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Dilute-acid Hydrolysis of Cellulose to Glucose from Sugarcane Bagasse

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As the main component of lignocelluloses materials, cellulose is a biopolymer consisting of many glucose units connected through β-1,4-glycosidic bonds. The breakage of the β-1,4-glycosidic bonds by acids leads to the hydrolysis of cellulose polymers, resulting in the sugar molecule glucose or oligosaccharides. Mineral acids, such as HCl and H_2SO_4, have been used in the hydrolysis of cellulose. The lignocellulosic materials usually require a first step of pretreatment due to the association between the three major components of plant cell wall (cellulose and hemicelluloses fractions and lignin) in order to make available the monomeric sugars found in these fractions, for fermentation to ethanol. Different procedures have been employed, for example, acid hydrolysis, alkali hydrolysis, steam explosion, among others. The pretreatment using dilute sulfuric acid (acid hydrolysis) is the most widely used for having high efficiency in the separating process of cell wall components resulting in hemicellulose hydrolysate and cellulignin. A second step for obtaining the cellulose present in the cellulignin, studies have shown a need for delignification stage using sodium hydroxide as catalyst. The cellulose is submitted to acid or enzymatic hydrolysis to solubilize the glucose (cellulose hydrolyzate). Regardless of intense research on cellulose hydrolysis process by enzymatic way, the amount of hydrolyzate obtained in this process is still less than the amount required for subsequent studies on the fermentation of these hydrolysates which opens the option of research for the use of chemical hydrolysis. In order to use sugarcane bagasse as a substrate for ethanol production, optimum conditions for acid hydrolysis of cellulose fraction were investigated. A 2^3 full factorial Central Composite Design (CCD), including three replications at the center point was applied to evaluate the effect of temperature, acid concentration and reaction time on extraction efficiency. In this study, the hydrolysis of cellulose conditions varied in terms of sulfuric acid (H_2SO_4) concentration (2-6 %, w/v), reaction time (10-30 min) and incubation temperature (155-175 ºC). The experiments were carried out using a 200-ml stainless-steel container (19 x 7 cm), which was tightly sealed and immersed in a silicone bath provided with electrical heating. The maximum extraction efficiency (E) was 71 % under the conditions of 2 % of H_2SO_4 at 155 ºC for 10 min, which the main components (in g L^{-1}) in the hydrolysate were glucose, 22.74; 5-hydroxymethylfurfural, 0.206; furfural, 0.145 and no xylose, arabinose and acetic acid formation was detected. Experiments will be performed to evaluate the fermentability of this hydrolysate to ethanol by *Scheffersomyces stipitis*.

1. Introduction

Sugarcane bagasse, previously considered a waste, is one of the most abundant co-product in the Brazilian sugar and ethanol industry, so that 1 ton of cane is able to produce 280 kg of bagasse (Unica). About 50 % of bagasse is burned to be used as a fuel for combined heat and power systems implemented to meet energy needs of the mills and the remainder is stored (Dantas et al., 2013). As there is a large amount of bagasse which is not used in the industry and this material has enormous potential for the ethanol production, it has been the main target of numerous studies aimed at producing ethanol from plant biomass (Rabelo et al., 2011). Sugarcane bagasse is a lignocellulosic biomass and therefore it has cell wall composed mainly of cellulose, hemicellulose and lignin. The high proportion of glucose and xylose

