Delignification of sugarcane bagasse using glycerol–water mixtures to produce pulps for saccharification

Lísias Pereira Novo [a], Leandro Vinícius Alves Gurgel [a], Karen Marabezi [a], Antonio Aprigio da Silva Curvelo [a,b,*]

[a] Grupo de Físico-química orgânica, Departamento de Físico-química, Instituto de Química de São Carlos, Universidade de São Paulo, Av. Trabalhador São Carlense 400, Caixa Postal 780, 13560-970 São Carlos, São Paulo, Brazil
[b] Laboratório Nacional de Ciência e Tecnologia do Bioetanol (CTBE), Centro de Pesquisa em Energia e Materiais (CNPEM), Caixa Postal 6179, 13083-970 Campinas, São Paulo, Brazil

ABSTRACT

This paper describes the organosolv delignification of depithed bagasse using glycerol–water mixtures without a catalyst. The experiments were performed using two separate experimental designs. In the first experiment, two temperatures (150 and 190 °C), two time periods (60 and 240 min) and two glycerol contents (20% and 80%, v/v) were used. In the second experiment, which was a central composite design, the glycerol content was maintained at 80%, and a range of temperatures (141.7–198.3 °C) and time (23–277 min) was used. The best result, obtained with a glycerol content of 80%, a reaction time of 150 min and a temperature of 198.3 °C, produced pulps with 54.4% pulp yield, 7.75% residual lignin, 81.4% delignification and 13.7% polyose content. The results showed that high contents of glycerol tend to produce pulps with higher delignification and higher polyoses content in relation to the pulps obtained from low glycerol content reactions. In addition, the proposed method shows potential as a pretreatment for cellulose saccharification.

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1. Introduction

Environmental issues have been widely discussed over the past few decades but have grown in importance recently with the development of ecological sustainability policies (Vieira and Celso, 2006). In 1975, the environmental policies of Brazil resulted in the development of a plan for the production of ethanol from sugarcane with the aim of either partially or completely replacing gasoline, diminishing the emissions of green house gases (Bastos, 2007).

The use of fossil fuels is one of the main sources of atmospheric pollution, as its combustion generates gases such as SO_2 and NO_x. Fossil fuels are used in many activities that include the generation of electricity (by combustion of coal, natural gas and oil) and as a fuel for vehicles that run on gasoline and diesel (Moore et al., 2010). The world's energy matrix is based on the use of oil-based fuels, which produce 55% of the world's energy (BNDES and CGEE, 2008). Therefore, the production of "green" fuels has been intensively studied and in this context the use of agricultural by-products has increased.

Agriculture has been one of the major economic sectors throughout Brazil's history (Perobelli et al., 2007) and is presently based on the production of sugarcane (about 720 million tons in 2010) for sugar and bioethanol production, and various grains, such as soy and corn (about 130 million tons in 2010), sources of vegetable oil that may be used for biodiesel production (Brazilian Institute of Geography and Statistics – IBGE, 2011).

Since 2008, Brazilian law has stated that 2% of biodiesel must be added to all diesel made from petroleum with the goal of elevating this quantity to 5% by 2013 (Brazilian National Program of Production and Use of Biodiesel – PNPUB). From 2005 to 2010, the production of biodiesel in Brazil has risen from approximately 700,000 liters to over 2 billion liters (Brazilian National Petrolium Agency – ANP, 2011). One of the most important by-products formed during the production of biodiesel from the reaction of vegetal oil with alcohol is glycerol (Adhikari et al., 2008). It is a high-boiling-point organic solvent, and its production accounts for up to 10% of the total biodiesel product, which is approximately 200 million liters per year in Brazil. The soaring price of oil has made the oleochemical production of compounds like biodiesel more appealing, and glycerol production has increased in Brazil, Europe and the United States. Consequently, this by-product has been generated on a scale larger than its demand on the world market, and its price has declined accordingly (O'Driscoll, 2007; Pichon, 2007).

According to Guragain et al. (2011), the rapid growth of global biodiesel production indicates that crude glycerol from biodiesel industry will be a cheap waste with a high cost to treat rather than a valuable by-product in the future. It also can be a serious

* Corresponding author at: Grupo de físico-química orgânica, Departamento de Físico-química, Instituto de Química de São Carlos, Universidade de São Paulo, Av. Trabalhador São Carlense 400, Caixa Postal 780, 13560-970 São Carlos, São Paulo, Brazil. Tel.: +55 16 3373 9938; fax: +55 16 3373 9952.
E-mail address: aprigio@iqsc.usp.br (A.A.S. Curvelo).

environmental problem. Therefore, the exploitation of crude glycerol for pretreatment of biomass has the potential to be an attractive economic route for the utilization of the by-product directly, leading to commercial success of both bioethanol and biodiesel (Guragain et al., 2011).

The large-scale production of sugar and alcohol from sugarcane in Brazil results in a high quantity of bagasse being generated as a by-product. Bagasse is burned in the boilers of the sugar and alcohol plants to produce steam and energy for the plant. Bagasse exhibits a high polysaccharide content, which is approximately 70% of the total dry mass (Triana et al., 1990). These polysaccharides could be a potential source of sugars for a second-generation ethanol production process. Therefore, in the context of the biorefinery process and taking into account the necessity to separate the three major components of bagasse, it is of great importance to study the pulping (lignin removal) and hydrothermal, or prehydrolysis (polyose removal), processes (Pasquini et al., 2005).

