The effect of organosolv pretreatment variables on enzymatic hydrolysis of sugarcane bagasse

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ABSTRACT

Sugarcane bagasse pretreated with dilute-acid was submitted to an organosolv ethanol process with NaOH under different operational conditions (pretreatment time, temperature, and ethanol concentration) aiming to maximize the glucose yield in the subsequent enzymatic hydrolysis stage. The different pretreatment conditions resulted in variations in the chemical composition of the solid residue as well as in the glucose recovered by enzymatic hydrolysis. All the studied variables presented significant ($p < 0.05$) influence on the process. The optimum organosolv pretreatment conditions consisted in using 30% (v/v) ethanol at 195 °C, during 60 min. Enzymatic hydrolysis of the residue then obtained produced 18.1 g/l glucose, correspondent to a yield of 29.1 g glucose/100 g sugarcane bagasse. The scale-up of this process, by performing the acid pretreatment in a 10-l semi-pilot reactor fed with direct steam, was successfully performed, being obtained a glucose yield similar to that found when the acid pretreatment was performed in autoclave.

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1. Introduction

The economic feasibility of second-generation bioethanol, i.e. ethanol produced from lignocelluloses, depends among other factors on the availability of cheap feedstocks [1]. In Cuba, sugarcane bagasse (the solid residue obtained after extraction of the sugarcane juice) is a residue available in large quantities and its use to produce fuels and chemicals would contribute to decrease the nation's dependence on oil importation. Currently, part of the sugarcane bagasse generated in the sugar-mills is used for producing steam and electricity required for the cane processing plant. However, large amounts still remain unused, and could be employed in many practical applications, such as raw material for ethanol production.

The conversion of lignocellulosic residues to ethanol is a topic of great interest nowadays. This process, which consist in a pretreatment of the raw material for hemicellulose sugars extraction, followed by a treatment (usually enzymatic) for the cellulose conversion to glucose that will be converted to ethanol by fermentation, has been strongly studied but there are some challenges to be overcome to achieve an efficient production on commercial scale [2]. The main techno-economic challenge is the development of cost-effective pretreatment methods to make cellulose

more accessible to enzymes. Pretreatment is non-trivial owing to the heterogeneity of the lignocellulosic materials and the tight three-dimensional structure of them due to the network of lignin, hemicellulose, and cellulose [3]. During the last decades, many pretreatment processes, including the use of dilute-acid [4,5], steam explosion [1,6], wet oxidation [7,8], organosolvents [9–11], among others, have been developed for decreasing the biomass recalcitrance, but only a few of them seem to be promising.

Among the pretreatment technologies, organosolv process has been considered as one of the most promising for second generation ethanol [12,13]. Treatment with organosolvents involves the use of an organic liquid (methanol, ethanol, acetone, ethylene glycol or triethylene glycol) and water, with or without addition of a catalyst agent (acid or base). This mixture partially hydrolyzes lignin bonds and lignin-carbohydrate bonds, resulting in a solid residue composed mainly by cellulose and some hemicellulose [14,15]. Organosolv pretreatments efficiently remove lignin from lignocellulosic materials but most of the hemicellulose sugars are also solubilized by this process. Therefore, a combined use of organosolv process with a previous stage of dilute-acid hydrolysis, to separate hemicellulose and lignin in two consecutive fractionation steps, would be useful to produce a pulp enriched in cellulose, avoiding losses of potential valuable sources from hemicellulose. Organosolv process is also reported to be able to produce a large amount of a high-quality lignin that is relatively pure, primarily unaltered, and less condensed than Kraft lignins. Such lignin is partially soluble in many organic solvents and could be applied in the fields of

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adhesives, films and biodegradable polymers [16]. The use of the lignin and hemicellulose fractions obtained during the lignocellulosic biomass fractionation is of large importance in a biorefinery concept.

