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Study of Sugarcane Bagasse Pretreatment with Sulfuric Acid as a Step of Cellulose Obtaining

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***Abstract*—**To produce sugar and ethanol, sugarcane processing generates several agricultural residues, being straw and bagasse is considered as the main among them. And what to do with this residues has been subject of many studies and experiences in an industry that, in recent years, highlighted by the ability to transform waste into valuable products such as electric power. Cellulose is the main component of these materials. It is the most common organic polymer and represents about 1.5 x 10^{12} tons of total production of biomass per year and is considered an almost inexhaustible source of raw material. Pretreatment with mineral acids is one of the most widely used as stage of cellulose extraction from lignocellulosic materials for solubilizing most of the hemicellulose content. This study had as goal to find the best reaction time of sugarcane bagasse pretreatment with sulfuric acid in order to minimize the losses of cellulose concomitantly with the highest possible removal of hemicellulose and lignin. It was found that the best time for this reaction was 40 minutes, in which it was reached a loss of hemicelluloses around 70% and lignin and cellulose, around 15%. Over this time, it was verified that the cellulose loss increased and there was no loss of lignin and hemicellulose.

***Keywords*—**cellulose, acid pretreatment, hemicellulose removal, sugarcane bagasse

I. Introduction

IN recent years, there has been an increasing trend towards more efficient utilization of lignocellulosic agro-industrial residues and among them, sugar cane bagasse, a waste in the process of sugar and ethanol extraction, is one of the most abundant low-cost lignocelullosic material [1]. In Brazil, during the 2010/2011 harvest, more than 625 million tons of sugarcane were crushed, which generated around 208 million tons of sugarcane bagasse [2]. An absolute minimum of about 70% of all bagasse is needed to generate heat and power to run the sugar milling process [3] and the remainder can be stockpiled. The stockpiled bagasse is of low economic value and constitutes an environmental problem to sugar mills and surrounding districts, especially if stockpiled for extended periods, due to the risk of spontaneous combustion occurring within the pile [4]. Thus, several processes and products that utilize bagasse as a raw material have been reported. Among them are include, pulp and paper production and products based on fermentation [5-7]

The sugarcane bagasse as well as any other type of plant biomass is composed by cellulose, hemicelluloses, lignin, and small amounts of extractives and mineral salts. The structural components are distributed in a lamellar structure [8]. The main component is cellulose, which is an inexhaustible and biodegradable natural raw material characterized by interesting properties such as hydrophilicity, chirality, broad chemical modification capacity and the formation of different polymorphs. Chemically, cellulose is a linear natural polymer of anhydroglucose units linked at the one and four carbon atoms by β-glycosidic bonds. The hierarchical structure of cellulose formed by networks of hydrogen bonds between the hydroxyl groups has been the subject of intense research for over 100 years, marked by frequent controversy over the results and a consistent supply of new insights. Directly from the beginning, the progress was connected to the introduction and continued development of methods for structure analysis [9]

Regardless of the application of lignocellulosic materials, it is required a preliminary processing to separate the three macromolecular fractions, particularly the lignin, which can be considered the main physical barrier for making the fibers of these materials (mainly cellulose) cemented together. These processes modify the lignocellulosic material by disruption of cell wall structure of plant biomass, removing, solubilizing or depolymerizing the lignin. The kinds of processes depend on the material used and the proposed purpose of lignocellulosic fractions utilization and may be mechanical, physical, biological or chemical. The development of pretreatment processes strong enough as to separate the cell wall arrangement and mild enough as to avoid a significant chemical degradation of biomass components is a challenge for today's chemical industry [10]. For the novel pretreatment methods it is advisable to use cheap and easily recoverable chemicals and low-cost equipment. The use of environmentally friendly and low energy-intensive approaches is highly desired. The chemical cooking process is the most efficient and most used to perform the separation of lignocellulosic components of vegetal biomass [11]. It results in enlargement of the inner surface area of substrate particles, accomplished by solubilization and/or degradation of hemicellulose and lignin. Prior research on the hydrolysis of bagasse and similar materials has concentrated on the use of low concentration mineral acids [12-19]. To carry out this process, the choice of

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acid and its concentration depend on the sample type and the purpose of the hydrolysis. The most utilized one is sulfuric acid.

