



Measurement of fiber conversion levels in reducing sugars from hydrolyzed sugarcane bagasse with acid catalysts

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Resumo: O objetivo deste trabalho foi determinar a eficiência de redução de fibras em detergente neutro (FDN) e a conversão em açúcares redutores através de hidrólises ácidas. Foram utilizados dois tipos de ácidos fortes como catalisadores das reações: ácido sulfúrico (H_2SO_4) nas concentrações de 0,05, 0,1 e 0,15M e ácido clorídrico (HCl) nas concentrações de 0,05%, 0,1% e 0,2%. Foram aplicados diferentes tempos (30, 45 e 60 minutos) e temperaturas (80, 100 e 120 °C). Para o H_2SO_4 , a melhor hidrólise obtida foi no ensaio de número 4 com tempo de 60 minutos, temperatura de 120 °C e concentração de H_2SO_4 de 0,05 mol.L⁻¹ com conversão em açúcares redutores de 93,54%. Para o HCl, o melhor resultado foi observado no ensaio de número 4 com tempo de 60 minutos, temperatura de 120 °C e concentração de HCl de 0,05% com valor de 94,14%. A hidrólise com HCl foi a que obteve os melhores resultados, alcançando os menores teores de fibras e maiores valores de eficiência de conversão em açúcares redutores. Os resultados obtidos foram de grande valia, sendo possível sua utilização em projetos futuros, visando à otimização das hidrólises para concluir o processo de digestão anaeróbia.

Palavras-chave: Digestão anaeróbia, hidrólise ácida, matéria-prima.

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Abstract: This study aimed at determining the efficiency of neutral detergent fiber (NDF) reduction and conversion to reducing sugars by acid hydrolysis. Two kinds of strong acids as reaction catalysts were used: sulfuric acid (H_2SO_4) at 0.05, 0.1 and 0.15 M concentrations and hydrochloric acid (HCl) at 0.05%, 0.1% and 0.2% concentrations. Different times (30, 45 and 60 minutes) and temperatures (80, 100, 120 °C) were applied. For H_2SO_4 , the best hydrolysis was obtained in essay 4 with 60 minutes, at 120 °C and 0.05 mol L⁻¹ H_2SO_4 concentration with 93.54% conversion to reducing sugars. While for HCl, the best result was observed in essay 4 with 60 minutes, at 120 °C and 0.05% HCl concentration of 94.14%. Hydrolysis with HCl showed the best results, with the lowest levels of fiber and the highest conversion efficiency in reducing sugars. The results were quite important, since it will be possible to use them in future projects, aiming at optimizing the hydrolysis in order to complete the anaerobic digestion process.

Keywords: Anaerobic digestion, acid hydrolysis, raw material.

1. Introduction

Biomass has been used as the main material for studies concerning alternative energy production. The sugarcane bagasse has potential energy to produce biogas through anaerobic digestion. It is a lignocellulosic waste, a by-product of the sugarcane industry and it can also be generated from its juice extraction (BNDES; CGEE, 2008).

Its residue consists mainly of cellulose, hemicellulose and lignin, which has about 28% sugarcane weight. Hemicellulose and cellulose are the most structural fractions of sugarcane bagasse and represent a potential source of sugars such as xylose and glucose. However, this sugar obtaining requires the application of techniques that allow their selective extraction (Betancur and Pereira Jr, 2010).

Currently, studies involving anaerobic bagasse digestion for biogas production, in order to improve this process, have been under development, although such technology has been established at lower operating costs. Initially, during hydrolysis phase, complex organic compounds are converted into simpler compounds (Morais and Broetto, 2012). This conversion process begins with the transformation of more complex particulate organic matter in dissolved materials, by specific hydrolytic exoenzymes excreted by fermentative bacteria (Teixeira *et al.*, 2008).

Currently, the biomass treatment is accomplished by acid or enzymatic hydrolysis, which are technologies under improvement, although acid hydrolysis is the most prominent (Morais and Broetto, 2012). This consists of adding more diluted or concentrated acids and aims at solubilizing hemicellulose as well as making cellulose more accessible to digestion (Hamenlick *et al.*, 2005). This hydrolysis requires a shorter time of reaction as well as lower spending on chemicals and energy when compared to enzymatic hydrolysis (Ogeda and Petri, 2010).

