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Ethanol Production from Sugarcane Bagasse Pretreated by Steam Explosion

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ABSTRACT Bioethanol is an alternative renewable fuel that can be produced from cellulosic biomass through hydrolysis and fermentation based processes. Sugarcane bagasse constitutes a potential lignocellulosic substrate for bioethanol production, since it has high sugar content and is a renewable, cheap and readily available feedstock. In this work, steam explosion pretreatment at different temperatures (200°C, 215°C and 230°C) for 5 min was evaluated on sugarcane bagasse for ethanol production by simultaneous saccharification and fermentation (SSF). Sugar recovery in the solid and liquid fraction obtained after pretreatment and the enzymatic digestibility of the pretreated material were used to determine the optimum temperature, 215°C for 5 min, which resulted in an overall glucose yield of 86.8% of the content in raw material. The solid fraction of sugarcane bagasse pretreated at 215°C was submitted to SSF at increasing solid loading and the effect of xylanase supplementation and a presaccharification step was assessed. The highest final ethanol concentration (56.3 g L^{-1}) was achieved with SSF supplemented with xylanase enzyme at 20% of solid concentration. Ethanol yields exceeded 0.30 g per gram of glucose in all the conditions tested. The results obtained demonstrate the effectiveness of steam explosion in the treatment of sugarcane bagasse fiber.

KEYWORDS Lignocellulosic biomass; enzymatic hydrolysis; simultaneous saccharification and fermentation; hydrothermal pretreatment.

Introduction

Currently, energy demand is mainly supplied by fossil fuels which is shown to cause harmful changes in the climate. Burning of fossil fuels contributes to global warming and air quality degradation, which is disadvantageous for sustainable development. Due to the increasing demand for energy and serious concerns on greenhouse gas emissions, the search of renewable alternative energy sources is high-priority for researchers worldwide.

Bioethanol production offers a solution to conventional fuels. Combustion of ethanol results in relatively low emissions of compounds such as carbon monoxide and nitrogen oxides, and decreases CO_2 emissions in comparison to fossil fuels, thus contri-

buting to global warming avoidance [Bailey, 2005].

Commercial fuel-ethanol production is currently carried out by fermentation of sugars using yeast strains. The utilization of economically important crops such corn, wheat and sugarcane has many detractors because of competition with its use as a food source for humans, which affects the sustainability of the process [Gray et al., 2006].

Lignocellulosic biomass is an attractive raw material for ethanol production because it is an abundant, cheap and renewable resource on earth and includes various agricultural residues that can be converted to liquid transportation fuels [Chen et al., 2007; Dawnson and Boopathy, 2008]. Its structure is chiefly represented by the physical-chemical interaction of cellulose, a linear glucose polymer, with hemicellulose, a highly branched heteropolymer, and lignin, a very high molecular weight and cross-linked aromatic macromolecule [Himmel et al., 2007]. The general process for bioethanol production using lignocellulosic biomass involves pretreatment, hydrolysis and fermentation.

The lignocellulosic biomass is not susceptible to enzyme attack, which makes the pre-treatment an essential step to increase the reactivity of cellulose by disruption of its close association with hemicellulose and lignin [Taherzadeh and Karimi, 2007; Martin et al., 2008].

Several pretreatment methods have been developed for different lignocellulosic biomass [Hu et al., 2008; Lee et al., 2009; Saratale and Oh, 2012] with steam explosion (SE) being one of the most studied for fractionating biomass. SE consists of heating up lignocellulose by high-pressure steam followed by a sudden decompression. This pretreatment has been successfully applied to a great variety of lignocellulosic materials such as corn stover, sugarcane bagasse, wheat straw and woods [Ballesteros et al., 2006; García-Aparicio et al., 2006; Öhgren et al., 2007].

One disadvantage of SE technology is the formation of degradation compounds during pretreatment that can affect the enzymatic hydrolysis and reduce glucose conversion during fermentation thus affecting the ethanol yield at the end of the process [García-Aparicio et al., 2006]. The amount and the type of toxic compounds present depend on the source of lignocellulose, process conditions and other factors [Abril and Abril, 2009]. Therefore, it is important to monitor its production during pre-treatment experiments.

Sugarcane bagasse is the solid residue obtained after extraction of the juice from sugar cane (*Saccharum officinarum*) and can be a potential substrate for ethanol production. Bagasse could represent the main lignocellulosic biomass in many tropical countries since it is available at the sugar factory without additional cost and contains high sugar and low lignin content [Martin et al., 2002].