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that can be obtained from cellulose and hemicellulose fractions of bagasse and ability that micro-organisms have to convert these sugars into ethanol are factors that drive the use of this co-product for the production of biofuels such as ethanol (Gírio et al., 2010). For this it is necessary to submit the material to the processes of hydrolysis to release fermentable sugars, however, one of the main obstacles in bioconversion of lignocellulosic materials is the recalcitrance of crystalline cellulose to hydrolysis (Amiri and Karimi, 2013). Ethanol production from lignocellulosic biomass requires the separation of its fractions through pre-treatment methods. Pre-treatment breaks down the lignin protection and the crystalline cellulose structure creating the conditions for hydrolysis of polysaccharides (Dantas et al., 2013). The pre-treatment with H_2SO_4 is one of the widely employed procedures for structural plant cell wall depolymerization and solubilization of its sugars forming mainly xylose in the hemicellulosic fraction in the hemicelluloses hydrolysate and cellulignin. In a second step for obtaining the cellulose present in the cellulignin, studies have shown a need for delignification stage using sodium hydroxide as catalyst. After delignification, cellulose fibers become more exposed to subsequent acid or enzymatic hydrolysis (Hamelinck et al., 2005). In the case of acid hydrolysis of cellulose, despite of low cost, it has disadvantages such as the formation of byproducts. On one hand, the use of diluted acids is milder reaction requires a higher temperature that cause the glucose degradation to hydroxymethylfurfural (HMF). In contrast, the use of concentrated acids requires more moderate temperatures to achieve high yields similar to cellulase enzymes process (Botha and Blottnitz, 2006), however, it promotes the formation of the inhibitor compounds that affect microbial activity and reaction medium becomes strongly corrosive, which can be avoided with the use of reactors made from materials that show chemical resistance to acids and bases. Acid hydrolysis is an old process and in which the residual acid process is not usually recycled, it is only neutralized generating high amounts of salt, which difficult this process in economic and environmental terms. However, with recent research advances on the recovery and recycling of the acid in this process opens a possibility of using this method of deconstruction of the cell wall as a promising alternative to enzymatic hydrolysis (Laopaiboon et al., 2010). In order to obtain ethanol from cellulosic fraction most studies involve the application of enzymes and the costs of enzymes and processing makes the production of second generation cellulosic ethanol expensive compared to the production of ethanol from first generation (Chandra et al., 2010). Besides, the amount of hydrolysate obtained from enzymatic hydrolysis process is still less than the amount required for subsequent studies of fermentation of these hydrolysates which opens the possibility of a research on the use of chemical hydrolysis. Few studies have reported the dilute-acid hydrolysis of cellulose to glucose from sugarcane bagasse and the use of microorganism with ability to produce ethanol in this hydrolysates. In order to use sugarcane bagasse as a substrate for ethanol production, optimum conditions for acid hydrolysis of cellulose fraction were investigated.

2. Materials and methods

2.1 Pretreatment of the sugarcane bagasse and treatment of cellulose-lignin

The pretreatment of sugarcane bagasse was performed in a 250 l stainless steel reactor employing H_2SO_4 1 % (w/v), 1:10 solid-liquid ratio, at the temperature at 121 °C for 20 min. After hydrolysis, the resulting solid material (cellulose-lignin) was removed by filtration, washed and dried. After, cellulose-lignin was soaked in a solution of 1.5 % w/v NaOH, 1:20 solid-liquid ratio and temperature at 10°C for 1 h in a 50 L stainless steel reactor. Afterward, the mixture was filtered to collect the cellulose, washed thoroughly with water and dried.

2.2 Dilute-acid hydrolysis of cellulose

Initially, the cellulose (18 g) and the aqueous sulfuric acid solution (1:8 solid-liquid ratio) were loaded into a 200-ml stainless-steel container (19 X 7 cm), which was tightly sealed and immersed in a silicone bath provided with electrical heating. After removing the screw cap from the container, the cellulosic hydrolysate was quantitatively separated from the pretreated solids by filtration. The glucose extraction efficiency (E, %) was defined as Eq(1).

$$E\ (\%) = \frac{m_{glucose} * f_h}{m_{initial} * y_i} * 100 \quad (1)$$

Where, $m_{glucose}$ is mass of glucose in hydrolysate (g), $m_{initial}$ is initial mass of dry matter (g), y_i is cellulose in the biomass (%) and f_h is conversion factor (for cellulose is 0.9). In the experimental design, a complete 2^3 factorial experiment was performed with 3 variables (temperature, residence time and sulfuric acid concentration) in two levels, minimum and maximum, coded as “−1” and “+1”, respectively, and three

replicates of the center point, coded as “0” (Table 1). The response variables in experimental design were glucose extraction efficiency. The statistical treatment of the results was performed by the software STATISTICA version 8.0 (StatSoft, Inc).