During the hydrolysis occur the fibers conversion in reducing sugars. Carbohydrates are the most abundant macronutrients and are classified into mono, oligo and polysaccharides. Monosaccharides are non-hydrolysable simple sugars, i.e, reducing sugars (Silva *et al.*, 2003), however, in order to obtain these sugars, it is required the application of techniques to extract them, in this case, by acid hydrolysis. Combinations of acid concentration, temperature and reaction time can generate large amounts of sugar from this fraction. After the conversion of fibers in reducing sugars, these ones are recovered for subsequent fermentation to keep on the biogas production process.

Thus, the present study aimed at analyzing the effectiveness of acid hydrolysis with sulfuric and hydrochloric acid under different concentrations of sugarcane bagasse.

2. Material and methods

The material was collected in a place where we can get sugarcane juice and took to the laboratory to grind its residue into pieces of approximately 3 cm diameter and easy its

characterization. For the initial characterization, the evaluated parameters were neutral detergent fiber (NDF) and reducing sugar.

For hydrolysis, sulfuric acid (H₂SO₄) and hydrochloric acid (HCl) were the strong catalyst acids used. The applied concentrations were 0.05M, 0.1M and 0.15M for H₂SO₄ and 0.05%, 0.1% and 0.2% for HCl.

The trials of acid hydrolysis followed a factorial experimental design, with these independent variables: temperature, hydrolysis time and acid concentration. The following $2^n=2^3=8$ treatments were performed in which 'n' is the number of variables, where each one is at two coded levels as +1 and -1; a central treatment was repeated 4 times. All factors are at a medium level, coded as zero, totaling 12 experiments (Table 1).

Table 1. Experimental design at -1, 0 e +1.

		Levels		
Variables		-1	0	+1
Hydrolysis H ₂ SO ₄	Temperature (°C)	80	100	120
	Time (min)	30	45	60
	[] H ₂ SO ₄	0.05M	0.1M	0.15M
		Levels		
Variables		-1	0	+1
Hydrolysis HCl	Temperature (°C)	80	100	120
	Time (min)	30	45	60
	[] HCl	0.05%	0.1%	0.2%

The hydrolytic trials started with 10g samples, which corresponded to 0.435g dry matter. The samples were packed in 100 ml polyethylene bottles and added 50ml of acid solution for hydrolyses. Afterwards, they were placed in an oven or autoclave depending on the temperature required. The trials were carried out in a greenhouse at 80 °C and 100 °C temperatures with both air exchange and circulation. On the other hand, the trials at 120 °C were carried out at 1 atmosphere (atm) pressure in autoclave.

After each trial, the hydrolysate was filtered and collected in a 50 ml beaker. Then, it was stored in a test tube and kept at -18 °C until the reducing sugars analyses. The solid residue was taken to the greenhouse at 65 °C for drying and subsequent fiber analysis. During the fiber analyses, the NDF - Neutral Detergent Fiber - reagent was prepared according to the Brazilian Compendium of Animal Nutrition Protocol. For the reducing sugars analyses, SN-1 and SN-2 solutions were prepared according to the Embrapa protocol, Somogy and Nelson method (Maldonade et al., 2013)

After all the studied procedures, the hydrolysis efficiency was evaluated according to the fiber reduction (NDF) and the increasing concentration of reducing sugars. In order to determine the best treatment and the influence of applied variables, a statistical analysis at 5% probability was carried out with the STATISTICA ® software, version 7.0 (STATSOFT TM, USA).

3. Results and discussion

During the initial characterization, % NDF values and reducing sugar concentration were 84.81% and 936.91 mg L⁻¹, respectively. Carvalho et al. (2009) researched about the characterization of sugarcane bagasse, thus, NDF content was 63%, therefore, it was lower than the answer already obtained in this trial. While Mota et al. (2010) recorded that its studied bagasse showed 45.25% for initial NDF content parameter. Consequently, the following procedures conditions have influenced on temperature, time and moisture of bagasse. The values of NDF (%) and conversion efficiency on reducing sugars (%), quantified in both hydrolyses, are shown on Table 2.

Table 2. Values for NDF (%) and conversion efficiency in reducing sugars for hydrolyses with H₂SO₄ and HCl.