There are various pulping processes in which different kinds of delignification mechanisms are able to occur depending on the reagents/catalyst used. Actually, Kraft process is the most applied industrial pulping process for paper production. This process can be applied for the treatment of different raw materials and is performed using concentrated aqueous alkaline solutions of sodium hydroxide and sodium sulfide and operated at temperatures of approximately 170 °C. The recovery of inorganic reagents is a fundamental step of the process and can be economically performed only for very big mill installations. However, alternative methods have been tested in lower industrial scales such as Alcell™ process which involves organosolv pulping using ethanol/water as liquor with efficient delignification rates (Pye and Lora, 1991).

Organosolv processes can be employed for pretreatment as well as for pulping of different lignocellulosic raw materials and present advantages when compared with aqueous based processes. In particular, the recovery of lignins and polyoses from the liquor is easily performed by distillation with the simultaneously recycling of solvents. Lignins are separated as a solid material while polyose fraction is obtained in aqueous solution. However, organosolv process has some disadvantages such as (1) the pretreated solids need to be washed with organic solvent to avoid the reprecipitation of the dissolved lignin and (2) the reactions occur at higher pressures.

Different organic solvents and experimental conditions were already applied for the treatment of wood and agricultural byproducts. (Sun and Cheng, 2002; Zhu and Pan, 2010). The utilization of external catalyst enables the operation at lower temperatures but includes more sophisticated liquor remake (Aziz and Sarkanen, 1989; Johansson et al., 1987; Muurinen, 2000; Zhao et al., 2009).

The process of organosolv delignification has been study for decades, and ethanol is frequently used as a solvent in this process; however, there are few reports in the literature about the use of glycerol as the delignification agent. The use of glycerol as an organic solvent for pretreatment has some advantages such as (1) low solvent cost since crude glycerol produced in the transesterification process for biodiesel production can be applied instead of pure glycerol, (2) the pretreatment can be performed under atmospheric pressure, decreasing the energy consumption, and (3) due to its highly polar structure, glycerol can easily penetrate the bagasse tissue, providing an effective reaction medium for delignification. However, there are inherent drawbacks to the use of aqueous glycerol for pretreatment. The high energy consumption for solvent recovery may decrease the attractiveness (Saberikhah et al., 2011; Zhao et al., 2009). Two important literature contributions were those of Küçük and Demirbaş (1993); Demirbaş (1998), whose studies indicated that the treatment of a biomass with an aqueous glycerol organosolv for pulping yielded a high amount of delignification and negligible cellulose degradation. Sun and Chen (2007, 2008) also studied the use of aqueous glycerol for

the pretreatment of wheat straw as a method for enhancing enzymatic hydrolysis and demonstrated that the glycerol organosolv pretreatment without the addition of a catalyst led to the recovery of 95% of the cellulose and the removal of 70% of the lignin.

This paper aimed to study the organosolv delignification of depithed sugarcane bagasse by employing glycerol–water mixtures as the solvent system without the addition of a catalyst in order to produce pulps with low lignin content with high polysaccharides preservation. Especially, cellulose for saccharification what is indicated by high values of yield, and low Klason lignin or high delignification extent values.

2. Experimental

2.1. Sugarcane bagasse preparation and chemicals

The raw bagasse from the 2007 and 2008 harvests was collected in the Ipiranga sugar and alcohol plant in Descalvado, São Paulo, Brazil. The raw bagasse was washed at 70 °C for 1 h with constant stirring to remove residual sugars and dirt. Then, the raw bagasse was subjected to a wet depithing process using a sieve system containing two screens of 16 (1.19 mm) and 60 (0.250 mm) mesh. The 60-mesh strainer held the pith, while the 16-mesh strainer caught the bagasse fibers. The depithed bagasse was then air-dried until only 10% moisture remained.

Glycerol (99.5%) was purchased from Synth (Brazil) and used without further purification. The chemicals used in the analyses of the hydrolysates by high performance liquid chromatography (HPLC) were sulfuric acid (95–98%) (Merck), acetonitrile (Tedia) and glacial acetic acid (Merck). The chromatography standards used were D-cellobiose (Sigma cat no. C7252), D-glucose (Sigma cat no. G8270), D-xylose (Sigma cat no. 95729), L-arabinose (Sigma cat no. 10839), acetic acid (49–51%, Sigma cat no. 45754), formic acid (49–51%, Sigma cat no. 09676), 5-hydroxymethyl-2-furfaldehyde (HMF, Sigma cat no. W501808) and 2-furfaldehyde (furfural, Sigma cat no. 319910).