Low boiling point alcohols, mainly methanol and ethanol, seem to be the most suitable organic liquids for use in organosolv processes, due to their low cost and easy recovery. However, pretreatment with ethanol is safer because ethanol is less toxic than methanol [17]. In addition, substrates pretreated by organosolv ethanol process have been reported to have superior enzymatic digestibility over those pretreated by the other alternative processes [18]. The use of sodium hydroxide as catalyst agent during organosolv ethanol pretreatment greatly improves the ethanol selectivity with respect to lignin, i.e., improves the delignifying ability of ethanol [19]. Otherwise, ethanol also reduces the surface tension of the pulping liquor favoring the alkali penetration into the material structure, and the lignin removal, as a consequence [20].

The efficiency of the organosolv ethanol process with alkali may be significantly improved when the lignocellulosic material has been previously submitted to an acid catalyzed pre-hydrolysis [21]. Considering this fact and all the above mentioned reasons, the present work had as objective to evaluate the sugarcane bagasse fractionation by organosolv ethanol pretreatment with NaOH. More specifically, the effect of organosolv pretreatment variables on enzymatic hydrolysis of sugarcane bagasse was evaluated. Reactions were performed under different operational conditions (organosolv pretreatment time, temperature, and ethanol concentration), according to a 2^3 full-factorial design. The solid residue obtained in each experimental condition was enzymatically hydrolyzed, being the released glucose concentration and the glucose yield per gram of sugarcane bagasse determined. The design allowed to define the pretreatment variables of great influence on enzymatic hydrolysis of sugarcane bagasse and to define the conditions able to maximize the glucose recovery yield.

2. Material and methods

2.1. Raw material and dilute-acid pretreatment

Sugarcane bagasse was supplied by the sugar mill “Amancio Rodríguez” in Las Tunas, Cuba. Sugarcane bagasse was manually collected, depithed and dry packed. The moisture content was 8% (w/w) and the particles sizes were less than 1 cm. Prior to be used in the organosolv process, sugarcane bagasse was submitted to a dilute-acid pretreatment (0.2 M H_2SO_4 solution in a solid:liquid ratio of 1:5 w:w, at 120 °C for 40 min), which was performed in 100-ml flasks in an autoclave. After the reaction, the obtained solid residue was separated by filtration, washed with water until neutral pH and dried at 40 °C to attain around 5% moisture content. The original material and the acid pretreated sugarcane bagasse were chemically characterized to determine glucose, xylose and lignin contents. In a second stage, the dilute acid hydrolysis was carried out in a 10-l semi-pilot reactor, to evaluate the possibility of process scale-up. This process consisted in mixing 500 g of raw material with dilute acid inside the reactor, which was then heated by direct steam. The other operational conditions used in this stage were the same as used in the process in autoclave.

2.2. Organosolv ethanol pretreatment

The acid pretreated sugarcane bagasse was submitted to organosolv treatment, which was carried out in a 1000-ml Parr reactor. Reactions were performed using the acid pretreated sugarcane bagasse and the ethanol and NaOH solution in a solid:liquid

Table 1

Experimental range and levels of the process independent variables evaluated for organosolv ethanol pretreatment of acid-pretreated sugarcane bagasse, according to a 2^3 full factorial design.

Independent variable	Symbol	Range and levels		
		−1	0	+1
Pretreatment time (min)	x_1	20	40	60
Temperature (°C)	x_2	175	185	195
Ethanol concentration (% v/v)	x_3	10	20	30

