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DELIGNIFICATION OF SUGARCANE BAGASSE USING CRUDE GLYCEROL ORGANOSOLV PRETREATMENT

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ABSTRACT

The pretreatment of sugarcane bagasse with crude glycerol from biodiesel production was evaluated at three different times (30, 45, and 60 min), varying the temperature, glycerol, and solids concentrations. The pretreatment efficiency was evaluated based on delignification and fermentable sugars production. The results show, considering a 95% confidence level, that the main effect of temperature on lignin solubilization is significant and positive only when the pretreatment is performed for the shortest time considered (30 min). For all pretreatment times, the interaction between solids concentration and temperature is significant and higher than the main effect of solids concentration. Lignin solubilization was similar in all pretreatment conditions, which did not strongly affect cellulose conversion during the enzymatic hydrolysis stage.

Keywords: Circular economy. Fractionation. Lignin solubilization. Fermentable sugars.

1 INTRODUCTION

Biorefinery is considered a sustainable development technology in the biomass value chain, making it possible to improve the use of biomass by prioritizing the development of a specific platform depending on the market, socioeconomic needs, and environmental problems of a country[1]. In the case of Brazil, the sugarcane biorefinery is well developed, producing different types of sugar, first-generation ethanol (1G ethanol), and heat and bioelectricity (cogeneration) through the combustion of sugarcane bagasse and straw. This sector is self-sufficient in energy and still sells surplus production. Despite cogeneration, there is still a surplus of lignocellulosic biomass in this sector, and it can be incorporated into the chain to obtain other fuels, such as second-generation ethanol (2G ethanol) and biogas, for example, further strengthening the sector and expanding possibilities of integration of the 1G2G chain[2]. For use in these technologies, the deconstruction of biomass through pretreatment is a high-cost process but is essential for obtaining fermentable sugars after the enzymatic hydrolysis step. Several pretreatments have been evaluated over the last few years for the fractionation of lignocellulosic biomasses. Among these, organosolv processes demonstrate great attractiveness, including selectivity of components, highly digestible cellulose, low production of inhibitors, and use of noncorrosive and potentially recyclable solvents[3]. Therefore, crude glycerol becomes attractive because it promotes such effects on biomass, in addition to a low cost. In addition, the glycerol polar structure promotes fast penetration into the lignocellulosic biomass fiber, providing an effective condition for delignification[4]. Therefore, this work aims to evaluate the use of crude glycerol, a byproduct of the biodiesel chain, in the delignification of sugarcane bagasse and fermentable sugars production.

2 MATERIAL & METHODS

Raw sugarcane bagasse was provided by Usina Granelli, located in Charqueada-SP, Brazil. The material was dried at room temperature and stored in plastic bags. The chemical composition was determined by Sluiter et al.[5] methodology, comprising 47.68 ± 0.39% cellulose, 30.16 ± 0.36% hemicelluloses, 22.88% ± 0.81% lignin, 1.38 ± 0.39% ash, and 1.83 ± 0.06% extractives. Crude glycerol from transesterification was provided by a biodiesel production plant located in Montes Claros-MG, Brazil, and the composition was 76.5% glycerin, 18% water, 4.5% ash, and 1% other organic material, its specific mass was 1,237 kg/m³ and pH 6. The cellulolytic enzyme cocktail CellicCtec2 used in the enzymatic hydrolysis was provided by Novozymes (Bagsværd, Denmark).

For the evaluation of the pretreatment, three 2^3 factorial designs were performed for 30, 45, and 60 min of pretreatment (Table 1), considering triplicates at the central points. Each experiment was conducted by preparing the reactional media, which was bagasse (without washing, prior milling or sieving) mixed with glycerol and water solution in the amounts described in Table 1. The reaction media was then subjected to pretreatment in 500 mL stainless-steel reactors without stirring and immersed in a thermostatic bath (Marconi, MA 159/BB, Brazil) with glycerin as the thermal fluid.

Table 1 Factorial planning of the crude glycerol organosolv.