2.2. Organosolv treatment

All of the delignification experiments were performed in 195 cm^3 grade 304 stainless steel reactors (5.0 OD × 4.0 ID × 15.5 cm length). The reactors were equipped with a self-sealed o-ring closure made of polytetrafluoroethylene (PTFE), a fluoropolymer that is resistant to high temperatures (>250 °C). In this design, the sealing force on the gasket was provided by the pressure within the vessel itself. The cap screws were also made of grade 304 stainless steel. The reactors were heated in a thermostatic bath containing glycerol as the heating fluid and stirred mechanically. The pulping reactions were performed using 10 g (dry mass) of the depithed sugarcane bagasse and a solid to liquid ratio of 1:15 (w:v). Premade glycerol-water mixtures of 20:80, 50:50 and 80:20 (v/v) were used, and the reactors were submerged into the glycerol bath at the desired reaction temperature. After the reaction time, the reactors were cooled in an ice bath to stop the reaction.

After the pulping reaction, the pulps were submitted to a defiber process and washed with a 1% (w/v) sodium hydroxide solution to remove any adsorbed lignin on the cellulosic fibers (Pasquini et al., 2005). Afterwards, the pulps were washed with water until a neutral pH was obtained. After the washing, the pulps were filtered in a Büchner funnel and air-dried at 25 °C until only 10% moisture remained.

The pulping experiments were performed using DOE design as a tool to evaluate the effect of temperature, time, and glycerol content on delignification of depithed sugarcane bagasse. Considering that the aim of the present study is to delignify sugarcane bagasse, preserving the cellulose fraction that can be used for subsequent

saccharification. Therefore, DOE was used to optimize lignin extraction and cellulose preservation.

Initially, the DOE used was a cubic experimental design procedure with three factors of two levels each (2^3) and using the central point for error evaluation. The factors used were temperature (T), time (t) and glycerol content (GC). The resulting planning matrix is shown in Table 1. The analyzed responses were pulping yield (Y), Klason lignin quantity (KL), delignification extent and polyose content. This cubic experiment was designed to explore a wide range of possible responses to better understand the system's behavior. After the first experimental set was completed, a new experimental design was developed using the obtained results to optimize the delignification extent while still preserving the cellulose fraction. In addition, this new set of experiments was accomplished considering that an increase in the reaction temperature could increase the delignification extent.

2.3. Pulp analysis

The pulping yield was determined by taking the ratio between the dry weight of the obtained pulp and the dry weight of depithed bagasse (as a percentage). The chemical characterization of the pulp was obtained by determining the quantity of acid-insoluble lignin (the Klason lignin method) according to the Technical Association of the Pulp and Paper Industry (TAPPI) standard method T222 om-02 (TAPPI, 2002). In the Klason method, the depithed bagasse is hydrolyzed by a sulfuric acid solution, and the acid-insoluble lignin is separated from the hydrolysate through filtration using an American Society for Testing and Materials (ASTM) 10–15 sintered funnel. An aliquot of the hydrolysate was stored at -5 °C for subsequent determination of the carbohydrate composition using HPLC. The acid-insoluble lignin was oven-dried at 100 °C and weighed. The delignification extent was determined by Eq. (1) (Pasquini et al., 2005).

$$\text{Delignification(\%)} = \left(\frac{KL^{bagasse} - (KL^{pulp}\frac{Y^{pulp}}{100})}{KL^{bagasse}} \right) \times 100 \tag{1}$$

where $KL^{bagasse}$ is the quantity of insoluble Klason lignin in the depithed bagasse (%), KL^{pulp} is the quantity of the insoluble Klason lignin in the pulp (%) and Y^{pulp} is the yield of the pulping reaction (%).

Quantification of the carbohydrates was performed using a Shimadzu chromatograph model CR 7A equipped with an IR Shimadzu R10–6A detector with an Aminex HPX 87H (300 × 7.8 mm, BIO-RAD) column and eluted with a sulfuric acid solution (0.005 mol L^{-1}) at a flow rate of 0.6 mL min^{-1}. The peaks corresponding to cellobiose and glucose were converted into cellulose (conversion factor of 0.95 and 0.90, respectively), while those corresponding to xylose and arabinose were converted into polyoses (conversion factor of 0.88). The acetic acid peak indicated the presence of acetyl groups in the polyose fraction (conversion factor of 0.717).

The degradation of the sugar products was quantified using a Shimadzu chromatograph model CR 7A equipped with an UV–Vis detector set to 274 nm (Shimadzu, model SPD-10A) with a Hewlett–Packard C_{18} column, model RP 18, and eluted with an acetonitrile/water 1:8 (v/v) solution containing 1% acetic acid (v/v) at a flow rate of 0.8 mL min^{-1}. The peak corresponding to the 5-hydroxymethyl-2-furfuraldehyde (HMF) was converted into cellulose (conversion factor of 1.375) and the furfural peak was converted into polyoses (conversion factor of 1.286). The amount of residual cellulose left after the pulping reaction was determined by Eq. (2).

$$\text{Residual cellulose(\%)} = \left(\frac{C^{pulp}\frac{Y^{pulp}}{100}}{C^{bagasse}} \right) \times 100 \tag{2}$$

where $C^{bagasse}$ is the cellulose content in the depithed bagasse (%), C^{pulp} is the cellulose content in the pulp (%) and Y^{pulp} is the yield of the pulping reaction (%).