Each structural component of the lignocellulosic material behaves differently in acidic media. The hemicelluloses are hydrolyzed much more rapidly in acidic media than cellulose, due to, mainly, the amorphous character of the hemicelluloses front of the crystalline nature of cellulose. Moreover, the furanosidic rings are hydrolyzed faster than the piranosidic rings on account of higher tension angle in the conformation of the rings with five carbon atoms as compared to the rings with six carbon atoms, that are free of tension. Under acid conditions, the degradation of lignin is produced by substitution reactions and broken links, usually accompanied by condensation reactions that prevent lignin dissolution and, consequently, it is eliminated in small proportions. Thus, the final product from an acid treatment is rich in cellulose and [8]. The aim of this study was to obtain the best reaction time for the pretreatment of sugarcane bagasse with sulfuric acid targeting a smaller loss of cellulose and a higher removal of hemicellulose and lignin.

II. Methodology

A. Pretreatment with H_2SO_4

In order to minimize losses of cellulose and increase the extraction of hemicellulose and lignin, a kinetic study was performed to determine the best parameters for this step. Bagasse (15,00 g) and H_2SO_4 (10% v/v) were placed, under a consistency of 10% (w/v), in a polypropylene beaker with a capacity of 4 L. The reaction was carried out in thermal bath with heating ramp and when the temperature reached 100 ° C, the time counting started. It was conducted reactions in the times of 0, 5, 10, 20, 30, 40, 50 and 60 min. At the end of each reaction time, the material was washed with distilled water until pH neutral and characterized for the determination of chemical composition and profiles of component and mass losses as a function of time.

B. Chemical Characterization

The chemical characterization of samples was performed using the analytical methodology developed for sugar cane bagasse by [20] and validated by [21]. Samples of 2.00 g (dry weight) of pretreated bagasse (ground to 20 mesh) were transferred to a beaker of 100 mL to be treated with 10 mL of 72% (w/w) H_2SO_4 under vigorous stirring in a bath at 45.00 ± 0.50 °C for 7 min. The reaction was stopped by adding 50 mL of distilled water, and the sample was transferred quantitatively to a 500 mL Erlenmeyer flask. Then, the volume of water inside the Erlenmeyer flask was raised to 275 mL. For a complete hydrolysis of the remaining oligomers, the flask was sealed with aluminum foil and autoclaved for 30 min at a pressure of 1.05 bar at 121°C. After decompression of the autoclave, the flask was removed from it, cooled to room temperature and the reaction mixture was filtered and transferred to a 500 mL volumetric flask which was completed using the water wash of the material retained on the filter. The volumetric flask containing the hydrolyzed material was stored for later analysis of carbohydrates and organic acids.

C. Determination of Carbohydrates

The levels of celobiose, glucose, xylose, arabinose and acetic acid present in the filtrated solution on sulfuric acid hydrolysis 72% of lignocellulosic materials were analyzed through HPLC. A small sample of the solution was filtered in a Sep Pak C18 cartridge and then examined for its contents of celobiose, glucose, xylose, arabinose and acetic acid through high performance liquid chromatography (HPLC), a chromatograph Shimadzu applying a model CR 7A refraction index detector Shimadzu model RID-6A, for a column in which was used Aminex HPX 87H (300 mm × 7.8 mm, BIO-RAD. The mobile phase was H_2SO_4 0.005 mol/L with a flow of 0.6 mL/min, at 45 ◦C. The concentrations of glucose and celobiose were converted into cellulosic pulp. The concentrations of xylose, arabinose and acetic acid were converted to hemicelluloses. The masses were divided due to their dry weight of the original material and multiplied by the hydrolysis factor. The conversion factors from glucose and celobiose to cellulosic pulp are 0.90 and 0.95, respectively. Similarly, xylose and arabinose were converted to hemicellulose using the factor 0.88. The conversion factor of acetic acid to acetyl group is 0.72. The concentrations of the compounds were determined due to the calibration curves drawn for each component.