Factors	Levels		
	-1	0	1
Temperature (°C)	180	195	210
Solid concentration (%)	10	20	30
Glycerol concentration (%)	30	50	70

After the reaction time, the reactors were removed from the glycerin bath and submerged in an ice bath to halt the reaction. The reactors were opened, and for pretreatments with 20 and 30% solids (w/w), 10 mL water/g raw bagasse was added before all the material was centrifuged to separate the solid and liquid. For pretreatment with 10% solids, centrifugation occurred without adding water. The remaining solid fraction was washed by adding 10 mL with a 1.0% (w/w) NaOH solution/g of raw bagasse. The mixture was stirred at 150 rpm in a shaker (Marconi MA 832, Brazil) at room temperature for 40 min, and after this time, the mixture was centrifuged to remove the liquor. The procedure described was repeated four times. Last, the bagasse was washed with water until neutral pH and dried at room temperature to chemical characterization[5] and enzymatic hydrolysis assays.

Enzymatic hydrolysis was performed in flasks incubated in an orbital shaker (Marconi MA 832, Brazil) at 150 rpm for 72 h, 50°C, and 15 FPU/g bagasse of Cellic Ctec 2 (Novozymes). Hydrolysis was performed in a 0.05 mol/L citrate buffer solution with pH 4.8 and a solids concentration of 10% (w/w). The conversion of cellulose into glucose was calculated from the composition of the pretreated bagasse and the sugar concentration present at the end of the enzymatic hydrolysis. The quantification of sugars obtained after enzymatic hydrolysis was performed by a 1260 Infinity HPLC system (Agilent, Santa Clara, USA) with a refractive index (RI) detector. The stationary phase used was an Aminex HPX-87H column (300 mm × 7.8 mm, 5 µm) with Micro-Guard Cation PC H Refill Cartridges (Bio-Rad Laboratories, Hercules, USA). The mobile phase consisted of sulfuric acid (5 mmol/L) flowing at a rate of 0.6 mL/min.

3 RESULTS & DISCUSSION

Figure 1 shows the delignification of each pretreatment conditions. The values of the central points of the 30, 45, and 60 min pretreatments (195°C, 20% solids (w/w) and 50% glycerol (w/w)) are presented as the triplicate mean. The standard deviation of the central points was used as the standard deviation of the design. In the pretreatments performed, the lignin solubilization various from 62,1% ± 0,4 to 88,82% ± 0,4%. High lignin solubilization is desirable since not only is the access of the enzymes used in enzymatic hydrolysis to cellulose facilitated, but the added value of the biomass is also maximized by the recovery of lignin from the biorefinery point of view[6]. It can also be noted that, for most of the experiments, pretreatment time had no influence on lignin solubilization.
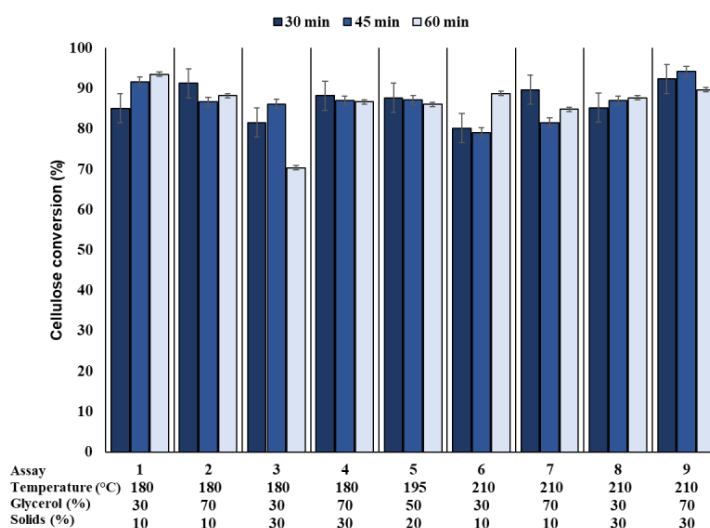

Figure 1. Solubilization of lignin for the assays of the factorial designs at 30, 45, and 60 min. The central point was triplicated and employed as the measure of standard deviation within the experimental design.