From the more appropriated results obtained from the cubic set of experiments (2^3) and considering the aim of the study, another set of experiments was designed using a central composite design of only two factors with two levels. This experimental design, 2^2, is rotated 45° in relation to the painted area shown in Supplementary Fig. S1, resulting in a star-shaped design. The corresponding planning matrix for this design is shown in Table 2.

The experimental results were analyzed using the *Statistica 7.0* software to obtain Pareto diagrams and an ANOVA table as well as to estimate the effects and predict the values of the studied variables. Models for the system were developed from the cubic experimental design, and a polynomial equation was generated for each response.

$$A = a_0 + a_1T + a_2t + a_3GC + a_4Tt + a_5TGC + a_6tGC \tag{3}$$

where A is the desired response; a_0, a_1, a_2, a_3, a_4, a_5 and a_6 are the regression coefficients obtained by fitting to experimental data; T is the temperature (°C); t is the reaction time (min) and GC is the glycerol content (%).

Further models were developed from the central composite experimental design, providing the following equation for each response:

$$R = r_0 + r_1T + r_2T^2 + r_3t + r_4t^2 + r_5Tt \tag{4}$$

where R is the desired response; r_0, r_1, r_2, r_3, r_4, and r_5 are the regression coefficients obtained by fitting to experimental data; T is the temperature (°C); and t is the reaction time (min).

3. Results and discussion

3.1. Cubic design of experiments

The depithed bagasse used in this study contained 22.69% lignin, 49.49% cellulose, and 24.34% polyose (dry mass). The

Table 1
Cubic experimental design (2^3) and obtained results from a glycerol–water pulping system.

Experiment	Temperature (°C)		Time (min)		Glycerol content (%)		Pulp yield (%)	Klason lignin (%)	Delignification (%)	Polyose content (%)	Residual cellulose (%)
1	150	(−1)	60	(−1)	20	(−1)	87.6	19.17 ± 0.08	26.0 ± 0.3	28.33 ± 0.39	91.39 ± 1.46
2	190	(+1)	60	(−1)	20	(−1)	52.5	12.22 ± 0.23	71.7 ± 0.5	7.48 ± 0.06	82.96 ± 0.42
3	150	(−1)	240	(+1)	20	(−1)	67.8	17.40 ± 0.07	48.0 ± 0.2	18.17 ± 0.25	86.10 ± 1.44
4	190	(+1)	240	(+1)	20	(−1)	51.7	18.15 ± 0.16	58.6 ± 0.4	2.51 ± 0.01	83.79 ± 0.30
5	150	(−1)	60	(−1)	80	(+1)	93.0	18.42 ± 0.35	24.5 ± 1.4	27.58 ± 0.37	100.52 ± 0.69
6	190	(+1)	60	(−1)	80	(+1)	66.3	10.32 ± 0.23	69.8 ± 0.7	18.67 ± 0.95	91.69 ± 0.01
7	150	(−1)	240	(+1)	80	(+1)	85.5	17.74 ± 0.58	33.1 ± 2.2	22.81 ± 1.42	101.13 ± 1.53
8	190	(+1)	240	(+1)	80	(+1)	57.8	7.88 ± 0.05	79.9 ± 0.1	14.18 ± 0.01	91.95 ± 0.04
9 (Central)	170	(0)	150	(0)	50	(0)	61.5	13.15 ± 0.39	64.4 ± 1.1	14.53 ± 0.54	88.38 ± 1.01
10 (Central)	170	(0)	150	(0)	50	(0)	62.5	13.31 ± 0.61	63.3 ± 1.7	14.59 ± 0.25	90.81 ± 1.31

Table 2
Central composite experimental design and obtained results from a glycerol–water pulping system with a glycerol content of 80%.

Experiment	Temperature (°C)		Time (min)		Pulp yield (%)	Klason lignin (%)	Delignification (%)	Polyose content (%)	Residual cellulose (%)
1	150	(−1)	60	(−1)	93.0	18.42 ± 0.35	24.5 ± 1.4	27.58 ± 0.37	100.52 ± 0.69
2	150	(−1)	240	(+1)	85.5	17.74 ± 0.58	33.1 ± 2.2	22.81 ± 1.42	101.13 ± 1.53
3	190	(+1)	60	(−1)	66.3	10.32 ± 0.23	69.8 ± 0.7	18.67 ± 0.95	91.69 ± 0.01
4	190	(+1)	240	(+1)	57.8	7.88 ± 0.05	79.9 ± 0.1	14.18 ± 0.01	91.95 ± 0.04
5	141.7	(−√2)	150	(0)	93.8	19.11 ± 0.39	21.0 ± 1.6	26.05 ± 0.62	100.49 ± 1.98
6	198.3	(+√2)	150	(0)	54.4	7.75 ± 0.11	81.4 ± 0.3	13.72 ± 0.40	80.65 ± 0.98
7	170	(0)	23	(−√2)	95.9	18.64 ± 1.03	21.2 ± 4.4	26.25 ± 0.02	105.18 ± 7.22
8	170	(0)	277	(+√2)	65.5	13.28 ± 0.82	61.7 ± 2.4	18.03 ± 0.08	89.59 ± 3.62
9 (Central)	170	(0)	150	(0)	72.2	14.38 ± 0.63	54.2 ± 2.0	19.91 ± 1.39	80.59 ± 3.28
10 (Central)	170	(0)	150	(0)	71.9	15.22 ± 1.08	51.8 ± 3.4	20.78 ± 0.60	83.17 ± 2.45

remaining minor components are extractives and ash. The results from the cubic experimental design are shown in Table 1 and indicate that pulping experiment 8 produced the best results in terms of the amount of insoluble lignin (7.78%) and the delignification extent (79.9%). It is also interesting to note that higher values of residual cellulose (>91%) were attained with higher glycerol concentrations (80%, v/v). The best compromise between lignin removal and cellulose preservation was obtained at a high glycerol content and reaction temperature.