ratio of 1:7 w:w, at different pretreatment times, temperatures and ethanol concentrations, according to a 2^3 full factorial design (Table 1). A 3% (w/w on dry fiber) NaOH concentration was used in the solutions of all the experiments. At the end of the reactions, the reactors were immediately cooled in ice bath, and the obtained hydrolysate was separated from the residual solid by filtration. The pretreated solids were washed with water to remove residual ethanol and alkali, dried at 40 °C, and a sample of each one of them was analyzed to determine the remaining glucose, xylose and lignin contents. All the reactions were carried out in duplicate.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis of the solid residues obtained after organosolv ethanol pretreatment was performed by using a commercial cellulase concentrate (Celluclast 1.5 L) supplemented with β -glucosidase (Novozym 188), both from Novozymes (A/S Bagsvaerd, Denmark). For the reactions, a cellulase loading of 15 filter paper units (FPU)/g substrate, and a β -glucosidase loading of 15 international units (IU)/g substrate were added to 50 mM sodium citrate buffer (pH 4.8) and them mixed to the solid substrate to give a 5% (w/v) consistency. The enzymatic hydrolysis experiments were performed in shaker at 50 °C, 150 rpm for 24 h. Glucose content released in the reactions was quantified by HPLC. The glucose yield was expressed as the ratio between the amount of glucose released in the enzymatic hydrolysis and the amount of initial raw material.

2.4. Experimental design

A 2^3 full-factorial design with two experiments to each condition and four at the midpoint leading to 12 sets of experiments was made to evaluate the effect of the variables: pretreatment time (x_1), temperature (x_2) and ethanol concentration (x_3) during the sugarcane bagasse pretreatment by organosolv ethanol process. For statistical analysis, the variables were coded according to Eq. (1), where each independent variable is represented by x_i (coded value), X_i (real value), X_0 (real value at the midpoint), and ΔX_i (step change value). The range and levels of the variables investigated in this study are given in Table 1. Low and high factors were coded as −1 and +1; the midpoint was coded as 0.

$$x_i = \frac{(X_i - X_0)}{\Delta X_i} \quad (1)$$

Four assays at the midpoint of the design were carried out to estimate the random error needed for the analysis of variance, as well as to examine the presence of curvature in the response surface. The glucose concentration in the enzymatic hydrolysates and the glucose yield (g glucose/100 g initial raw material) were taken as the dependent variables or responses of the design experiments. The results were subjected to an analysis of variance (ANOVA), and the responses and variables (in coded unit) were correlated by the response surface analysis to obtain the coefficients of Eq. (2).

$$\hat{y}_i = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{23}x_2x_3 \quad (2)$$

Table 2

Composition of the main components of sugarcane bagasse (in the original form and after the pretreatment with dilute sulfuric acid), recovered mass and removal of each component after the acid pretreatment.

Component	Composition of original (untreated) sugarcane bagasse (g/100 g)	Recovered mass after the acid pretreatment (g) ^a	Removal after the acid pretreatment (% w/w)	Composition of acid pretreated sugarcane bagasse (% w/w)
Glucose	44.94	44.78	0.36	61.83
Xylose	28.24	7.60	73.09	10.60
Lignin	18.93	18.77	0.85	25.92
Others ^b	7.89	1.28	83.78	1.65
Total	100	72.43	27.57	100

^a Values correspondent to the mass recovered from each 100 g of the original sugarcane bagasse, calculated by the percentage of each fraction in 72.43 g of the pretreated material (total recovered mass after the acid pretreatment).

^b Other components include ashes, proteins, and extractives.

In Eq. (2), $\hat{y}_i$ represents the response or dependent variable; a_0 is the interception coefficient; x_1 , x_2 and x_3 are the coded levels of the variables (pretreatment time, temperature, and ethanol concentration), and a_1 , a_2 , a_3 , a_{12} , a_{13} , and a_{23} are the regression coefficients. The statistical significance of the regression coefficients was determined by Student's *t*-test, and the proportion of variance explained by the models was given by the multiple coefficient of determination, R^2 . Statistica 5.0 was the software used for regression and graphical analyses of the data.