The aim of this work was to test steam-explosion technique as pre-treatment on sugarcane bagasse and to study ethanol production from steam-exploded biomass by a simultaneous saccharification and fermentation process. Three different temperatures, 200, 215 and 230°C, during 5 minutes residence time were tested and pretreatment effectiveness was assessed in terms of carbohydrate recovery after pretreatment and enzymatic hydrolysis yield of the pretreated material. Subsequently, bagasse pretreated at optimum temperature was tested in simultaneous saccharification and fermentation (SSF) process for ethanol production at increasing solid concentration (16 to 20% consistency) in different process configurations, including a fed-batch addition of enzymes and substrate, xylanase supplementation and with a presaccharification step.

Materials and methods

Raw material

Sugarcane bagasse used in this work was provided by the "Heriberto Duquerne" sugar mill (Villa Clara, Cuba) with a particle size of 1-2 cm and moisture content of 12.6%. A portion of the bagasse was milled at 1-2 mm particle size for biomass composition analysis.

The content of total solids, ash, extractives, acid insoluble lignin, acid soluble lignin, and structural

carbohydrates in biomass was determined using the standard laboratory analytical procedures for biomass analysis provided by the National Renewable Energy Laboratory [NREL, 2006]. All measurements were conducted in triplicate.

Steam explosion pretreatment (SE)

SE experiments were performed by applying Masonite technology in a 2L-SE pilot unit with 42 kg cm^{-2} of maximum operating pressure [Ruiz et al., 2008]. 200 g of bagasse (dry weight basis, dwb) were loaded into the reactor and the material was treated by saturated steam at 200°C, 215°C and 230°C during 5 min. Subsequently the material (slurry) was recovered in a cyclone connected to the outlet of the reactor and filtered to separate two fractions: the water-insoluble solid (WIS) and the liquid fraction or prehydrolyzate (PH). The WIS fraction was washed with water, weighed and dried at 45°C for storage. Solid recovery (SR) was calculated as dry weight of WIS remaining after pretreatment referred to 100 g of raw material introduced in the reactor.

Liquid fractions were analyzed for carbohydrate content through high-performance liquid chromatography with refractive index detector (HPLC-IR) as described in other works [Negro et al., 2006]. WIS fraction was analyzed for carbohydrates and acid insoluble lignin content as for raw material and used as substrate in enzymatic hydrolysis and SSF tests.

Results of sugar composition of filtrate and WIS were used to calculate sugar recovery yields (SRY) in both fractions, referring those values to the sugar content in untreated raw material. Moreover, in this work, the amount of glucose released and recovered in the filtrate is used to calculate overall glucose yield (OGY), which refers to the amount glucose released in both pretreatment and enzymatic hydrolysis referred to as the amount of sugar in raw material (see below).

The concentration of by-products such as acetic acid, furfural, hydroxymethylfurfural, formic acid, 4-hydroxybenzaldehyde, vanillin, coumaric acid and ferulic acid was measured in the liquid fraction by HPLC-IR with photodiode array detector, as described in other studies [Cara et al., 2008].

Enzymatic hydrolysis (EH)

The WIS fraction was enzymatically hydrolyzed to determine glucose yield for each pre-treatment condition. The assays were carried out in 100 mL Erlenmeyer flasks with a final reaction volume of 20 mL. EH tests were carried out at 5% (w/w) WIS loading, using cellulase mixture (NS 50013) supplemented with fungal β-glucosidase (NS 50010) kindly provided by Novozymes A/S (Denmark). Enzyme loading was 15 FPU of cellulase mixture and 15 UI of ß-glucosidase /g dry WIS, dissolved in 0.05 M acetate buffer (pH 4.8). The flasks were placed on a rotary shaker at 150 rpm and 50°C for 72 h. All experiments were performed in duplicate and hydrolysis of untreated bagasse was used as control.

EH yield was calculated as the ratio of g glucose released in the EH/ potential glucose in WIS, expressed in percentage.