Table 2 shows the effect of the factorial design on lignin solubilization.

Table 2 Effects of the factorial design on delignification (%). Effects in bold are significant at the 95% confidence level ($p<0.05$) considering residual error.

Time (min)	30		45		60	
Effects (%)	Lignin solubilization (%)	p-value	Lignin solubilization (%)	p-value	Lignin solubilization (%)	p-value
(1) Temperature	**11.4**	**0.003**	0.8	0.747	6.1	0.174
(2) % Glycerol	1.8	0.342	2.5	0.358	7.1	0.130
(3) % Solids	**-5.1**	**0.041**	3.6	0.202	1.6	0.697
(1)(2)	1.5	0.431	5.6	0.078	3.4	0.412
(1)(3)	**8.1**	**0.009**	**9.0**	**0.019**	**12.4**	**0.029**
(2)(3)	-1.0	0.598	-4.7	0.117	1.1	0.780

At the 95% confidence level, the main effect of temperature on lignin solubilization is significant and positive only when the pretreatment is performed for the shortest time considered (30 min). For all pretreatment times, the interaction between solids concentration and temperature is significant and stronger than the main effect of solids concentration alone. This interaction has the greatest impact on lignin solubilization, except for the pretreatment conducted for 30 min, where the main effect of temperature is most pronounced. The concentration of glycerol showed no significant influence on lignin solubilization at the 95% confidence level.

After the pretreatments, the pulps were hydrolyzed to determine the conversion of cellulose for each of the pretreatment assays, considering 72 h of hydrolysis (Figure 2).
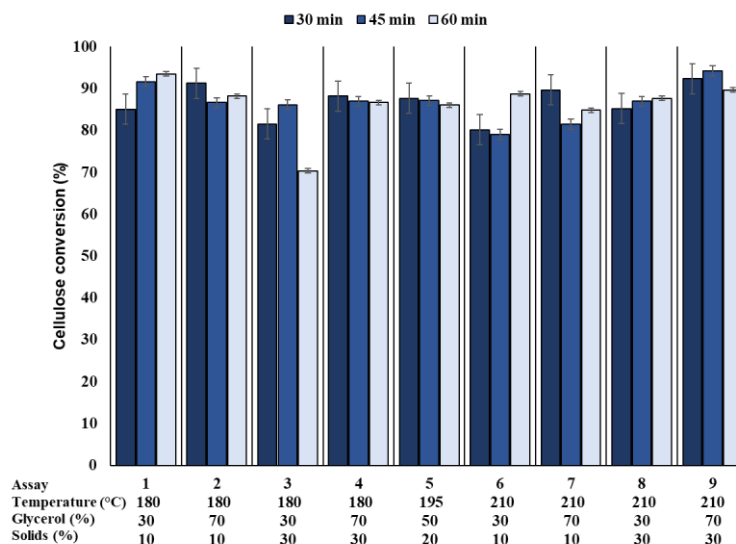

Figure 2 Cellulose conversion into glucose after 72 h of enzymatic hydrolysis. The central point was triplicated and employed as the measure of standard deviation within the experimental design.

It can be seen from Figure 2 that all pretreatment conditions led to similar cellulose conversions, which means that the susceptibility of the pretreated biomass to enzymatic attack is similar for all the conditions studied, making the pulps equally hydrolysable. According to Sun et al.[7] the high conversion of cellulose is achieved due to the characteristics of pretreatment with glycerol, which produces a significant modification of the physicochemical characteristics of sugarcane bagasse.

4 CONCLUSION

The glycerol organosolv pretreatment step demonstrated efficient solubilization of lignin, showcasing the effective interaction between crude glycerol and sugarcane bagasse fiber, even under milder conditions. This process resulted in low concentrations of inhibitors and enhanced the hydrolysis of carbohydrates present in the pulp.

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