Essay	t(min)	T (°C)	[H ₂ SO ₄] (mol L ⁻¹)	NDF (%)	Reducing Sugars Conversion (%)	[HCl] (mol L ⁻¹)	NDF (%)	Reducing Sugars Conversion (%)
1	30	80	0.05	84.85	91.91	0.05	79.84	89.74
2	30	120	0.05	76.09	92.43	0.05	72.35	92.84
3	60	80	0.05	87.87	75.89	0.05	75.75	90.59
4	60	120	0.05	76.35	93.54	0.05	81.00	94.14
5	30	80	0.15	86.68	80.30	0.20	86.79	93.03
6	30	120	0.15	78.26	92.24	0.20	70.74	92.77
7	60	80	0.15	85.61	91.89	0.20	72.81	92.03
8	60	120	0.15	81.68	91.95	0.20	70.80	92.56
9	45	100	0.10	87.45	92.03	0.10	82.71	91.44
10	45	100	0.10	89.47	91.79	0.10	83.89	92.65
11	45	100	0.10	81.17	91.51	0.10	79.37	92.80
12	45	100	0.10	85.07	91.74	0.10	82.98	91.98

Hydrolysis with H₂SO₄ showed high levels of fiber even after the hydrolytic process, an average NDF value of 83.38% and ±4.59% standard deviation. The best result, with the lowest percentage of fiber, was observed in the essay number 2, at which a 30-minute time, 120 °C and 0.05 mol.L⁻¹ H₂SO₄ concentration were used. Essay number 10 showed that the highest NDF content after hydrolysis, one of the central point replicas, at which 45-minute time, 100 °C, 0.10 mol.L⁻¹ acid concentration were used, and whose NDF value was 89.47%.

In this same hydrolysis, there were high conversion values of reducing sugars, with an average 89.77% answer and a standard deviation of ± 5.56%. The best result was observed in essay number 4, at which 60-minute time, 120 °C and 0.05 mol.L⁻¹ sulfuric acid concentration were used. Essay number 3 showed the lowest conversion of reducing sugars after hydrolysis, with the same time and the same acid concentration, however, with lower temperature (80 °C), whose obtained value for conversion sugars was 75.89%.

Aguilar et al. (2002) in sugar cane bagasse hydrolysis with H_2SO_4 at concentrations of 2, 4 and 6% determined as optimum conditions the concentration of 2% H_2SO_4 at a temperature of 122 °C and time of 24 minutes, taking approximately 90 % hemicellulose hydrolyzate.

Morais and Broetto (2012) evaluated the acid pre-hydrolysis of sugarcane bagasse in hydrolysis times of 15, 30, 45 and 60 minutes. The authors used 7.0 and 9.0% H_2SO_4 concentration and found out that the best treatment for the release of reducing sugars occurred at 30 and 45 minutes, with 7.0 and 9.0% H_2SO_4 concentrations. So, 6.89 and 6.60 gL^{-1} reducing sugars were released, respectively. These data showed an increase of 22.4 and 17.1 times in relation to the reducing sugars concentration.

According to Gurgel et al. (2012) the maximum glucose yields reached 67.6% (200 °C, 0.07% H_2SO_4 , 30 min), 69.8% (210 °C, 0.14% H_2SO_4 , 10 min) and 67.3% (210 °C, 0.28% H_2SO_4 , 6 min) in acid hydrolysis of sugar cane bagasse.

Acid hydrolysis with HCl showed acceptable values of fibers even after the hydrolytic process, with 78.25% NDF average value and $\pm 5.33\%$ standard deviation. The best results, the lowest percentage of fibers, was observed in essay number 6, at which 30-minute time, 120 °C and 0.20% hydrochloric acid concentration were used and obtained 70.74% NDF. The essay number 5 showed that the highest NDF content after hydrolysis, at which 30-minute time, 80 °C and 0.20% acid concentration were used and 86.79% NDF value was obtained.

On the other hand, hydrolysis with HCl showed 92.21% average for converting reducing sugars, considered as high values, and $\pm 1.12\%$ standard deviation. The best result was observed in essay number 4, at which 60-minute time, 120 °C and 0.05% hydrochloric acid concentration were used. While, essay number 1 showed the lowest reducing sugar conversion after hydrolysis, at which 30-minute time, 80 °C and 0.05% acid concentration were used, and the obtained value for sugar conversion was 89.74%.

Aguilar et al. (2002) describe the acids commonly used in the process of hydrolysis release protons that act on glycosidic bonds that occur between sugar monomers in polymer chains. With the breaking of these bonds several compounds are released, especially sugars like glucose and xylose.

In Pareto diagram (Figure 1), it can be seen the variables influence on hydrolysis process of sugarcane bagasse for the NDF dependent variable.