Table 1: Factors and levels used in the experimental 2^3 factorial design for glucose extraction efficiency.

Factors	minimum value	center point	maximum value
	-1	0	+1
A, Temperature (ºC)	155	165	175
B, Residence time (min)	10	20	30
C, Sulfuric acid concentration (w/v %)	2	4	6

2.3 Fermentation

Fermentations were carried out in 125 mL Erlenmeyer flask using a yeast strain of *Sc. stipitis* NRRL Y-7124. The cellulosic hydrolysates were destoxified with activated charcoral and supplemented with yeast extract 3.0 g L^{-1}, ammonium sulfate 2.0 g L^{-1}, calcium chlorate 0.1 g L^{-1} and peptone 5.0 g L^{-1}, inoculated with 1.0 g L^{-1} yeast, and cultivated at 30 °C, 200 rpm for 72 h. The yield of ethanol ($Y_{P/S}$, g/g) was defined as the ratio of produced ethanol (g) over the consumed sugar (g) and the ethanol productivity (Q_P, g L^{-1} h^{-1}) was determined by the ratio of ethanol concentration (g L^{-1}) to fermentation time (h).

2.4 Analytical Methods

All samples were analyzed by HPLC, equipped with a RI detector (Agilent Technologies 1200, EU). Glucose, ethanol and acetic acid was monitored on an Aminex HPX-87H column (Bio-Rad, CA, USA) at 45±0.5°C. Sulfuric acid (0.005 M) was used as the mobile phase at a flow rate of 0.6 mL/min. Furfural and HMF were analyzed on a Zorbax Eclipse Plus C18 column at 30 °C with 0.8 mL/min eluent of acetonitrile:water (1:8, v/v) with 1% of acetic acid. Detection was done at 276 nm in a UV-VIS detector. Cell concentrations were determined by absorbance at 600 nm and correlated with the cell dry weight through a calibration curve.

3. Results and discussion

The chemical composition of the sugarcane bagasse sample, before and after pretreatment, used in the current study, was determined according to the method validated by Gouveia et al. (2009) and it can be seen in Table 2. The amount of cellulose and lignin after pretreatment of bagasse “in natura” with acid increased, indicating a proportional increase of these fractions due to the high solubility of the constituents hemicellulose sugars (90.87%). This indicates that the hydrolysis conditions used were adequate to extract the sugars contained in this fraction. The delignification process was efficient and it can be observed that cellulose fraction showed a solubilization of 96.39 %. The temperature, residence time and sulfuric acid concentration variables reported to influence the hydrolysis of different cellulosic materials, were varied according to a full factorial design. As can be seen in Table 3, the highest glucose extraction efficiency (70 %) was observed when the treatment was performed with 2 % w/v acid at 155 ºC for 10 min. Such conditions led to extensive hydrolysis of cellulose to glucose.

Table 2: Composition of sugarcane bagasse: “in natura”, cellulignin (after pretreatment with sulfuric acid), cellulose pulp (after delignification with sodium hydroxide) and final residue (after hydrolysis with dilute sulfuric acid)

Composition (% w/w)	Sugarcane bagasse “in natura”	Cellulignin	Cellulose pulp	Final residue
Cellulose	39,37	50,15	72,56	2,62
Hemicellulose	27,39	4,05	-	-
Lignin	22,42	24,43	18,77	80,28
Ashes	7,87	5,66	5,62	6,89
Others	2,95	15,71	3,05	10,21

Table 3: Matrix of experimental 2^3 factorial design for efficiency and corresponding results of glucose concentration