D. Determination of furfural and hydroxymethylfurfural

The furfural and hydroxymethylfurfural in the filtered material for the sulfuric acid hydrolysis with 72% of lignocellulosic materials were analyzed through HPLC using a chromatograph Shimadzu model C-R7A detector with an UV visible Shimadzu SPD model – applying a column RP 10A-18 (C-18) of 125 mm × 4 mm (Hawlett-Packard), and in the mobile phase a solution of acetonitrile–water 1:8 (v/v) with 1% of acid acetic, at a flow of 0.8 mL/min to 25 ◦C. The middle areas of chromatograms for furfural and hydroxymethylfurfural were converted into cellulose and hemicellulose using the conversion factors 1.37 and 1.29, respectively (experimental values).

E. Determination of insoluble lignin

The insoluble material retained on the filter paper was washed with about 1.5 L of distilled water to remove residual acid (until pH near 7) and dry in oven at 105°C until constant mass. The percentage of insoluble lignin was calculated in relation to the mass of dry lignocellulosic material discounting the mass of ashes (inorganic compounds).

F. Determination of ash content

The materials resulting from the step of determining insoluble lignin were placed in a previously calcined and weighed porcelain crucible. Subsequently, the crucibles containing the samples were capped and pre-calcined at a temperature of 800°C for approximately 1 h. Then, the lid was removed and the material was calcined for 2 h at 800°C. After

calcination, the crucible was cooled in a desiccator and the mass of ash was determined by weight difference.

G.Determination of soluble lignin

The amount of soluble lignin was determined by measuring absorbance at 280 nm in a UV-visible spectrophotometer Perkin Elmer model Lambda 25. An aliquot of 5.00 mL of the hydrolyzate obtained in the step of chemical characterization was transferred to a 100.00 mL volumetric flask with 50.0 mL of distilled water and 2.00 mL of NaOH 6.50 mol/L (final pH near 12). After shaking, the volume was completed with distilled water and the resulting mixture was analyzed in the spectrophotometer.

III. Results and Discussion

A. Loss of weight

The first assessment after any stage of lignocellulosic materials treatment was the yield measurement, which is a parameter for the process classification and crucially dependent on raw material and on the conditions employed in the treatment. The total yield might be measured as the ratio between the mass of material obtained after the treatment stage and the initial mass used to perform the same. Industrially it is more appropriate to assess the sorted or cleaned yield, i.e., the yield of the material after the removal of wastes (material not shredded) and impurities of vegetable origin (sand, stones and metals) [22]. However, in this work, due mainly to the small mass used in the reactions, it was determined only the total yields of the treatments, i.e., it was taken into consideration the amount of waste. In Table 1 is showed the yields and loss of mass obtained for each reaction of bagasse in the different times carried out.

TABLE I
YIELDS AND LOSS OF WEIGHT IN THE DIFFERENT TIMES OF ACID PRETREATMENT OF SUGARCANE BAGASSE

Time reaction (min)	Initial mass (g)	Final mass (g)	Yield (%)	Loss of mass (%)
0	15.00	13.35	88.99	11.01
5	15.00	12.07	80.44	19.56
10	15.00	11.58	77.23	22.77
20	15.00	11.30	75.36	24.64
30	15.00	11.24	74.91	25.09
40	15.00	10.89	72.62	27.38
50	15.00	10.55	70.30	29.70
60	15.00	10.41	69.37	30.63

Analyzing Table 1, it was verified that there was a decline in yields, and hence, an increased loss of weight in function of reaction times. In other words, a higher reaction time provided a higher removal of components. Nevertheless, this loss of weight may be considered small when compared with other treatments that use chemicals such the ones that utilize NaOH or the kraft process, whose losses of weight might reach around 50%. This indicates that acid pretreatment is more selective than the others processes. In acid medium occurs, preferably, the removal of hemicelluloses. They have lower molecular weight and are present in lesser extent in the lignocellulosic materials than cellulose and lignin, i.e., their removal do not affect significantly the final yield and this can explain the low rate of weight of loss in the pretreatment reactions. The graph of Fig. 1 makes clear the phenomenon of weight loss versus time. It shows that the relationship between weight loss and reaction time was not linear, mainly because of structural complexity presented by the bagasse. The way in which cell wall components are organized and their physical structures do not allow that their elimination follow a pattern, mainly on account of access to these components by the sulfuric acid.