2.5. Analytical procedures

The chemical composition (glucose, xylose, and lignin) of sugarcane bagasse in the starting, acid-pretreated and organosolv pretreated forms was determined by performing a two-steps sequential acid hydrolysis, based on the material reaction with 72% (w/w) H_2SO_4 at 30 °C for 1 h. After this pretreatment, distilled water was added to the mixture to dilute H_2SO_4 to 4% (w/w) and autoclaved at 121 °C for 1 h [22]. Glucose and xylose concentrations were determined by HPLC using a Varian Prostar liquid chromatograph equipped with a RI detector and an Aminex HPX-87P column at 80 °C, deionized water as mobile phase under a flow rate of 0.4 ml/min. Hydroxymethylfurfural (HMF) and furfural concentrations were also analyzed by HPLC but using a UV detector and a Bio-Rad HPX-87H column at 65 °C, 5 mM H_2SO_4 as eluent at a flow rate of 0.5 ml/min.

The activity of the cellulase concentrate was determined using the filter paper assay and expressed in FPU [23], while the β -glucosidase activity was determined using *p*-nitrophenyl- β -D-glucoside as substrate, and expressed in IU [24]. All analytical determinations were performed in duplicate (average results are shown).

3. Results and discussion

3.1. Acid pretreatment

To be used in the organosolv ethanol process, sugarcane bagasse was initially pretreated with dilute sulfuric acid to solubilize the hemicellulose fraction, since it has been reported an improvement in the efficiency of the organosolv process by the removal of this fraction from the lignocellulosic structure in a first stage [21]. The previous removal of the hemicellulose is also of interest from economical and technological viewpoints, since contributes to decrease the use of chemicals in the next pretreatment stage (in our case, the organosolv ethanol process), and facilitates the diffusion of the chemical agents due to the porosity that its removal causes on the material structure. In addition, the solubilized hemicellulose sugars can be used as substrates for the production of different value-added compounds that is of great importance in a biorefinery concept.

Dilute-acid hydrolysis was the technique chosen for this pretreatment because it does not require the use of drastic conditions (temperature is usually between 120 °C and 160 °C, and the acid concentration varies between 1 and 4%) and is efficient for the hemicellulose sugars solubilization, promoting little sugar decomposition [25,26]. In fact, chemical analyses of the original and pretreated sugarcane bagasse (Table 2) revealed that xylose, which is the main hemicellulose sugar in sugarcane bagasse, was removed more than 50% from the material structure during the acid pretreatment. In addition, furfural that is a compound generated from the pentose sugars degradation [25] was found in low concentration (0.76 g/l) in the obtained liquid phase (hemicellulose hydrolysate), being an indicative of the little xylose degradation during the acid pretreatment.

The selectivity of this pretreatment for hemicellulose removal is confirmed by the results of glucose and lignin shown in Table 2. Note that glucose, sugar proceeding from the cellulose structure, was removed less than 1% (w/w) from the original raw material. Degradation of the released glucose to HMF practically did not occur, since this compound was found in the hemicellulosic hydrolysate in a concentration of 0.01 g/l, only. Lignin was even less attacked than cellulose during the acid pretreatment. In this case, only 0.85% (w/w) of this fraction was removed from the sugarcane bagasse structure as a consequence of the acid pretreatment. Cellulose and lignin have been well cited as being more resistant to attack by dilute acids than hemicelluloses [5,27].

Besides hemicellulose, the fraction correspondent to other components (including ashes, proteins and extractives) was also removed in large amount during the acid pretreatment (Table 2). Such fact has also been observed during the dilute-acid hydrolysis of other lignocellulosic materials, such as brewer's spent grains [28]. As a consequence of the removal of these two fractions, an enrichment of the cellulose and lignin contents in the pretreated material was verified.

3.2. Organosolv ethanol pretreatment with NaOH and enzymatic hydrolysis

Acid pretreated sugarcane bagasse was submitted to organosolv ethanol reactions with NaOH, which were performed under different operational conditions. The solid residue obtained after each reaction was chemically characterized and subsequently utilized as substrate for enzymatic hydrolysis aiming to recover glucose. The chemical composition of organosolv pretreated sugarcane bagasse and the correspondent glucose yields after their enzymatic hydrolysis are shown in Table 3. When compared to the glucose content present in the acid pretreated sugarcane bagasse (61.83% (w/w), Table 2), it can be observed that the glucose amount in the organosolv pretreated solid residue was increased for all the evaluated reaction conditions. However, the obtained values were different to each assay, suggesting that the process variables affected by different ways (according to the employed levels) the solu-

Table 3

Experimental matrix with the coded levels of the variables used for organosolv ethanol pretreatment of sugarcane bagasse, composition of the solid residue obtained after each pretreatment, and glucose concentration and yield after their enzymatic hydrolysis.