Run	Temperature	Residence time	Sulfuric acid concentration	Efficiency (%)	Glucose (g L^{-1})	5-HMF (g L^{-1})	Furfural (g L^{-1})	Phenols (g L^{-1})
1	-1	-1	-1	70.91	22.74	0.206	0.145	3.694
2	1	-1	-1	31.15	9.99	0.209	0.134	3.260
3	-1	1	-1	5.43	1.74	0.088	0.095	3.596
4	1	1	-1	6.42	2.06	0.084	0.095	2.527
5	-1	-1	1	63.36	20.32	0.176	0.125	3.057
6	1	-1	1	39	12.51	0.220	0.143	6.223
7	-1	1	1	3.58	1.15	0.006	0.001	3.528
8	1	1	1	3.18	1.02	0.018	0.020	4.377
9 (C)	0	0	0	7.61	2.44	0.083	0.083	3.452
10 (C)	0	0	0	4.46	1.43	0.052	0.056	3.522
11 (C)	0	0	0	5.76	1.85	0.016	0.062	3.166

Although cellulose was extensively hydrolysated in this condition and the amount of glucose recovered in the cellulosic hydrolysate was 22.74 g L^{-1} and did not exceed 70 % of glucose extraction efficiency, presented furfural 0.206 g L^{-1}, HMF 0.145 g L^{-1} and phenolic compounds 3.694 g L^{-1} and no xylose, arabinose and acetic acid formation were detected (Table 3). In this context, it can be observed that the hydrolysis of cellulose with dilute acid usually requires high temperature and low residence time, Harris and Beglinger (1946) reported a treating process in which wood was treated with 0.5 w/v % H_2SO_4 in a continuous reaction. The degradation of products was minimized for the short residence time of cellulose in the reactor. Another successful process using an isothermal plug flow reactor was reported by (Thompson and Grethlein, 1979) They used 1 w/v % H_2SO_4 in the continuous process at 240 °C with a short residence time of 0.22 min; 50 % glucose was obtained at last. A few years later, Harris et al. (1985) used a two stage system containing dilute H_2SO_4 in the saccharification of wood. After the hemicellulose was extracted, cellulose was transferred to hydrolysis and a high purity of glucose was obtained. On the other hand, harsh conditions used in treatment create a variety of toxic compounds that inhibit the fermentation performance. Inhibitors have been previously categorized by Olsson and Hahn-Hägerdal (1996). Specifically, furan derivatives include furfural and HMF, which result from pentose and hexose degradation, respectively, and phenolic compounds are derived from lignin (Ulbricht et al., 1984). The highest inhibitors concentration can be observed in the experiment that use the maximum values of temperature and sulfuric acid concentration at 175 ºC and 6 % w/v, respectively, demonstrated that harsh conditions of temperature and acid concentration caused the degradation of the sugar present in the hydrolysate (Table 3). In addition, we can observe in Table 3 the lowest glucose extraction efficiency (3.18 %) was observed when hydrolysis was performed in the maximum values of temperature, residence time and sulfuric acid concentration, 175 ºC, 30 min and 6 % w/v, respectively. This result confirms the importance of analysis of this variables during treatment of lignocellulosic materials to improve cellulose hydrolysis. Table 3 also shows the results of the 2^3 factorial design. The glucose concentration ranged from 23.0 to 1.0 g L^{-1} and the glucose extraction efficiency from 71 % to 3 %. These results allowed the drafting of the Pareto chart (Figure 1), which provided important data about the statistical relevance of the factors as well as of their interactions. From these charts, it can be seen that residence time and temperature had significant relevance to glucose extraction efficiency.

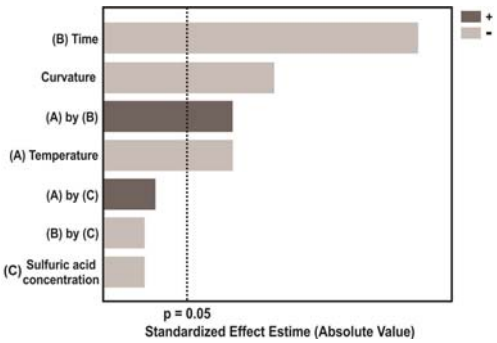

Figure 1: Pareto chart for glucose extraction efficiency in 2^3 factorial design.

