

## Pretreatment of sugar cane bagasse with phosphoric and sulfuric diluted acid for fermentable sugars production by enzymatic hydrolysis

Gómez Sandra M R, Andrade Rafael R, Santander Carlos G, Costa Aline C, Maciel Filho Rubens

School of Chemical Engineering, University of Campinas, UNICAMP  
Av. Albert Einstein, 500 - CEP 13083-852 – P. O. Box 6066 Campinas - SP – Brazil

In the process of enzymatic conversion of cellulose to ethanol, a pretreatment is required to break the lignocellulosic biomass structure. This work presents the glucose production by enzymatic hydrolysis of sugar cane bagasse pretreated with two diluted acids. The best values of the variables of diluted acid pretreatment that influence glucose production in the hydrolysis enzymatic, such as acid concentration and pretreatment time were evaluated. An experimental design was used to compare the following factors: sulfuric and phosphoric acid concentrations (0.5 %wt to 3.5%wt) and reaction time (15 to 180 minutes). Pretreatment temperature and the solids concentration were maintained constant at 130° C and 10 %wt of bagasse. The maximum glucose concentration achieved was 404,5 mg glucose/g raw bagasse for the bagasse pretreated with phosphoric acid and 414,9 mg glucose/g raw bagasse for the bagasse pretreated with sulfuric acid.

### 1. Introduction

Ethanol can be produced through the fermentation of agricultural products such as sugarcane bagasse, rice straw, silver grass, corn stover among others. This process involves four basic steps: feedstock pretreatment, enzymatic or acid hydrolysis, sugars fermentation, and ethanol recovery (Saha and Hayashi, 2004). Positive impacts of ethanol use are the elimination of lead compounds from gasoline and the reduction of noxious emissions. There is also the reduction of CO<sub>2</sub> emissions, since sugarcane ethanol requires only a small amount of fossil fuels for its production, being thus a renewable fuel (Goldemberg et al. 2008).

Sugarcane bagasse is the solid byproduct from sugar refining based on sugarcane. Currently, it is mainly used as a boiler fuel in order to provide energy for sugar mills. Possible biotechnological applications of bagasse have been reviewed recently (Pandey et al. 2000). In Brazil, sugarcane (*Saccharum* sp.) is one of the most important agro-industrial products. According to data of São Paulo State Research Foundation, about 60–90% of the bagasse generated from milled sugar cane in the country is used as fuel for steam and energy production and, between 10 and 40% is not used, representing

about 5–12 million ton annually. In 2003, 340 million ton of sugarcane were produced and, consequently, 91.8 million ton of bagasse were generated. Because of the high carbohydrate content, sugar cane bagasse can be potentially used for bioethanol production and/or others products, within the context of biorefinery (Fapesp, 2004).

An effective pretreatment is characterized by several criteria. It should avoid the need for reducing the size of biomass particles, preserve the pentose (hemicellulose) fractions, limit formation of degradation products that inhibit growth of fermentative microorganism, minimizes energy demands, and limit costs. Also, the pretreatment agent should have low cost and inexpensive recycle (Mosier et al. 2005). Among all the pretreatment methods, dilute acid pretreatments have been widely studied because it is effective and inexpensive. The dilute sulfuric acid pretreatment can effectively solubilize hemicellulose into monomeric sugars (arabinose, galactose, glucose, mannose, and xylose) and soluble oligomers, thus improving cellulose conversion (Cadoche and López, 1989).

The goal of this study was to determine the effects of variables of the diluted acid pretreatment on the fermentable sugars production from sugar cane bagasse. The effect of acid concentration and pretreatment time on the conversion of cellulose to glucose during enzymatic hydrolysis was also studied using surface response methodology.

## 2. Materials and methods

### 2.1 Raw Material

The raw material for this analysis was sugar cane bagasse milled, non-burned, provided by mechanical harvesting, in October 2007, from the Usina da Pedra, located in Serrana, Sao Paulo, Brazil. It was dried at room temperature for four days, milled in a knife mill and sieved using Tyler 35 sieve (around 0.5 mm) for all analysis. The material was stored in plastic bags and kept in a freezer.

*Table 1. Chemical composition of sugar cane bagasse*

Component	%
Glucan	37,35 ±0,02
Xilan	23,66± 0,9
Lignin	25,10± 0,5
Extractives	3,25± 0,2
Ash	1,79 ±0,02

### 2.2 Chemical analysis

The compositions of the raw and pretreated material were determined by a standard analysis procedure, which was modified by the National Renewable Energy Laboratory (NREL) analytic methods (Wen-Hua et al. 2008). The sugar content was determined based on monomer content measured after a two-step acid hydrolysis procedure. The samples were treated with 72% wt H<sub>2</sub>SO<sub>4</sub> at 30 °C for 1 h in the first step. The reaction mixture was then diluted to 4% wt H<sub>2</sub>SO<sub>4</sub> and autoclaved at 121°C for 1 h. The hydrolysis solution was filtered and analyzed for sugar content and acetyl content by HPLC and for acid soluble lignin (ASL) from absorbance at 280 nm. The remaining

solid residue was dried overnight at 105°C and used to calculate the content of acid insoluble lignin (AIL). The chemical composition of bagasse is shown in the table 1.