Assay	Independent variables ^a			Organosolv pretreatment stage			Enzymatic hydrolysis stage	
	x_1	x_2	x_3	Sugarcane bagasse composition after pretreatment (% w/w)			Glucose concentration (g/l)	Glucose yield (g/100 g) ^b
				Glucose	Xylose	Lignin		
1	−1	+1	+1	66.2 ± 1.0	7.6 ± 0.4	25.3 ± 0.2	15.4 ± 0.2	26.4 ± 0.3
2	+1	+1	+1	67.3 ± 1.5	6.1 ± 0.7	26.6 ± 0.8	18.1 ± 0.3	29.1 ± 0.4
3	+1	−1	−1	62.9 ± 0.9	8.2 ± 0.8	27.9 ± 0.3	12.3 ± 0.2	20.1 ± 0.3
4	−1	+1	−1	67.2 ± 1.6	6.5 ± 0.8	27.1 ± 1.5	13.3 ± 0.6	22.1 ± 1.0
5	+1	+1	−1	66.2 ± 1.1	4.9 ± 0.1	28.8 ± 0.9	15.8 ± 0.1	24.7 ± 0.2
6	+1	−1	+1	63.5 ± 1.0	7.9 ± 0.3	27.6 ± 0.5	12.7 ± 0.2	20.2 ± 0.3
7	−1	−1	+1	68.0 ± 1.1	10.1 ± 0.2	25.7 ± 0.6	11.9 ± 0.4	20.8 ± 0.7
8	−1	−1	−1	64.3 ± 0.5	8.4 ± 0.9	27.9 ± 0.3	11.3 ± 0.6	20.2 ± 1.1
9	0	0	0	67.1	6.9	28.1	14.3	24.4
10	0	0	0	67.3	7.1	29.9	15.5	26.2
11	0	0	0	67.1	6.9	28.1	14.3	24.4
12	0	0	0	66.9	6.7	26.3	13.1	22.6

^a x_1 , pretreatment time; x_2 , temperature; x_3 , ethanol concentration.

^b g glucose/100 g initial raw material.

bilization of the other fractions (hemicellulose, lignin, and other components) from pretreated sugarcane bagasse. In fact, xylose and lignin contents were present in different proportions in each residual solid obtained. Similar residual contents of these two fractions were obtained during the alkaline treatment of other lignocellulosic materials (maize stems, rye straw, rice straw, and brewer's spent grains) to obtain cellulose pulps [28,29]. Considering that a residual fraction of the lignin and xylose is very difficult to be removed from the lignocellulose structure because part of these fractions is strongly bound to the cellulose, being thus very resistant to hydrolysis [30], more efforts were not given to reduce even more the contents of these fractions during the organosolv pretreatment, since the energy consumption involved with the use of a major temperature and reaction time, probably would not justify the little increase in the cellulose content or the little decrease in the residual lignin content [28].

As a consequence of the different conditions used for organosolv ethanol pretreatment and their influence on the chemical composition and structure of sugarcane bagasse, the enzymatic hydrolysis of the solid residual material also gave different glucose recovered values (Table 3). A statistical analysis was then performed to identify the organosolv process variables that had the greatest influence on the responses of glucose concentration and yield obtained after enzymatic hydrolysis. According to this analysis (Table 4), all the studied variables presented significant individual effects ($p < 0.05$) on glucose concentration in the enzymatic hydrolysate. All these effects were of positive signal suggesting that the glucose concentration was higher when the values of the variables were increased. Among the variables, temperature had the most pronounced effect since the increase in the value of this variable from 175 °C to 195 °C