Statistical analysis of the experimental data showed that two independent variables (residence time and temperature) significantly influenced the hydrolysis of cellulose, after hemicelullose and lignin removed, from sugarcane bagasse. Using the analysis of variance (ANOVA; Table 4), significant effects ($p \leq 0.05$) of temperature, residence time and both interaction were observed. Also, interaction between sulfuric acid concentration has not been observed. This experimental design showed curvature. Thus, relationship between the factors and the dependent variable showed non-linearity. The correlation coefficient (R^2) showed a good fit to the experimental results with values higher than 99 %.

Table 4: Analysis of variance (ANOVA) of glucose extraction efficiency

Source of variation	Sum of squares	Degrees of freedom	Mean square	F	p_{value}
Curvature	1049.809	1	1049.809	78.2473	0.003045
(A) Temperature	504.508	1	504.508	37.6034	0.008720
(B) Residence Time	4315.670	1	4315.670	321.6674	0.000378
(C) Sulfuric acid concentration	2.868	1	2.868	0.2138	0.675300
A by B	523.423	1	523.423	39.0132	0.008279
A by C	24.535	1	24.535	1.8287	0.269186
B by C	3.632	1	3.632	0.2707	0.638836
Lack of fit	35.238	1	35.238	14.062	0.064326
Pure Error	5.012	2	2.506		
Total	6464.693	10			

The desirability surface plots are presented in Figure 2. Then the desirability surface was used to determine and compare the optimal formulation domain for each condition. A zero level of desirability implies that performances of the corresponding response may not be acceptable, while a desirability of level one can be considered as the most satisfactory level of the response's performance. The analysis of Figure 2a shows that glucose extraction efficiency could be increased with a temperature and residence time in a minimum value (155 °C and 10 min). The temperature and residence time were a parameters of great influence during diluted-acid hydrolisys of cellulose after hemicellulosic and lignin removal from sugarcane bagasse. The temperature, residence time and sulfuric acid concentration values were 155 ºC, 10 min and 2 % w/v, which provided the best glucose concentration (22.74 g L^{-1}) in the current study. Two additional experiments were then performed at these selected conditions, in order to validate the models. The results obtained in these supplementary pretreatments were compatible with the expected values. The cellulosic hydrolysates obtained in the validation experiments, with 2 % w/v sulfuric acid at 155 ºC for 10 min, were 4-fold concentrated and destoxified with activated charcoal for the reduction of inhibitors concentration (components (in g L^{-1}): glucose 55.6; furfural 0.012; HMF 0.379; phenolic compounds 11.04) and supplemented with all necessary nutrients they were used as a source of sugars to produce ethanol with a yeast *Sc.* NRRL Y-7124. After a period of 72 h for fermentation it was observed a consumed glucose of 60 %, 16.8 g L^{-1} of ethanol concentration with yield ($Y_{P/S}$) and productivity (Q_P) of 0.38 g g^{-1} and 0.23 g L^{-1} h^{-1}, respectively. These results indicate the fermentability of this hydrolysate by *S. stipitis*, but more experiments are still needed to increase the productivity of this bioprocess.

4. Conclusions

Sugarcane bagasse, after hemicellulose and lignin removal, could be hydrolysed by dilute acid (2.0 % of H_2SO_4) at 155 ºC for 10 min and glucose was obtained as the main fermentable sugar (22,74 g L^{-1}). This treatment process generated inhibitory compounds, and the detoxification was required for removing these compounds found in the hydrolysate. The detoxified cellulosic hydrolysate was used as a fermentation medium by *Sc. stipitis*, an excellent ethanol producer from lignocelullosic hydrolysates, resulting in good values of fermentative parameters ($Y_{P/S}$ 0.38 g g^{-1} and Q_P 0.23 g L^{-1} h^{-1}), indicating that this hydrolysate is a potential substrate for ethanol production.