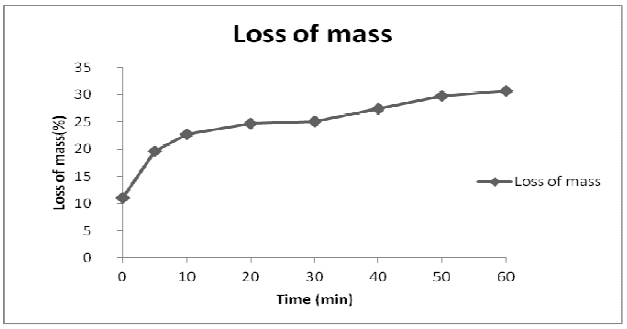

Fig. 1 Graph of loss of weight versus the reaction time.

B. Chemical characterization

Table II presents the values of the chemical composition of the materials produced at different times of pretreatment.

TABLE II
CHEMICAL COMPOSITON OF THE MATERIALS OBTAINED IN THE DIFFERENT TIMES OF BAGASSE PRETREATMENT WITH SULFURIC ACID

Time reaction (min)	Cellulose (%)	Insoluble lignin (%)	Soluble lignin (%)	Hemicellulose (%)
0	47.44	23.43	7.31	21.20
5	50.17	23.47	6.38	18.38
10	51.98	24.58	6.55	15.96
20	53.88	26.02	5.72	13.93
30	53.96	26.18	5.59	12.68
40	53.78	25.90	6.87	10.57
50	55.06	29.57	5.71	9.18
60	53.21	28.37	6.07	11.37

The chemical characterization of the materials shows that there was a growing decline in the percentage of hemicelluloses and, only at the last time tested (60 min), this percentage increased, indicating that there was no loss of this component. Such behavior was different for cellulose and lignin, whose percentage values presented a growing profile up to 50 minutes. The lack of structural similarity among the three

components is responsible for the behavior difference. The hemicelluloses were more easily degraded by sulfuric acid because they are amorphous molecules. The cellulose has a majority crystalline character and so, its structure is not so easily broken by a mineral acid, especially when it's at low concentrations, as in this work. The low lignin removal was caused for the condensation reactions that occur under acid condition. These reactions are undesirable because they prevent the lignin solubilization in acid medium. Proof of this was that the insoluble lignin content was increasing in most of the times studied, meaning that it was not very modified during treatment with sulfuric acid. This culminated in a major loss of hemicellulose than cellulose and lignin, as can be observed in Fig. 2. Hemicelluloses reached loss of more than 70% (50 and 60 minutes), while for the other two components, the biggest loss was approximately 20%, also in the highest times employed. Considering the purpose of this work, the loss of cellulose can be considered satisfactory until the time of 50 minutes, since with 60 minutes there was an increased in the amount of loss, that, until then, remained nearly constant. On the other hand, there was no alteration in the value of lignin loss from 40 minutes of reaction, i.e., this is the time limit for removal of lignin. Reactions with longer times would be unsuccessful. Considering the lignin as the main barrier in the process of cellulose obtaining, for the step of pre-treatment with sulfuric acid, the reaction time of 40 minutes would be ideal.