Overall glucose yield (OGY)

Overall glucose yield (OGY) refers to the amount glucose released in both pretreatment and enzymatic hydrolysis referred to the amount of sugar in raw material, expressed in percentage. It is calculated by the following formula:

$$\%OGY = [(g\ glucose\ in\ EH \div g\ WIS\ (dwb)) \times \%SR \div 100) + (g\ glucose\ in\ PH \div g\ raw\ material(dwb))\ g\ glucose\ in\ raw\ material]\ 100$$

where:

%SR : % solid recovery
EH : Enzymatic hydrolysis assay
PH : Liquid fraction or prehydrolyzate

Table 1 Summary of the SSF experiments with initial solid concentration between 16-20% at different enzyme dose/g dry WIS and process configurations. E1, cellulase; E2, β-glucosidase, E3, xylanase.

% dry WIS	Enzyme loading/g substrate			PS (24 h)	Exp. nº
	E_1(FPU)	E_2(IU)	E_3(IU)		
16	20	20	-	NO	1
	20	20	-	YES	2
	10+10(1)	10+10(1)	-	YES	3
	20	20	75	YES	4
	20	20	75	NO	5
18	20	20	75	YES	6
16+2(1)	20	20	75	YES	7
20	20	20	75	YES	8
18+2(1)	20	20	75	YES	9

(1) Addition by a pulse at 24 h interval. (PS) Presaccharification step

Ethanol production

Microorganisms and growth condition

Sacharomyces cerevisiae (EthanolRed) from Fermentis (France) was used in SSF experiments. The microorganism was previously grown in a medium containing (g L^{-1}): yeast extract, 5; NH_4CL, 2; KH_2PO_4, 1; $MgSO_4.7H_2O$, 0.3 and glucose, 30, at 150 rpm and 35°C during 16 h.

SSF Experiments

A summary of the tested SSF conditions is shown in Table 1. Different SSF tests were performed at 16, 18 and 20% solids (w/w). First experiments at 16% solids content were performed to test the effect of the presaccharification step of 24 h at 50°C, the supplementation of xylanase to the cellulase cocktail and the addition of enzymes by pulses (Table 1). Then, a series of experiments were performed on 18 and 20% solid loading with cellulase supplemented with xylanase to test the addition of substrate by pulses. The objective was to avoid product inhibition and improve the rheology of the mixture.

SSF experiments were carried out in 250 mL Erlenmeyer flasks with a final reaction volume of 50 mL. The fermentation medium used was the same as described above but without glucose. Enzymatic loading used in the reactions was 20 FPU g^{-1} substrate of Novozymes 50013 (cellulase) (E1) and 20 UI g^{-1} substrate of Novozymes 50010 (β-glucosidase) (E_2). The effect of xylanase addition (75 UI g^{-1} substrate of Novozymes 50030) (E_3) was also evaluated. To start SSF, flasks were inoculated with 0.4 g L^{-1} yeast culture except for experiments with a 24 h presaccharification step, and subsequently the flasks were inoculated after that period. In all experiments ethanol content in SSF media was measured every 8 h during 144 h, and ethanol yield was calculated at 72 hours fermentation. Ethanol yield is expressed as g ethanol/g potential glucose in pretreated substrate.

The ethanol yield was referred to as the theoretical yield, which assumes that all the potential glucose in the WIS fraction is available for fermentation (100%

Table 2 Chemical composition of sugarcane bagasse. Values are shown in percentages on dry matter basis. Standard deviation shown in parentheses.

Component	Percentage on dry weight basis
Cellulose	**38.2 (1.60)**
Glucose	41.9 (1.76)
Hemicellulose	**25 (0.78)**
Xylose	27.3 (0.85)
Arabinose	0.9 (0.03)
Lignin	**24.0 (0.29)**
Extractives	**8.3 (1.07)**
Ash	**1.0 (0.07)**
Acetyl groups	**3.1 (0.05)**

hydrolysis yield), and a fermentation yield of 0.51 g ethanol g^{-1} glucose.

Statistical analysis

All statistical analysis was carried out using Statgraphics Centurión XV II (Statpoint Technologies Inc., Warrenton, VA). Data were analysed by analysis of variance (ANOVA) followed by Fisher LSD test to determine significant differences ($p \leq 0.05$) between the means.

Results and discussion

Composition analysis of raw material

Table 2 shows the composition of the sugarcane bagasse used in this study. Carbohydrate content of sugarcane bagasse biomass, which accounts for about 65% of dry mass, makes this material an interesting feedstock for ethanol production. Moreover, it has been reported that minor differences in the chemical composition of bagasse between the different varieties of sugarcane exist [Gastón et al., 2000], which can be considered an advantage, given that the composition is to some extent homogeneous when using bagasse from different sources.