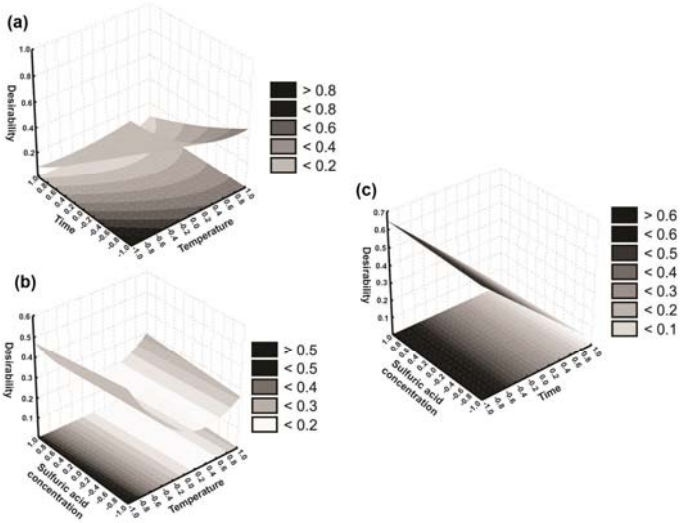

Figure 2: Desirability surface of glucose extraction efficiency.(a) Time vrs. Temperature, (b) Sulfuric acid concentration vrs. Temperature and (c) Sulfuric acid concentration vrs. Time

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References

Amiri H., Karimi K., 2013, Efficient Dilute-Acid Hydrolysis of Cellulose Using Solvent Pretreatment, Industrial & Engineering Chemistry Research, 52, 11494-11501
Botha T., Blottnitz H., 2006, A comparison of the environmental benefits of bagasse-derived electricity and fuel ethanol on a life-cycle basis, Energy Policy, 34, 2654-2661
Chandra M., Kalra A., Sharma P.K., Kumar H., Sangwan R.S., 2010, Optimization of cellulases production by Trichoderma citrinoviride on marc of Artemisia annua and its application for bioconversion process, Biomass and Bioenergy, 34, 805-811
Dantas G.A., Legey L.F.L., Mazzone A., 2013, Energy from sugarcane bagasse in Brazil: An assessment of the productivity and cost of different technological routes, Renewable and Sustainable Energy Reviews, 21, 356-364
Gírio F.M., Fonseca C., Carvalheiro F., Duarte L.C., Marques S., Bogel-Łukasik R., 2010, Hemicelluloses for fuel ethanol: A review, Bioresource Technology, 101, 4775-4800
Gouveia E.R., Nascimento R.T.d., Souto-Maior A.M., Rocha G.J.d.M., 2009, Validação de metodologia para a caracterização química de bagaço de cana-de-açúcar, Química Nova, 32, 1500-1503
Hamelinck C.N., Hooijdonk G., Faaij A.P.C., 2005, Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle- and long-term, Biomass and Bioenergy, 28, 384-410
Harris E.E., Beglinger E., 1946, Madison Wood Sugar Process, Industrial & Engineering Chemistry, 38, 890-895
Harris J.F., Baker A.J., Conner A.H., Jeffries T.W., Minor J.L., Pettersen R.C., Scott R.W., Springer E.L., Wegner T.H., Zerbe J.I., 1985, Two-Stage, Dilute Sulfuric Acid Hydrolysis of Wood: An Investigation of Fundamentals, Madison, WI., pp. 77.
Laopaiboon P., Thani A., Leelavatcharamas V., Laopaiboon L., 2010, Acid hydrolysis of sugarcane bagasse for lactic acid production, Bioresource Technology, 101, 1036-1043
Olsson L., Hahn-Hägerdal B., 1996, Fermentation of lignocellulosic hydrolysates for ethanol production, Enzyme and Microbial Technology, 18, 312-331
Rabelo S.C., Carrere H., Maciel Filho R., Costa A.C., 2011, Production of bioethanol, methane and heat from sugarcane bagasse in a biorefinery concept, Bioresource Technology, 102, 7887-7895
Thompson D.R., Grethlein H.E., 1979, Design and Evaluation of a Plug Flow Reactor for Acid Hydrolysis of Cellulose, Industrial & Engineering Chemistry Product Research and Development, 18, 166-169
Ulbricht R.J., Northup S.J., Thomas J.A., 1984, A review of 5-hydroxymethylfurfural (HMF) in parenteral solutions, Fundamental and Applied Toxicology, 4, 843-853
Unica, 2012, União da Agroindústria Canavieira de São Paulo <http://www.unica.com.br> accessed 20.11.2012.