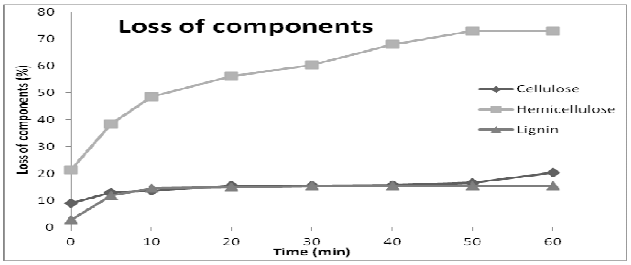

Fig. 2 Graphic of loss of components in function of reaction time

C. Hydrolyzate characterization

The results in Table 3 confirm what was observed in the analysis of loss of components (Fig. 1). The concentration of xylose in the hydrolysates was always higher than that of other sugars, showing the greatest loss of hemicellulose than cellulose during treatment with sulfuric acid. Taking into account that the arabinose, acetic acid and furfural are also generated by the breakdown of hemicellulose chains, this difference is even greater. It can be seen that the concentration of xylose increases relatively rapidly until 50 minutes. This period has been interpreted as representing the hydrolysis of the easy-to-hydrolysis fraction of the xylan. The hydrolysis then proceeded at a slower but measurable rate. This period has been interpreted as representing the hydrolysis of the hard-to-hydrolysis fraction of the xylan [23]. This fact confirms that from 50 minutes of reaction the hemicellulosic chain is no longer affected. The same phenomenon can also be noticed in the results obtained for arabinose and acetic acid, whose concentrations also remained almost constant after this time. The concentration of acetic acid, which is generated for the hydrolysis of the acetyl groups of the hemicelluloses, showed a fast increase at the start of hydrolysis (until 30 minutes) and then, it tended to stability. In the case of glucose, with 50 minutes of reaction, its concentration doubled; with another big increase of this value in 60 minutes. This behavior allows affirming with more certainty that the best reaction time, aiming a low cellulose removal concomitantly with the highest possible removal of hemicellulose, is 40 minutes. Furfural is one of the decomposition products from the hydrolysis of arabinose and xylose. Previous researcher data have indicated that the rate of xylose degradation is much slower than that leading to the formation of furfural [24-26]. In the present work the formation of furfural was low in the first times of reactions. Nevertheless, after 30 minutes, the rate of furfural formation started increasing faster, indicating that the xylose and arabinose degradation is higher in the highest time of acid treatment. It was not detected the presence of hydroxymethylfurfural (product of glucose degradation) and others sugars and carboxylic acids.One aspect that should be emphasized is that the high concentrations achieved, especially for the xylose in the highest reaction times employed, was due to the scale used. The average volume of hydrolysates was 50.00 mL, making the sugars stay more concentrated. At larger scales, the concentration values would be lower because the volume of hydrolyzed would be greater.

TABLE III
COMPOSITION OF THE HYDROLIZATES

Time reaction (min)	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Acetic acid (g/L)	Furfural (g/L)
0	0.68	1.91	1.96	1.97	0.00
5	1.51	10.70	3.80	5.74	0.36
10	1.80	21.73	4.36	7.64	0.45
20	2.59	41.24	5.25	8.72	0.68
30	3.10	51.54	5.82	9.17	0.74
40	4.37	64.31	6.50	9.19	1.06
50	9.91	81.23	6.89	9.25	2.30
60	15.10	81.50	7.04	9.31	2.60

IV. CONCLUSION

The best time of reaction found to pretreatment of sugarcane bagasse with sulfuric acid was 40 minutes. Though it had occurred removal of hemicelluloses in longer reaction times, the lignin loss became constant with 40 minutes, i.e., the removal of hemicellulose and lignin after this time would not be satisfactory and it would not be possible to increase significantly the purity of cellulose in the material. Besides, the cellulose loss raised with 60 minutes of reaction.