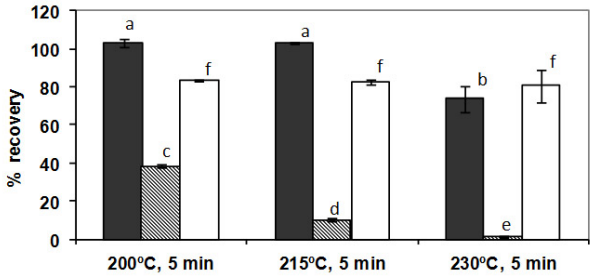

Figure 1 Recovery of the main components in the WIS fraction after pre-treatment, % w/w. Dark bars, cellulose; lined bars, hemicellulose; light bars, lignin. *n=3*. Error bars represent standard deviations. Common letters signify no significant differences according to least significant difference test (LSD) Fisher.

SE pretreatment

Analysis of the solid and liquid fraction

The values of solid recovery yield after pretreatment (SR) and percentage (on dwb) of main components in WIS fraction at different pretreatment conditions are shown in Table 3. Regarding WIS composition, cellulose is concentrated in relation to untreated material (38.2%) in all conditions tested. Pretreatment at 215°C resulted in the WIS fraction containing the highest value of cellulose content of all pretreated materials, 63.9% dwb. However, when pretreatment temperature increased to 230°C, cellulose content of the resulting WIS fraction dropped to 55%. These results are consistent with those obtained by Martin et al. [2008] for the bagasse treated with SE in similar process conditions, who found a drop of the cellulose content during pre-treatment at elevated temperature in comparison to milder conditions.

If the recovery of main components in WIS is calculated in relation to initial raw material considering the % of solids recovery, it can be observed that cellulose recovery was 100% in runs performed at 200°C and 215°C (Fig. 1). In the most severe condition, cellulose recovery was 73%, indicating solubilisation of this component at this temperature.

In a steam explosion process, part of the hemicellulose is removed from the solid fraction and solubilised into the liquid fraction or prehydrolyzate. In the experiments carried out for this study, at the lowest

Table 3 Solid recovery (SR) and WIS fraction composition (on dry weight basis) of bagasse pretreated at different temperatures (residence time 5 minutes) *n=3*. Common letters signify no significant differences according to least significant difference test (LSD) Fisher.

Temperature (°C)	% SR	WIS composition (% dwb)		
		Cellulose	Hemicellulose	Lignin
200°C	70.7 (a)	58.1	14.0	28.5
215°C	61.5 (a,b)	63.9	4.1	32.4
230°C	50.9 (b)	55.0	0.5	38.1

Table 4 Recovery of free monosaccharides in the liquid fraction obtained after filtration of slurry in SE pre- treatment at different temperatures. Values in g/100 g raw material.

Liquid fraction		Recovery of free monosaccharides (%)				
Temperature (°C)	Time (min)	Glucose	Xylose	Galactose	Arabinose	Mannose
200°C	5	2.0	14.5	0.5	0.8	0.09
215°C	5	2.2	11.0	0.6	0.5	0.20
230°C	5	2.7	3.3	0.3	0.2	0.16

temperature of 200°C, the content of hemicellulose in WIS accounted for 14.06%, which corresponded to 56% of the content in untreated material. Fig. 1 shows that the hemicellulose recovery in WIS was lower than 40% in all temperatures tested, with statistically significant differences between them ($p<0.05$).

In all conditions tested, lignin concentration in WIS increased in relation to the initial raw material due to the solubilisation of other biomass components such as hemicellulose (Table 3). When lignin values in WIS are referred to the content in untreated raw material, using the % SR value, losses between 16 and 17% of the initial content are found at all temperatures tested. These results are in agreement with those obtained by George et al. [2012] using steam explosion pretreatment of sugarcane bagasse followed by alkaline delignification. Steam explosion of lignocellulosic materials is described as resulting in extensive hemicelluloses solubilization with lignins being scarcely affected [Mosier et al., 2005]. However, the these data indicate that in the conditions tested in the present work, a minor part of lignin can also be solubilised to the liquid fraction due to the pre-treatment effect.

The analysis of the sugar composition of the liquid fraction (Table 4) shows that it basically consists of xylose and a minor amount of glucose. The recovery of free C_5 sugars in the liquid fraction at 215°C and 230°C was lower than that expected for these temperatures if the value of hemicelluloses solubilization from the analysis of the solid fraction is considered, due to sugars degradation by the action of elevated temperatures [Saha et al., 2003].