As shown in Table 1, higher water content in the pulping liquor promoted better removal of the polyoses than the lignin, especially at elevated temperatures; however, under these conditions, the cellulosic fraction began to degrade. This behavior is characteristic of the autohydrolysis/hydrothermal process and has a similar mechanism to the dilute acid hydrolysis that has been well discussed by Carvalheiro et al. (2008). As shown in Table 1, the efficiency of lignin removal can be explained by considering the variations in the composition of the glycerol–water mixtures. Autohydrolysis proceeds via a two-step mechanism. During the first step, the hydrolysis catalyst comes from the autoionization of water at high temperatures and depolymerizes hemicellulose through the selective hydrolysis of both the glycosidic bonds and the acetyl groups. In the second step, the hydronium ions come from the ionization of acetic acid and act as a catalyst for the hydrolytic cleavage of the ether bonds present in the lignin, improving delignification. It is evident that the contribution of the hydronium ions from acetic acid is higher than that of water autoionization (Carrasco, 1989; Carvalheiro et al., 2008; Heitz et al., 1986). The water portion of the glycerol–water mixtures is very important to the reaction due to its nucleophilic behavior during the hydrolysis of the ether bonds (α–O–4 and β–O–4) during the second step, while the glycerol is responsible for solubilizing the lignin fragments produced by cleavage of the ether linkages and then transporting them from the plant cell to the solution.

Fig. 1 shows Pareto diagrams of the responses from the cubic experimental design. In Fig. 1, T denotes the temperature, t the time and GC the glycerol content. As shown in Fig. 1, in all cases, the factor with the greatest effect was the reaction temperature. The Pareto diagrams also indicated that most of the other factors and interactions made no significant contribution to the model. For a confidence level of 95% (p-value of 0.05), the only factor of significance was the reaction temperature, except in the case of polyose content, which also had time and glycerol content as significant factors. This finding indicated that the proposed model does not offer a complete explanation of the system, likely because the model was constructed to include a wide range of possibilities and was only an exploratory model.

To obtain additional information, a new experimental design was created. For this design, one face of the cube was chosen to be the basis for a central composite experimental design of only two factors. The chosen face included the better results for lignin removal, which was described by a lower quantity of Klason lignin

or a higher extent of delignification. The higher delignification extents were attained at glycerol:water mixtures of 80:20 (79.9%) and 20:80 (71.7%) as shown in Table 1. According to Sun and Chen (2008), the crude glycerol recovered from biodiesel production has 70–80% purity. The pulping reactions were performed using bidistilled glycerol (99.5%), which is usually processed through neutralization, desalination, condensation, vacuum distillation, and discoloration from crude glycerol in the oleochemical industries. Supplementary Fig. S1 shows the results for the amount of insoluble Klason lignin and extent of delignification obtained from the cubic experimental design. The painted area corresponds to the cube face chosen as the base for the central composite design. Since the temperature exhibited an elevated response level in the previous model, this variable was maintained in the new experimental design. Taking into account the higher delignification extent attained and the potential of utilizing crude glycerol to reduce the purification costs, the glycerol–water mixture of 80:20 was chosen for the following studies, what makes the process economically feasible.

3.2. Central composite design of experiments

The results and conditions for the central composite experimental design are shown in Table 2. As shown in Table 2, a higher delignification extent was obtained in experiment 6, which yielded an insoluble Klason lignin value of 7.75%, an extent of delignification of 81.4% and a residual cellulose value of 80.7%. In this trial, the increase of the reaction temperature from 190 to 198.3 °C resulted in a very similar response to that of the cubic design (experiment 8, Table 1), but with a reduction of 90 min to the reaction time. However, the amount of residual cellulose also decreased from 92% to 80.7%. The decrease of residual cellulose content might be attributed not only to the increase of reaction temperature, but also to the decrease of pH through the formation of organic acids. During the treatment the organic acids are released from polyose (acetic acid from acetyl groups, formic acid and levulinic acid from sugar degradation) and dissociate into hydrogen ions and promoted acid hydrolysis of cellulose at high temperatures (Yang and Wyman, 2008).