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REFERENCES

[1] Cardona, C. A., Quintero, J. A., & Paz, I. C. Production of bioethanol from sugarcane bagasse: Status and perspectives. Bioresource Technology, v.101, 2010, p.4754–4766.
[2] CONAB (Companhia Nacional de Abastecimento). Producão de cana no Brasil terá novo recorde. Available at: http//www. conab.gov.br. [Accessed Oct 23, 2011].
[3] Edwards B. Energy use in cane and beet factories. Proceedings of the Australian Society of Sugar Cane Technologists,1991. p. 227–9.
[4] Dawson M,Dixon T,Inkerman P. Moisture loss from baled bagasse during storage. Proceedings of the Australian Society of Sugar Cane Technologists,1990. p. 199–206.
[5] Pandey, A., Soccol, C.R., Nigam, P., Soccol, V.T. Biotechnological potential of agro-industrial residues. I: sugarcane bagasse. Bioresource Technology, v. 74, n. 1, 2000, p. 69-80.
[6] Ojeda, K., Ávila, O, Suárez, J., Kafarov, V. Evaluation of technological alternatives for process integration of sugarcane bagasse for sustainable biofuels production –part 1. Chemical Engineering Research and Design, v. 89, n. 3, 2011, p. 270-279.
[7] Zhao, X., Wu, R., Liu, D. Production of pulp, ethanol and lignin from sugarcane bagasse by alkali-peracetic acid delignification. Biomass and Bioenergy, v.35, n.7, 2011, p. 2874-2882.
[8] Fengel, D.; Wegener, G. Wood Chemistry, Ultrastructure, Reactions, Berlin: Walter de Gruyter, 1989.
[9] Klemm, D., Heublein, B., Fink, H.P., Bonh, A. Cellulose: fascinating biopolymer and sustainable raw material. Angewandte Chemie International Edition, v. 44, n.22, 2005, p. 3358-3393.
[10] Canetieri, E., Rocha, G. J. M., de Carvalho, J. R., Silva, J. B. A. Optimization of acid hydrolysis from the hemicellulosic fraction of Eucalyptus grandis residue using response surface methodology. Bioresource Technology, 2007, p.422-8.
[11] Fernandez, N. Pulp and paper development from sugarcane bagasse. In: Third Internacional Non-wood Fiber Pulping and Papermaking Conference, Proceedings, Pequim, v. 1, 1996, p. 231-240.
[12] Marton, G., Dencs, J., Szokonya, L. Principles of biomass refining. In: Handbook of heat and mass transfer. Houston: Gulf Publishing,1989. p. 609–52.
[13] Tsao, G. Ethanol and chemicals from cellulosics. Proceedings of the International Symposium: Alternative Sources of Energy for Agriculture,1984. p. 177–85.
[14] Trickett, R., Neytzell-de Wilde, F. Dilute acid hydrolysis of bagasse hemicellulose. Chemsa, 1982; March: p.11–5.
[15] Youssef, K., Ghareib, M., Nour El Dein, M. Improvement of the biodegredation of some cellulosic wastes by acid pretreatment. Acta Microbiologica Polonica, 1991,p.187–95.
[16] Torget, R., Werdene, P., Himmel, M., Grohmann, K. Dilute acid pretreatment of short rotation woody and herbaceous crops. Applied Biochemistry and Biotechnology, 1990, p.115–26.
[17] El-Taaboulsi, M. ,Nassar, M., Abd El-Rehim, E. A modified method of nitric acid pulping of bagasse. Journal of Chemical Technology and Biotechnology, 1983, p.87–96.
[18] Fontana, J., Corea, J., Duarte, J., Barbosa, A., Blumel, M. Aqueous phosphoric acid hydrolysis of hemicelluloses from sugarcane and sorghum bagasses. Biotechnology and Bioengineering Symposium No. 14, 1984. p. 175–85.
[19] Springer. Hydrolysis of aspenwood xylan with aqueous solutions of hydrochloric acid. TAPPI Journal, 1966, p.102–6.
[20] Rocha, G. J. M., Silva, F. T., Araújo, G. T., Curvelo, A. A. S. A fast and accurate method for determination of cellulose and polyoses by HPLC. In: Brazilian Symposium on the chemistry of Lignin and Other Wood Components, Curitiba. Proceedings, v.5, 1997.
[21] Gouveia, E. R.; Nascimento, R. T.; Souto-maior, A. M.; Rocha, G. J. M. Validação de metodologia para a caracterização química de bagaço de cana-de-açúcar. Química Nova, 2009, p.1-4.
[22] Koga, M, E. T. Cellulose and paper. Technology of celullosic pulp production. São Paulo: IPT, Senai, v.1, Ed. 2, 1998.
[23] B. P. Lavarack,G. J. Griffin, D. Rodman. The acid hydrolysis of sugarcane bagasse hemicellulose to produce xylose, arabinose, glucose and other products. Biomass and Bioenergy. v. 23, 2002, p.367-380.
[24] Trickett, R. Utilisation of bagasse for the production of C5-and C6-sugar,Masters thesis,University of Natal,Durban, 1984.
[25] Koukios, E., Sidiras, D. The role of pre-hydrolysis in refining lignocellulosic biomass. Cellulose Chemistry and Technology. 1995, p. 435–50.
[26] Szokonya, L., Marton, G., Havas-Dencs, J., Kovacs, M. Chemical processing of biomass I- Production of D-xylose by acidic hydrolysis of plant materials. Hungarian Journal of Industrial Chemistry. 1988, p.11–20.