At 200°C the liquid fraction contained the highest concentrations of xylose, accounting for 53% of the content in raw material (14.5 g/100 g raw material). However, at this condition 40% of the content in raw material still remained in the solid fraction, which indicates a limited hydrolysis of hemicellulose during pre-treatment at 200°C. Recovery of glucose in the liquid was less than 6% in all conditions, which indicates very low solubilisation of cellulose at the pre-treatment conditions tested.

Table 5 By-product formation in the liquid fraction SE pre-treatment at different temperatures (residence time = 5 minutes). Values in g/100 g raw material..

	Compound							
Temp (°C)	Acetic acid	Formic acid	HMF	furfural	4HBenOH	vanillin	cumaric acid	ferulic acid
200	0.91	0.36	0.03	0.19	0.02	0.01	0.01	0.005
215	2.26	0.77	0.09	1.12	0.03	0.02	0.14	0.02
230	3.11	0.91	0.56	1.72	0.03	0.03	0.08	0.007

Formation of degradation products in the liquid fraction

In the present experiments, by-products produced by degradation of the sugars (furfural and hydroxymethylfurfural HMF), aliphatic acids (mainly acetic and formic) and phenolic compounds produced by the solubilization of the lignin were found in the prehydrolyzate from SE runs (Table 5). The highest concentrations of by-products were obtained at higher temperatures in agreement with previous reports [Kaar et al., 1998; Sendelius, 2005], reporting increasing concentrations as severity of pretreatment rises. The significant production of acetic acid is noteworthy, which was derived from the hydrolysis of acetyl groups linked to hemicellulose chains due to the elevated temperatures of pretreatment. Furfural is formed by dehydration of pentoses under thermal and acid conditions [Kholkin, 1989]. The furfural concentrations found in the liquid fraction could explain in part the loss of hemicellulosic sugars in the pretreatment.

Enzymatic hydrolysis (EH)

The effectiveness of the pretreatment with respect to digestibility of cellulose in the pretreated material was evaluated through enzymatic hydrolysis tests on WIS. EH yield was expressed as percentage of glucose released during hydrolysis at 72 h in relation to potential glucose in WIS fraction.

It has been described that SE pre-treatment provokes the removal or reduction of more easily available amorphous cellulose and a level off of the cellulose degree of polymerization, both affecting the crystalline structure of cellulose [Mansfield et al., 1999]. A rupture of cellulose and hemicellulose association due to the solubilization of the latter occurs, thus increasing the accessibility of the treated material to enzymatic attack [Alvira et al., 2010]. Results obtained in the present work clearly support this fact since a remarkable positive effect of steam-explosion pretreatment on enzymatic digestibility of pretreated substrate in relation to the untreated material is found (Fig. 2). A relevant aspect of this finding is that in SE only water is used, unlike other pretreatment techniques that need to employ an acidic or basic catalyst to improve cellulose digestibility.

Increasing pretreatment temperature results in EH yields raising from 60% of theoretical at 200°C to the highest yield of 90% achieved at 230°C, which indicates a highly digestible pretreated substrate at this temperature. However, it is important to take into account that at this condition, solid recovery yield decreases in comparison to lower severity conditions, which will affect the yield of glucose release by

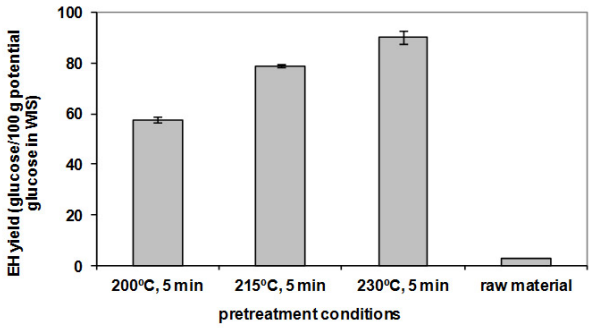

Figure 2 Enzymatic hydrolysis yield of pretreated bagasse reported as g of glucose obtained in EH/100g potential glucose in WIS