3.3. Design of experiments analysis

Statistica software was used to analyze the data and create a model to describe the process under the applied conditions. The p-values for the different factors and interactions for each response are listed in Table 3, where T corresponds to the temperature and t corresponds to the time. These values were obtained from an ANOVA table. The p-value parameter indicates the significance of the factor or interaction to the model. If a p-value is lower than the stipulated minimum, 0.05 and 0.10 (confidence levels of 95% and 90%, respectively) for example, then the factor is relevant to the model, and if not, then the factor may be excluded after analysis.
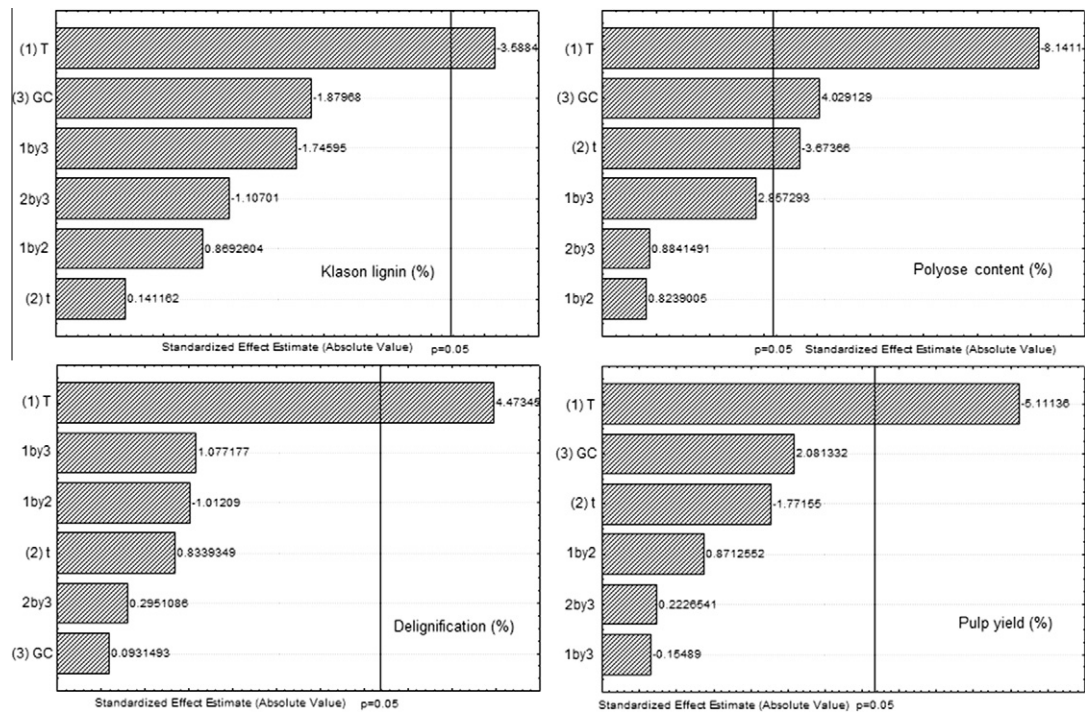
Fig. 1. Pareto diagrams of the responses from the cubic experimental design (2^3).

Table 3
ANOVA table for the studied responses from the central composite design.

Source of variation	p-values before exclusion			
	Pulp yield	Klason lignin	Delignification	Polyose content
T	0.0014	0.0005	0.0014	0.0000
T^2	0.8108	0.1605	0.9036	0.3278
t	0.0134	0.0340	0.0280	0.0002
t^2	0.1673	0.6159	0.3041	0.0283
Tt	0.9145	0.5028	0.9343	0.8078
Source of variation	p-values after exclusion			
	Pulp yield	Klason lignin	Delignification	Polyose content
T	0.0001	0.0000	0.0001	0.0000
T^2	–	0.0528	–	–
t	0.0022	0.0124	0.0062	0.0000
t^2	0.0789	–	0.1466	0.0040
Tt	–	–	–	–

Thus, the lower a value is, the higher its significance. Elimination of a factor was accomplished by removing any p-values higher than 0.15 (confidence level of 85%) one at a time until only lower values remained. The model with only the significant effects for all responses is shown in Table 3.

For all of the measured responses, the most significant effect was from changes to the linear reaction temperature, possessing a confidence level equal to or higher than 99%, as was expected from the results obtained during the cubic experimental design. The second-most significant effect on the responses was caused by the linear reaction time, possessing p-values lower than 0.02 (98%).

Most p-values were very low, which indicates that the generated models were more consistent than the previous ones. Further evidence of this consistency is that the plot of predicted versus observed data, shown in Fig. 2, demonstrated a good correlation between the predicted responses and those observed after taking into account the values established previously.

Table 4 shows the estimated coefficients for the models from the cubic experimental design and the central composite experimental designs. Table 4 clearly shows the parameters that established the polynomial equation for the models. Recapitulating, the aim of this study was to remove the lignin while preserving the polysaccharides, especially cellulose. Therefore, the optimum pulping system, taking into account the pulping yield, delignification extent, and polyose content, is one resulting in a high pulping yield and a low quantity of insoluble Klason lignin or a high delignification extent. By analyzing whether the coefficient parameters for the factor or the interaction between factors is positive or negative, it is possible to infer the quality of the pulping process. As shown in Table 4, for the cubic experimental design, the reaction temperature was the most significant factor for all responses. The coefficient, a_1, contributed negatively for pulping yield and polyose content and positively for the quantity of insoluble Klason lignin and delignification extent. The signs of the coefficient a_3 showed that a high glycerol content preserves the polysaccharide fraction and promotes lignin removal, producing high pulping yields.