caused an increase of 3.6 g/l in the glucose content. The pretreatment time increase from 20 to 60 min caused an increment of 1.75 g/l in the glucose concentration, while the ethanol concentration increase from 10 to 30% (v/v) increased the glucose content in 1.35 g/l. Several works report that the use of low ethanol concentration (around 30% (v/v)) is favorable for use on ethanol organosolv process [31,32]. In a recent study, Macfarlane [31] demonstrated that the use of 30% (v/v) ethanol concentration during the organosolv pulping of willow promoted elevated delignification of the material, and the use of ethanol concentrations higher than 30% (v/v) had no effect on the rate of lignin reaction and dissolution. The increase in the lignin delignification favors the glucose extraction in the following step, as it was observed in the present study.

The temperature had also the most pronounced individual effect for the glucose yield response (Table 4), which was significant at 95% confidence level and had a positive signal, promoting an increase of 5.25 g glucose/100 g bagasse when the temperature was increased from 175 °C to 195 °C. The individual effect of the ethanol concentration was significant at $p < 0.1$ for this response, and no statistical significance of the pretreatment time was observed in the studied range of values. Interactions among the variables were not significant at 95% confidence level for any of the evaluated responses.

An analysis of variance with estimation of the curvature revealed that this parameter was not significant ($p < 0.05$) for both responses. This means that a first-order polynomial equation is the most suitable to explain the glucose concentration and glucose yield variations as function of the evaluated variables in the studied region. A multiple regression analysis was then performed to fit the experimental data to polynomial equations (Eqs. (3) and (4)).

Table 4

Effect estimates (EE), standard errors (SE) and level of significance (p) for glucose concentration and yield during the enzymatic hydrolysis of organosolv ethanol pretreated sugarcane bagasse, according to a 2^3 full-factorial design.

Variables and interactions	Glucose concentration			Glucose yield		
	EE	SE	p	EE	SE	p
x_1	1.750	±0.421	0.025**	1.150	±0.916	0.298
x_2	3.600	±0.421	0.003**	5.250	±0.916	0.011**
x_3	1.350	±0.421	0.049**	2.350	±0.916	0.083*
x_1x_2	0.850	±0.421	0.137	1.500	±0.916	0.200
x_1x_3	0.000	±0.421	1.000	−0.100	±0.916	0.920
x_2x_3	0.850	±0.421	0.137	2.000	±0.916	0.117

x_1 : pretreatment time; x_2 : temperature; x_3 : ethanol concentration.

* Significant at 90% confidence level.

** Significant at 95% confidence level.

Fig. 1. Response surface fitted to the experimental data points corresponding to the glucose concentration (g/l) and the glucose yield (g/100 g initial raw material) obtained by enzymatic hydrolysis of the organosolv ethanol pretreated sugarcane bagasse.

The interaction between pretreatment time and ethanol concentration (x_1x_3), which was the less significant among the studied variables/interactions (Table 4), was excluded from the models without damage in the R^2 coefficients. In fact, the models obtained for the two responses presented elevated values of R^2 (≥ 0.94), which indicate that the models are suitable for the process, showing a close agreement between the experimental results and the theoretical values predicted by the equations. Three-dimensional response surfaces described by the above-mentioned first-order polynomials were fitted to the experimental data points concerning the glucose concentration and glucose yield (Fig. 1A and B, respectively). It can be noted that both responses were well-fitted to a flat surface, the highest values being achieved by performing the organosolv ethanol pretreatment with NaOH under the highest temperature (195 °C), pretreatment time (60 min) and with the most elevated ethanol concentration (30% (v/v)).

$$\text{Glucose (g/l)} = 13.94 + 0.88x_1 + 1.80x_2 + 0.68x_3 + 0.43x_1x_2 + 0.43x_2x_3 \quad R^2 = 0.97 \quad (3)$$

$$\text{Glucose (g/100 g)} = 23.24 + 0.58x_1 + 2.63x_2 + 1.18x_3 + 0.75x_1x_2 + 1.00x_2x_3 \quad R^2 = 0.94 \quad (4)$$