### 2.3 Pretreatment

Sugar cane bagasse was dried at 105 °C overnight to ensure low moisture content prior to pretreatment. A biomass sample of 10 g on a dry basis was treated with 100 ml of the pretreatment solution in 500 ml flask in an autoclave reactor at 130°C. the acid concentration of the pretreatment solutions and the duration of the reaction were determined by a factorial designed. The extracted lignocellulosic fraction was delignified with 1,5% NaOH solution at 100°C for 1 hour. The cooled samples were immediately separated into solid and liquid fractions by filtration and then underwent an enzymatic hydrolysis test.

### 2.4 Enzymatic hydrolysis

The pretreated bagasse was hydrolyzed in an orbital shaker (Marconi MA-832) agitated at 150 rpm. The enzymatic hydrolysis was carried out in 250-mL flasks, temperature of 50°C and pH 4,8 (citrate buffer). One gram of pretreated bagasse was hydrolyzed with a commercially available cellulase produced by *Trichoderma reesei* (Sigma) and  $\beta$ -glucosidase from *Aspergillus niger* (Sigma) with concentrations of 7 FPU/g dry pretreated biomass and 3,5 IU/g dry pretreated biomass, respectively. Enzymes activities were 45,227 FPU/mL for Cellulases and 578 CBU/mL for  $\beta$ -glucosidase. Aliquots were taken periodically during 72h and analyzed for glucose production.

### 2.5 Experimental design

The response surface methodology (RSM) was chosen to study 2 factors: acid concentration (AC) and pretreatment time (t). The experiments were carried out according to a 2<sup>2</sup> complete factorial design plus three central points. The response was glucose concentration after enzymatic hydrolysis. The Statistica software version 7.0 from Statsoft Co. was used in this study.

*Table 2. Coded factor levels and real values for independent variables*

Factors	Levels		
	-1	0	1
%wt acid	0,5	2	3,5
time min	15	97,5	180

## 3. Results and Discussion

The results obtained by utilizing the factorial design were analyzed for glucose production. The glucose concentration was expressed as g glucose/g dry raw bagasse. Table 2 shows the factor levels used.

Tables 3 and 4 shows the design matrix with the glucose yield obtained at the end of enzymatic hydrolysis for each assay. It can be seen from Tables 3 and 4 that, in the operational conditions used in this work, the maximum glucose release was obtained in assay 4 for the phosphoric acid pretreatment (404,5 mg/g dry bagasse) and in assay 1 for the sulfuric acid pretreatment (414,9 mg/g dry bagasse).

Table 3. Design matrix presenting mass of glucose released after hydrolysis of phosphoric acid pretreated bagasse

Assay	%wt phosphoric acid	time min	g glucose/g dry raw biomass	% yield
1	0,5	15	0,2482	59,81
2	0,5	180	0,2764	66,60
3	3,5	15	0,2391	57,59
4	<b>3,5</b>	<b>180</b>	<b>0,4045</b>	<b>97,47</b>
5c	2	97,5	0,2725	65,66
6c	2	97,5	0,2792	67,28
7c	2	97,5	0,2584	62,27

Table 4. Design matrix presenting mass of glucose released after hydrolysis of sulfuric acid pretreated bagasse

Assay	%wt sulfuric acid	time min	g glucose/g dry raw biomass	% yield
1	<b>0,5</b>	<b>15</b>	<b>0,4149</b>	<b>99,99</b>
2	0,5	180	0,2425	58,43
3	3,5	15	0,2521	60,75
4	3,5	180	0,2372	57,16
5c	2	97,5	0,3425	82,53
6c	2	97,5	0,3201	77,13
7c	2	97,5	0,3224	77,69

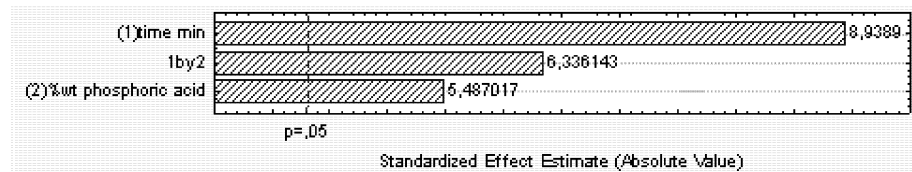


Figure 1(a)

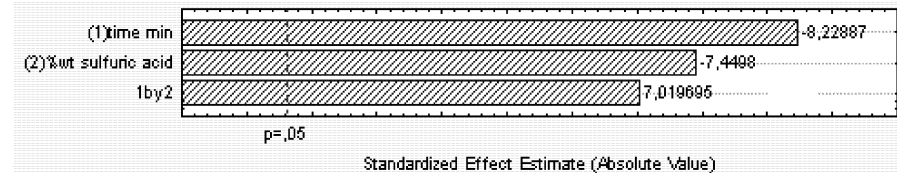


Figure 1(b)

Figure 1. Pareto chart of standardized effects for glucose yield (mg/g raw bagasse). (a) Phosphoric acid pretreatment, (b) Sulfuric acid pretreatment.