The central composite experimental design portion of Table 4 shows that with an increase in temperature for both the linear and the quadratic cases, the Klason lignin and the delignification extent approached optimum values, while the pulping yield and polyose content deviated further from the optimum values. The reaction time, as opposed to the temperature, exhibited opposite trends for the linear and the quadratic cases when a significant response was observed. Thus, the contribution of each response depends on the value of its magnitude. For both the pulping yield and polyose content responses, the linear response to the time factor results in positive values for the negative levels and negative values for the positive levels, while the quadratic response provides positive values for all levels. Therefore, in the data ranges studied, the linear time response has a greater contribution than the quadratic time response, which indicates that, for these responses, a lower reaction time provides better results, as shown
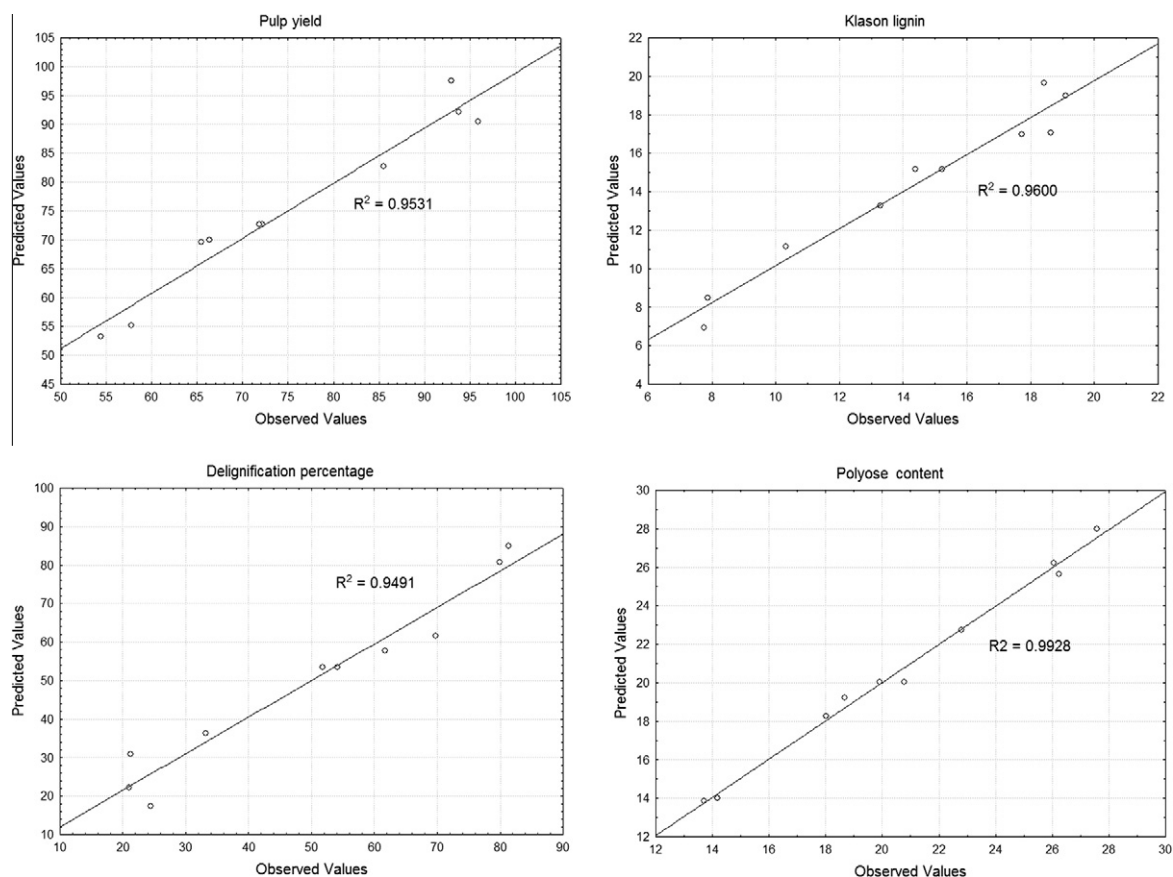
Fig. 2. Predicted versus observed data and *R*-squared values for each of the studied responses.

Table 4
Estimated coefficients for both models.