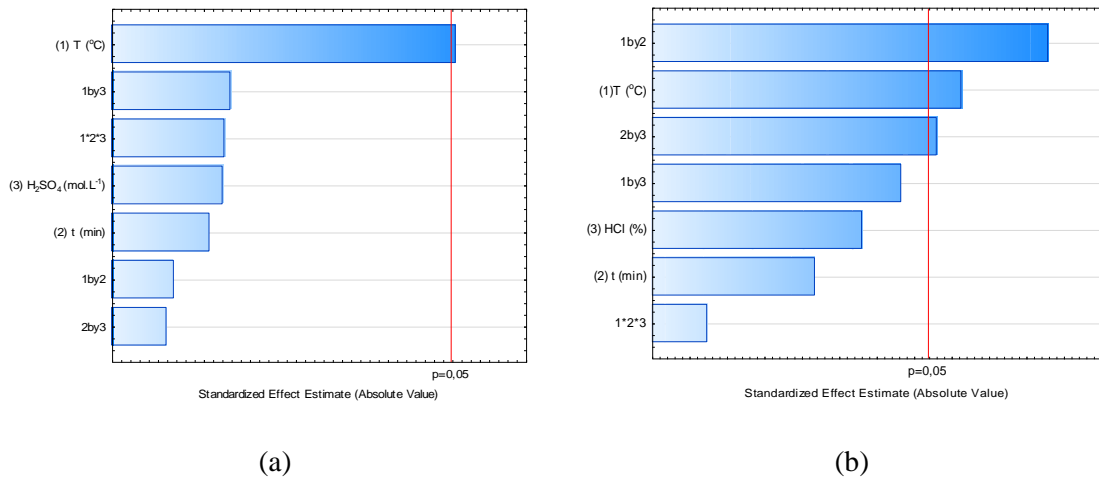


Figure 1. Pareto diagram for NDF variable in hydrolysis with H₂SO₄ (a) and HCl (b)

According to Figure 1a, it is observed that only the temperature has a significant effect during the hydrolysis process, with no other significant variables. On Figure 1b, the most important variable is the combination between temperature and time for the hydrolysis process, followed by temperature and the combination between time and concentration. Despite the reactions complexity, the pondering factor during the hydrolysis process is not the kinetics reaction, but the difficulty in reaching the reaction region of cellulose molecule by catalysts. The strong bond among cellulose, hemicellulose and lignin is characterized as one of the factors that controlled this access (Rao et al., 2006).

The behavior of the hydrolysis process in NDF reducing with H₂SO₄ and HCl are shown in Figure 2. As can only display the response surface only two independent variables, chose the variables that showed greater significant effect.

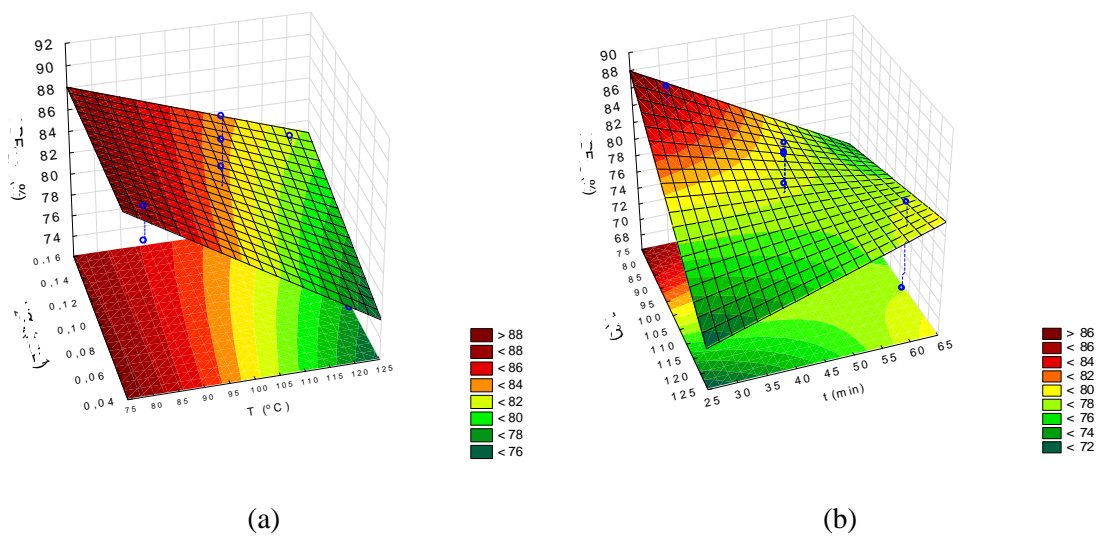


Figure 2 Response surface NDF conversion efficiency with H₂SO₄ (a) and HCl (b)

The rise in temperature has a negative effect on the variable NDF conversion, wherein the concentration of H_2SO_4 (Figure 2a) does not influence this variable. For the conversion of NDF with HCl (Figure 2b) it is observed that maintaining the concentration at the midpoint the shorter times and lower temperatures have better conversion efficiency.