3.3. Mass balance

A mass balance of the overall process used to recover glucose from sugarcane bagasse was performed. The chemical composition of pretreated materials considering their mass balance is presented in Fig. 2. As previously mentioned, the biomass recovered yield after the dilute-acid pretreatment was 72.43 g from 100 g original sugarcane bagasse. The biomass recovered yield after organosolv ethanol pretreatment under the optimum operational conditions was 80.5 g from 100 g acid-pretreated sugarcane bagasse, i.e. 58.33 g/100 g original sugarcane bagasse. From this amount, 67.3% (w/w) corresponded to the glucose content. In fact, the residual mass recovered after the two sequential pretreatment steps, was enriched in glucose (most predominant fraction) due to the removal of the other fractions from the lignocellulose structure during the pretreatment

stages. Xylose, for example, was removed in 87.5% (w/w) by performing the sequential acid and organosolv pretreatments. The major part (73.1% (w/w)) was removed during the first pretreatment stage, but the organosolv ethanol process removed also a significant portion of xylose (53.4% (w/w)) from the acid-pretreated residue. Similar to xylose, most of the content (83.8% (w/w)) correspondent to other components (including ashes, proteins and extractives) was removed during the dilute-acid pretreatment, and the organosolv pretreatment removed totally the residual amount of this fraction present in the pretreated material. Such results are similar to those obtained during the dilute-acid hydrolysis of brewer's spent grains, where hemicellulose fraction was removed in 86.5% (w/w), and the other components were removed in 84% (w/w) [28]. Unlike xylose, lignin from sugarcane bagasse was mainly removed (17.1% (w/w)) during the organosolv ethanol process, being only a few amount (0.85% (w/w)) solubilized during the acid pretreatment.

3.4. Glucose production by pretreatment in a semi-pilot scale

After optimizing the conditions for organosolv ethanol pretreatment with NaOH, the global process for the glucose recovery from sugarcane bagasse was repeated but performing the acid-pretreatment in a 10-l semi-pilot reactor fed with direct steam, from an industrial plant in Cuba. This study aimed to evaluate the possibility of performing this process in a larger scale. Although the previous optimized conditions for the organosolv ethanol process consisted in using a temperature of 195 °C; the maximum temperature possible to be employed in the industrial plant was 185 °C, and was then used in this stage. Enzymatic hydrolysis of the solid residue obtained after the two pretreatment stages under

Fig. 2. Schematic representation of mass balance obtained during the sugarcane bagasse pretreatment with dilute-acid and subsequent pretreatment by organosolv ethanol process with NaOH under the optimum operational conditions.

the described conditions gave a liquid fraction containing 22.2 g glucose/l, which corresponded to 29.7 g glucose/100 g original sugarcane bagasse. Such values are slightly higher than those obtained during the acid pretreatment in autoclave (18.1 g/l and 29.1 g/100 g, respectively). The use of steam in the semi-pilot reactor caused a higher rupture on the material fibers, changing its physical properties such as the crystallinity, which would have favored the enzymatic hydrolysis in the subsequent step. These values demonstrate a successful scale-up of the process for the glucose recovery from sugarcane bagasse.

4. Conclusion

Combination of a dilute-acid pretreatment followed by the organosolv pretreatment with NaOH under optimized conditions (60 min, 195 °C, using 30% (v/v) ethanol) was an efficient technique for sugarcane bagasse fractionation for subsequent use on enzymatic hydrolysis process, since yielded a residual solid material containing 67.3% (w/w) glucose, which was easily recovered by enzymatic hydrolysis. The scale-up of this process by performing the acid pretreatment in a 10-l semi-pilot reactor fed with direct steam was successfully performed, giving glucose concentration and yield values similar to those found when the acid pretreatment was performed in autoclave.

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