Parameter	Cubic experimental design			
	Pulp yield	Klason lignin	Delignification	Polyose content
a_0	53.95 ± 3.71	14.78 ± 0.75	16.88 ± 0.74	68.62 ± 2.31
a_1	18.56 ± 4.15	−3.02 ± 0.84	−6.76 ± 0.83	−13.20 ± 2.58
a_2	3.46 ± 4.15	0.12 ± 0.84	−3.05 ± 0.83	−4.57 ± 2.58
a_3	0.39 ± 4.15	−1.58 ± 0.84	3.34 ± 0.83	5.37 ± 2.58
a_4	−4.20 ± 4.15	0.73 ± 0.84	0.68 ± 0.83	2.25 ± 2.58
a_5	4.47 ± 4.15	−1.47 ± 0.84	2.37 ± 0.83	−0.40 ± 2.58
a_6	1.22 ± 4.15	−0.93 ± 0.84	0.73 ± 0.83	0.57 ± 2.58
	Central composite experimental design			
Parameter	Pulp yield	Klason lignin	Delignification	Polyose content
r_0	72.72 ± 1.89	15.15 ± 0.50	53.55 ± 3.02	20.02 ± 0.23
r_1	−13.76 ± 1.44	−4.25 ± 0.38	22.18 ± 2.31	−4.37 ± 0.18
r_2	–	−1.09 ± 0.45	–	–
r_3	−7.38 ± 1.44	−1.34 ± 0.38	9.51 ± 2.31	−2.61 ± 0.18
r_4	3.64 ± 1.72	–	−4.60 ± 2.76	0.97 ± 0.21
r_5	–	–	–	–

in Supplementary Fig. S2. The results for delignification indicate that the opposite occurs: the linear time is negative for the negative levels and positive for the positive levels, while the quadratic time is negative for all levels. Thus, for the data range studied, the magnitude of the effect of linear time is higher than that of quadratic time, indicating that increased reaction times will have a greater delignification extent, as indicated in Supplementary Fig. S2. The responses that approached the optimum case for increasing the reaction time are Klason lignin and delignification extent, while the polyose content and the pulping yield deviate from the optimum.

3.4. Treatment applicability

To obtain the optimum results for the glycerol organosolv pulping process, which requires a high delignification extent and residual cellulose, it is necessary to use a high ratio of glycerol in the pulping liquor, a high temperature and intermediate reaction times. Intermediate reaction times are especially important if there is interest in preserving the cellulosic fraction.

Ruzene et al. (2007) obtained pulps from sugarcane bagasse using an ethanol–water solution as the solvent and employing 0.02 mol L^{-1} H_2SO_4 and 5 wt.% NaOH as catalysts at 160 °C, which yielded a Kappa-number of 41.1 and 46.2, respectively, or Klason lignin quantities of 8.2% and 9.2%, respectively (giving a Klason to Kappa ratio equal to 0.2). Sun and Chen (2007) studied the pretreatment of wheat straw with glycerol (15 g/g based on dry feedstock) at 240 °C for 4 h and attained a delignification extent closer to 70%. Pasquini et al. (2005) obtained pulps from depithed bagasse using a 1-butanol/water (60/40) solvent system at 190 °C for 105 min with CO_2 at subcritical conditions, which yielded the best recorded results with a delignification extent of 94.5%. The best results obtained in the present study are very close to those reported by Ruzene et al. (2007) and are better than those reported by Sun and Chen (2007); however, the results reported by Pasquini et al. (2005) are better than those obtained here: 94.5% delignification versus 81.4%; however, these results were obtained using an external catalyst, such as a mineral acid/base or high pressure, whereas in the present study, no external catalysts were used, and milder conditions were employed. One of the main advantages of the proposed autocatalyzed glycerol

treatment is the significant cost savings in equipment relative to the acid catalyzed treatment.

One of the most important applications of organosolv pulps with regards to a biorefinery is enzymatic saccharification to produce fermentable sugars for bioethanol and other high value chemicals. According to Mooney et al. (1998), who studied the effect of lignin content on the enzymatic hydrolysis of softwood, a decrease in the lignin content does not necessarily lead to a higher accessibility of cellulose. In some cases, redeposition of lignin on the cellulose surface occurs, leading to a pulp with lower accessibility. In addition, the results reported by Chang and Holtzapple (2000) indicated that an effective lignocellulosic pretreatment process should remove all of the acetyl groups and reduce the lignin content to approximately 10%. Further reduction incurs an extra cost and, thus, is not justifiable for enzymatic hydrolysis (Kim and Holtzapple, 2006).

Therefore, a higher removal of lignin is not necessary for the enzymatic hydrolysis of the lignocellulosic biomass. The pulps obtained in this paper contain composition characteristics close to those recommended by Chang and Holtzapple (2000) for use in enzymatic hydrolysis, i.e., the absence of acetyl groups and a low lignin content (<8%).

Considering the possible use of pulps for enzymatic saccharification the better treatment conditions taking into account lignin removal are: (1) 198.3 °C, 150 min, and glycerol:water content of 80:20 and (2) 190 °C, 240 min, and glycerol:water content of 80:20. The choice of the best treatment condition related to energy requirement and time is the first option because an increase of 4.4% in the reaction temperature results in a time economy of 90 min, which increases the amount of biomass processed.

4. Conclusions

The organosolv process studied showed potential as method of delignification that still preserves the cellulose fraction. The best results obtained in the present study correspond to following pulp quality: residual lignin amount less than 8%; extent of delignification close to 80%; and residual cellulose content higher than 80%.

The studies developed showed that pulping experiments with higher glycerol content liquors exhibited better results for amounts of delignification and residual cellulose than those using higher water content solutions.

Furthermore, the obtained results demonstrated the possibility of applying crude glycerol directly from biodiesel production as delignification agent, making the process economically feasible.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2011.08.050.

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