In Pareto diagram (Figure 3), it can be observed the influence of variables in the hydrolysis process of sugarcane bagasse for the dependent variable conversion of sugars.

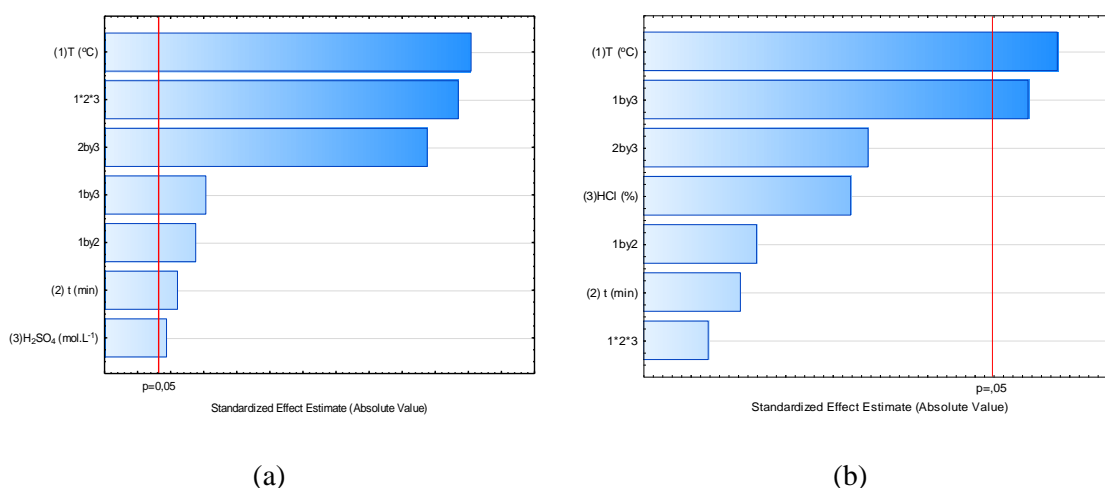


Figure 3. Pareto diagram for the variable sugars conversion in hydrolysis with H_2SO_4 (a) and HCl (b)

To H_2SO_4 (Figure 3a) all variables influence significantly the process at the 95% significance level, and temperature, the interaction between the three independent variables (temperature, time and acid concentration) and the interaction time and acid concentration had greater effect. For sugars conversion with HCl (Figure 3b) the variable temperature and the interaction acid concentration and temperature were the ones that influenced the process.

Morais and Broetto (2012) found out that during the pre-treatment of sugarcane bagasse hydrolysis for 60 minutes, the determined concentrations of reducing sugars did not increase when compared with the values obtained in the hydrolysis for 45 minutes. Thus, the 15-minute increase in autoclaving did not increase the obtained sugar production. A shorter time for the heat treatment indicates some cost reduction during the process.

The behavior of the hydrolysis process for sugars conversion with H_2SO_4 and HCl are shown in Figure 4. For shorter times and lower acid concentrations and longer times combined with higher concentrations of H_2SO_4 (Figure 4a), were obtained the best results. With the use of HCl (Figure 4b) the highest values of temperature and acid concentration promoted a higher sugars conversion.