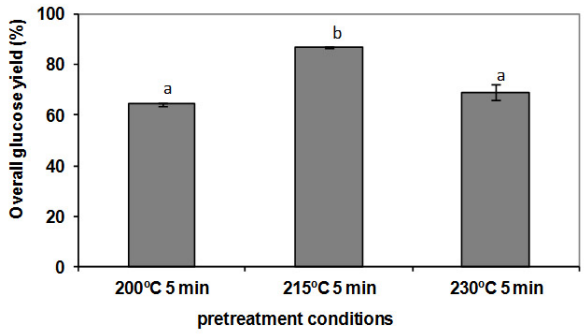

Figure 3 Overall glucose yield in steam explosion pre-treatment of sugarcane bagasse at different temperature conditions. Results in g glucose/100 g glucose in raw material. n=3. Common letters signify no significant differences according to least significant difference test (LSD) Fisher. Error bars represent standard deviations.

enzymatic hydrolysis when referred to raw material. Therefore, a balance between the enzymatic digestibility and the recovery of carbohydrates in the solid fraction must be established in the pretreatment evaluation to ensure high yields in glucose production from raw material. To evaluate this, the overall yield of glucose, which takes into account the amount of glucose released by enzymatic hydrolysis and that recovered in the liquid fraction referred to the glucose content in raw material, was calculated for different pre-treatment temperatures (Fig. 3). This is a significant parameter since it integrates the efficiency of both steps: pretreatment and enzymatic hydrolysis, to release sugars for fermentation.

At 215°C the highest overall glucose yield was obtained (86.8%), making it the most effective condition to achieve high yields of ethanol when taking into account all potentially fermentable glucose, coming from pretreatment and enzymatic hydrolysis steps. This value is similar to that reported by Ferreira-Leitão et al. (2010) who obtained a maximum overall glucose yield of 86.6% working with steam pretreated CO_2-impregnated sugarcane bagasse. The pretreatment at 230°C would not be adequate due to sugar losses at this condition, which could have a negative impact on final ethanol production process yield.

Simultaneous saccharification and fermentation

The SSF process combines cellulose hydrolysis and sugar fermentation in the same vessel, with one of the main advantages being the avoidance of the inhibitory effect of increasing glucose release on cellobiose and cellulase activities [Xiao et al., 2004]. SSF experiments were carried out on material pretreated at 215°C at different assay conditions regarding solid content, the addition of xylanase to the enzyme cocktail and the testing of a presaccharification step. The aim of the prehydrolysis step is to partially hydrolyze the cellulose to glucose prior to the yeast addition, which would increase the ethanol production rate during the initial part of SSF [Lin and Tanaka, 2006]. Another important aspect of a prehydrolysis step prior to SSF is to facilitate the mixing during fermentation, especially at high solid consistencies [Jørgensen et al., 2007].

Regarding the results of experiments at 16% consistency (Table 6A), neither the 24 h presaccharification nor the addition of enzymes by pulses resulted in an enhancement of ethanol production when compared to SSF performed with the addition of all enzymes at the beginning of the reaction and without prehydrolysis. The lack of positive effect of a prehydrolysis step has been reported for SSF on other materials as barley straw and corn cover [Linde et al., 2007; Öhgren et al., 2007], which could be attributed to enzyme deactivation caused by increased temperature during prehydrolysis. Notwithstanding, it is proven to be very effective to facilitate mixing at high consistencies and so, it was included in subsequent experiments at 18 and 20% solids.

On the other hand, results demonstrate the positive effect of xylanase addition, with statistically significant differences ($p<0.05$) on ethanol concentration in SSF media, in tests with xylanase in both process configurations, with and without presaccharification. The highest concentrations of ethanol were obtained when xylanase was used in the enzymatic hydrolysis tests without the presaccharification step reaching about 45 g L^{-1} in the SSF media, which corresponds

Table 6 Ethanol concentration (g L^{-1}) and ethanol yield (in g/g glucose and in % of theoretical yield of 0.51 g ethanol/g glucose) in 72 h SSF. (A) experiments at 16% dry WIS of steam-pretreated sugarcane bagasse (B) 18% and 20% dry WIS of steam-pretreated sugarcane bagasse *n=3*. Common letters signify no significant differences according to least significant difference test (LSD) Fisher. Details of different experiment conditions in Table 1.

