 5, all scenarios displayed an energy profit ranging from 63.93 to 142.67 MJ/kg_{SCB}. The fractionation strategy that resulted in the highest energy profit was that of the experiment using crude glycerol at 80% (v/v); whilst the fractionation strategy that resulted in the lowest energy profit was that of the experiment 13. The critical factor in the latter scenario was the decrease in the glycerol content (68.2%) compared to other scenarios evaluated, which employed 80% glycerol in GOD. An energy profit was only possible considering the high heat of combustion of pure or crude glycerol (ranging from 16 to 21 MJ/kg (Mattos, 2014)), which could release a huge amount of energy. The strategy of combustion of the residual glycerol in the black liquor was chosen due to its low market value. According to the Independent Chemical Information Service (ICIS, 2014), glycerol with a purity of 80% was estimated to value an average of USD $292.50/ton. Based on this information, the residual glycerol (boiling point of 290 °C) from the GOD is considered a costly waste to be recovered. In addition, its recovery could involve high distillation costs due to its high boiling point, and its storage within an integrated 1G and 2G bioethanol plant would also be difficult. Therefore, experiment 13 was not considered to be an energetically feasible strategy in comparison to the other fractionation strategies.

Another great advantage of using crude glycerol as an organic solvent in organosolv process is the reduction of water usage. It has been demonstrated that crude glycerol coming straight from biodiesel production can be used directly in the organosolv process, and its combustion in the black liquor, together with the lignin, and all other residual carbohydrates, drastically improved the economic feasibility of the process.

Although the results for enzymatic hydrolysis reported in the present study are in the same order of magnitude in comparison to those obtained by organosolv fractionating pretreatment of several lignocellulose biomass feedstocks for improving enzymatic hydrolysis of cellulose (Zhao et al., 2017), it should be noted that the process proposed in the present study is environmentally friendly and energetically sustainable, as the liquid stream (black liquor) of the process containing lignin and glycerol can be used as a fuel in an cogeneration energy system. On the other hand, the pretreatment processes that use other chemicals, such as ammonia and sodium hydroxide for delignification, need to have foreseen in their processing plants a unit for recovering such compounds, which implies in a greater energy demand (Gomes et al., 2014). Another point to be emphasized in the present study is the use of a lower enzyme loading (10 FPU/$g_{drymatter}$) and a lower water content (*LSR* = 10 mL/g) in the enzymatic hydrolysis, which could significantly increase the glucose concentration in the hydrolysate and ethanol concentration in the subsequent fermentation broth (Zhao et al., 2017).

4. Conclusions

Glycerol organosolv delignification (GOD) of sugarcane bagasse (SCB) pretreated by autohydrolysis (AH) showed to be an efficient fractionation method for preparing cellulose rich solids for enzymatic hydrolysis and 2G bioethanol production. The results of the AH pretreatment of SCB under three different conditions showed that an increase in the pretreatment severity led to an increase in the acid-insoluble lignin to acid-soluble lignin ratio, suggesting that changes in lignin structure occurred. The delignification results showed that an increase in the AH pretreatment severity led to a decrease in the delignification extents (*DE*), suggesting that the pretreatment severity defined the values of *DE* obtained. Nevertheless, a *DE* of ~64% was obtained under high temperatures and glycerol contents, while still preserving residual cellulose (> 80%). Crude glycerol displayed similar delignification properties as pure glycerol, suggesting its direct applicability in GOD to improve the economic feasibility of two-stage fractionation process proposed in this study. The fractionation strategy suggested in this study proved to be energetically viable considering that the black liquor containing glycerol, lignin and carbohydrates are burnt.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.indcrop.2017.06.049.

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