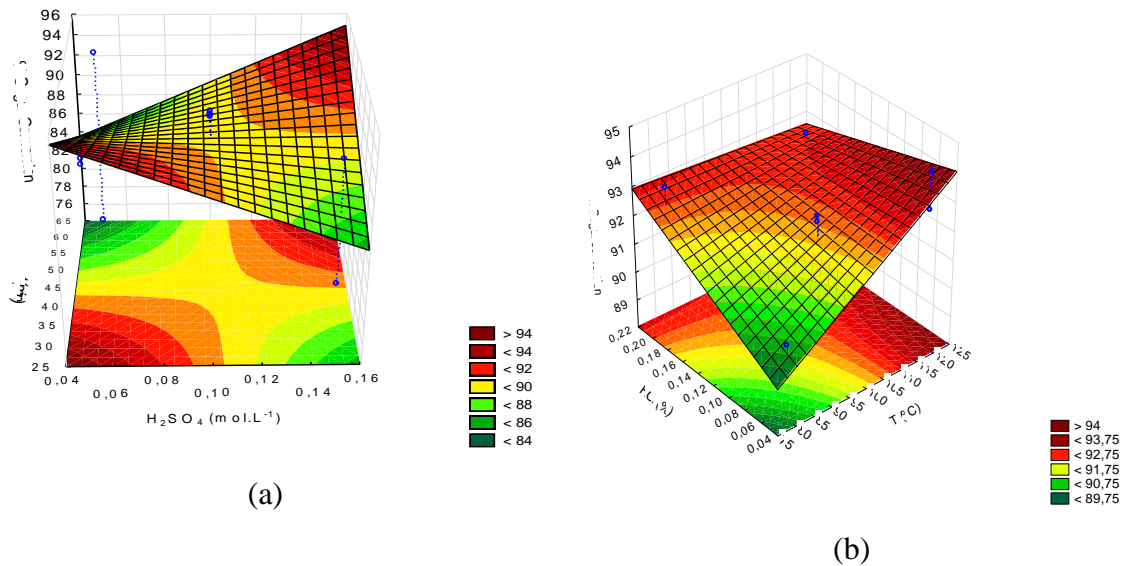


Figure 4. Response surface of sugars conversion efficiency with H_2SO_4 (a) and HCl (b)

In Table 3 are presented the equations, the coefficients of determination (R^2) and the values of F ratio ($F_{\text{cal}}/F_{\text{tab}}$) for NDF reduction (%) and reducing sugars conversion (%), in combinations H_2SO_4 and HCl with time and temperature. To obtaining the equations 1 to 4 took into account only the factors that influenced significantly, ie, those with $p\text{-value} < 0,05$.

Table 3. Regression models obtained for NDF reduction (%) and reducing sugars conversion (%), in combinations H_2SO_4 e HCl with time and temperature.

H_2SO_4		
Regression Model	R^2	$F_{\text{cal}}/F_{\text{tab}}$
(Eq. 1) FDN reduction (%) = $83,38 - 8,16 T$	0,58	2,11
(Eq. 2) Sugars conversion (%) = $89,77 + 7,54 T - 0,90 t + 0,65 [\text{H}_2\text{SO}_4] + 1,31 T.t - 1,54 T. [\text{H}_2\text{SO}_4] - 6,55 t. [\text{H}_2\text{SO}_4] - 7,25 T.t. [\text{H}_2\text{SO}_4]$	0,93	1155,10
HCl		
Regression Model	R^2	$F_{\text{cal}}/F_{\text{tab}}$
(Eq. 3) FDN reduction (%) = $78,25 - 5,08 T + 6,70 T.t - 4,62 t. [\text{HCl}]$	0,54	11,56
(Eq. 4) Sugars conversion (%) = $92,21 + 1,73 T - 1,59 T. [\text{HCl}]$	0,73	6,53

The coefficient of determination R^2 quantifies the adjustment quality, because it provides a measure of variance proportion, explained by the regression equation in relation to the total variation of responses. Thus it can be seen that the models found to NDF not exhibit satisfactory adjustment, being 58 and 54% for hydrolysis with H_2SO_4 and HCl , respectively. As for variable sugars conversion the model provides a good fit for both hydrolysis, this variable being explained in 93% when used H_2SO_4 as a catalyst and 73% when the catalyst is HCl .

The F test shows the ratio between the F calculated and F tabulated and, when this ratio exceeds 1, the regression is considered statistically significant, with the relationship between the independent and dependent variables. However, that model is useful for predicting, the value of this ratio should exceed 4 (Barros Neto, 2007). In the cases represented by the equations 1 to 4 all models

can be considered statistically significant and only the model for NDF using H₂SO₄ as catalyst (equation 1) cannot be considered predictive, because presents relation Fcal/Ftab equal to 2.11 < 4.

4. Conclusion

Concerning the initial characterization of sugarcane bagasse, the obtained values for NDF and reducing sugar were 84.81% and 936.91 mg L⁻¹, respectively.

The hydrolysis with HCl showed the best results with the lowest levels of fiber and higher conversion efficiency in reducing sugars.

According to the obtained results, both improvement and optimization of hydrolysis for biogas production are aimed for future researches, since this is a promising area regarding better utilization of sugarcane bagasse.

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