A

Experiment nº	1	2	3	4	5
Solid % p/v	16	16	16	16	16
Potential glucose (g L^{-1})	135.5	135.5	135.5	135.5	135.5
Ethanol (g L^{-1})	43.62 (a)	41.65 (b)	42.18 (b)	44.08 (a c)	44.93 (c)
Ethanol yield (g g^{-1} glucose)	0.322 (d)	0.307 (e)	0.311 (d e)	0.325 (d)	0.332 (d)
Ethanol yield (% of theoretical yield)	63.13	60.19	60.98	63.72	65.09

B

Experiment nº	6	7	8	9
Solid % p/v	18	18	20	20
Potential glucose (g L^{-1})	154.5	154.5	171.6	171.6
Ethanol (g L^{-1})	51.2 (f)	51.0 (f)	56.3 (g)	56.1 (g)
Ethanol yield (g g^{-1} glucose)	0.33 (h)	0.33 (h)	0.33 (h)	0.32 (h)
Ethanol yield (% of theoretical yield)	64.9	64.7	64.3	63.9

to a 65% of the theoretical ethanol yield. The addition of xylanase to the cellulase cocktail has been shown to enhance the hydrolysis of cellulose owing to the creation of more accessible cellulosic regions that are readily acted upon by exo- and endoglucanases. The synergistic action of cellulase and xylanase has been found to be advantageous for hydrolysis of lignocellulosic substrates such as sorghum straw [Dogaris et al., 2009] or corn cobs [Kim et al., 2009], pretreated hydrothermally and by aqueous ammonia soaking. The results from this study support this finding showing that the supplementation of xylanase enhances glucose release in saccharification of steam pretreated bagasse.

However, when ethanol yields per gram of glucose results are analyzed, the use of xylanase did not impact statistically in SSF yield ($p>0.05$), except in the comparison of experiments with the presaccharification step. The comparison of experiments with and without presaccharification in the SSF showed no increase neither in ethanol concentration nor in the yields, which could be attributed to the loss of enzyme activity for longer time subjected to high temperature.

Results at 18 y 20% solids show an increase of ethanol concentration as the concentration of dry WIS rises in all SSF conditions analyzed ($p<0.05$) (Table 6B). No effect of gradual addition of WIS was found.

However, when results of ethanol yield per g/ glucose were analyzed, the differences in this case were not significant between conditions, as well as between the WIS concentrations tested ($p>0.05$). It is therefore demonstrated that ethanol concentration in SSF can be increased up to 51 g L^{-1} by using higher consistencies without affecting the efficiency of fermentation.

In a comparison of results among SSF tests at different conditions, it was concluded that the best results were obtained with the conventional SSF supplemented with xylanase, without presaccharification, for the three dry WIS concentrations used. The highest

ethanol values were achieved at 20% of solid consistency with statistically significant differences between means ($p<0.05$). If the kinetics of SSF at this condition are compared for the three consistencies, a delay in the generation of ethanol in the first hours of fermentation is observed as solid loading grows, which could be attributed to the lack of homogeneity in the fermentation mixture. Nevertheless, at 72 hours the ethanol yields are similar, exceeding 0.30 g per gram of glucose released, and showing no significant differences among the different WIS concentrations. Maximum ethanol concentration of 56.3 g L^{-1} was found at 20% WIS. This is a very important result since high solid concentration is needed to guarantee the economics of ethanol recovery through distillation.

The values of ethanol production as well as yields achieved in this work are comparable, and at some times higher than those obtained in other studies using sugarcane bagasse [Sendelius, 2005; Geddes et al., 2011] and other lignocellulosic biomass such as wheat straw and residue of the olive oil extraction [Ballesteros et al., 2002; Ballesteros et al., 2006]. It is demonstrated that steam-exploded sugarcane bagasse allows a high substrate concentration of 20% to be used in the conversion of this feedstock to ethanol by an SSF process.

Conclusions

The results obtained in this study indicate the effectiveness of steam explosion in the treatment of sugarcane bagasse fiber to enhance the enzymatic digestibility of pretreated substrate for conversion to ethanol by SSF process. The best pre-treatment condition was 215°C for 5 min, which is a balance between glucose recovery in the pretreatment and enzymatic hydrolysis yield. In SSF experiments at 20% solid consistency using cellulase NS 50013, β-glucosidase NS50010 and xylanase 50030 (Novozymes A/S, Denmark), maximum ethanol concentration levels of 56 g L^{-1} was attained. These results demonstrate the feasibility of using sugarcane bagasse pretreated by SE in an SSF process at high consistency conditions to produce ethanol with theoretical yields close to 65%. However, further experiments are needed to test process performance on a larger scale.

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