The results of this analysis are shown by using the Pareto charts. In these charts, the effect estimates divided by their standard errors are sorted from the largest absolute value to the smallest absolute value. The vertical line corresponds to a p value of 0.05, which implies in a 95% level of confidence (Barros, 2003). In the Figure 1(a), the results for the pretreatment with phosphoric acid show that the largest effect is the effect of pretreatment time, followed by the effect of phosphoric acid concentration, both

affecting positively glucose yield. The Pareto chart for glucose yield of the bagasse pretreated with sulfuric acid is shown in Figure 1(b). The highest effects are also the effects of time and acid concentration, both negative, although the interaction effect is positive.

Table 5 depicts the analysis of variance (ANOVA) for the models of glucose yield after hydrolysis. The models present high correlation coefficient and can be considered statistically significant with 95% of confidence according to the F test, as they presented calculated  $F_{\text{regression}}$  values greater than the listed ones and  $F_{\text{lack of fit}}$  less than the listed one (Barros, 2003).

Table 5. ANOVA for the models describing glucose yield. P (phosphoric acid) and S (sulfuric acid)

Source of variation	sum of squares (SQ)		Degree of freedom (DF)		Mean square (MS)		F calc *		F** Listed
	P	S	P	S	P	S	P	S	
Regression (R)	1,76E-2	2,62E-2	3	3	5,88E-3	8,73E-3	16,77	9,52	9,28
Residual (r)	1,05E-3	2,75E-3	3	3	3,50E-4	9,17E-4			
Lack of fit (Lf)	8,17E-4	2,45E-3	1	1	8,16E-4	2,45E-3	6,96	16,12	19,25
Pure error (Pe)	2,35E-4	3,04E-4	2	2	1,17E-4	1,52E-4			
R2	0,9437	0,905							

\*F test for statistical significance of the regression= $MSR/MSr$ . F test for lack of fit= $MSLf/MSPe$ . \*\*F Listed to 95% of confidence.

The model for the glucose production is represented by equation (1) for the bagasse pretreated with phosphoric acid and (2) for the bagasse pretreated with sulfuric acid:

$$\text{Yield (g glucose/g raw bagasse)} = 0,2827 + 0,0484 \times t + 0,0297 \times P + 0,034 \times t \times P \quad (1)$$

$$\text{Yield (g glucose/g raw bagasse)} = 0,3067 - 0,0507 \times t - 0,0459 \times S + 0,0433 \times t \times S \quad (2)$$

In these equations, t, P, and S are the coded values of pretreatment time, phosphoric acid concentration and sulfuric acid concentration, respectively. These models were built to establish the relationship of glucose yield with dilute sulfuric and phosphoric acid concentrations and pretreatment time and they are appropriate in the range of sulfuric and phosphoric acid concentrations and residence times used in the experiments.

The proposed models can be used to plot response surfaces and for prediction or optimization purposes. The effect of dilute acid concentration and pretreatment time on the hydrolysis and solubilization of the biomass is clearly shown in Figures 3 and 4. Maximum glucose yield is obtained with high pretreatment time, and phosphoric acid

loading (Fig. 3), but in Fig. 4 the opposite effect is observed, maximum glucose yield is obtained with low times and sulfuric acid concentration.

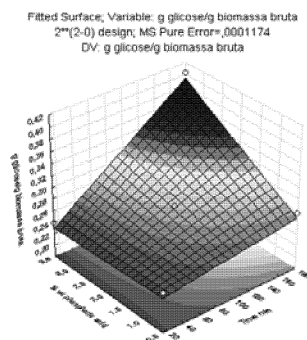


Figure 3. Glucose yield from phosphoric acid pretreated bagasse

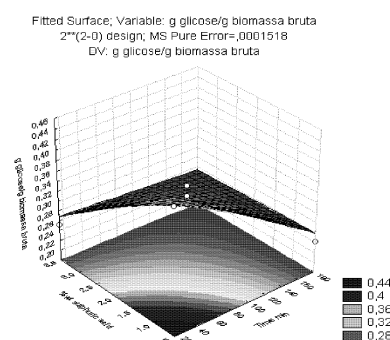


Figure 4. Glucose yield from sulfuric acid pretreated bagasse

#### 4. Conclusions

Dilute phosphoric and sulfuric acid pretreatment followed by the NaOH delignification were effective in solubilizing cellulose in the biomass. About 97% of glucan in the raw bagasse was hydrolyzed into monomeric glucose for 3,5%wt of phosphoric acid concentration and pretreatment time of 180 min. When the sulfuric acid concentration and pretreatment time were 0,5%wt and 15 min, respectively, 99,99 % of glucan were converted, what makes sulfuric acid pretreatment at best compared with phosphoric acid, as it allows higher yields in lower reaction times and concentration.

#### 5. References

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