Radiation Physics and Chemistry 81 (2012) 1008–1011

Contents lists available at SciVerse ScienceDirect

Radiation Physics and Chemistry

journal homepage: www.elsevier.com/locate/radphyschem

Electron beam combined with hydrothermal treatment for enhancing the enzymatic convertibility of sugarcane bagasse

C.L. Duarte [a,*], M.A. Ribeiro [a], H. Oikawa [a], M.N. Mori [a], C.M. Napolitano [a], C.A. Galvão [b]

[a] Energetic and Nuclear Research Institute (IPEN/CNEN—SP), Radiation Technology Center, Av. Professor Lineu Prestes 2242, 05508-000 Sao Paulo, SP Brazil
[b] Sugarcane Technology Center, CTC, 13400-970 2 Piracicaba, CP 16, SP Brazil

ARTICLE INFO

Article history:
Received 23 June 2011
Accepted 3 November 2011
Available online 23 November 2011

Keywords:
Sugarcane bagasse
Electron beam processing
Thermal hydrolysis
Acid hydrolysis
Enzymatic hydrolysis

ABSTRACT

The use of microbial cellulolytic enzymes is the most efficient process to liberate glucose from cellulose in biomass without the formation of fermentation inhibitors. A combination of pretreatment technologies is an alternative way to increase the access of enzymes to cellulose, and consequently, the conversion yield. In this way, the present study reports on the enzymatic hydrolysis of SCB submitted to three kinds of pretreatment: electron beam processing (EBP), and EBP followed by hydrothermal (TH) and diluted acid (AH) treatment. SCB samples were irradiated using a radiation dynamics electron beam accelerator, and then submitted to thermal and acid (0.1% sulfuric acid) hydrolysis for 40 and 60 min at 180 °C. These samples were submitted to enzymatic hydrolysis (EH) using commercial preparations, including Celluclast 1.5 L and beta-glycosidase. The addition of diluted acid improved TH treatment allowing for a shorter application time. EBP with 50 kGy increased the enzymatic hydrolysis yield of cellulose by 20% after TH and 30% after AH.

1. Introduction

Sugarcane bagasse is a renewable energy resource; however, to transform this resource into energy, the conversion of polysaccharides into free sugar is necessary. Pretreatment is the first step of the process of bioethanol production from biomass. Its main goal is to make cellulose more accessible to the enzymes that convert carbohydrate polymers into glucose. An effective pretreatment must preserve pentose (hemicellulose), limit the formation of degradation products that inhibit the growth of fermentative microorganisms, and minimize energy demand and costs. Pretreatment methods such as steam explosion and hydrothermal and dilute acid treatment are potential cost-effective methods (Mosier et al., 2005; Sanchez and Cardona, 2008).

Hydrothermal treatment consists of submitting the lignocellulosic material to high temperature and pressure in the presence of water to transform polysaccharides into low-molecular-weight, water-soluble products. The major drawbacks are the formation of byproducts such as carboxylic acids (mainly acetic and formic acid), formed by the oxidation and fragmentation of polysaccharides (cellulose and hemicelluloses), the formation of furfural and hydroxymethylfurfural by the dehydration reaction of xylose, and the risk to get diluted sugar solutions. Because of this fact, it is very important to keep a compromise between the temperature and time of the hydrolysis in order to get a maximum of glucose and celobiose liberation with the minimum byproducts formation (Yu et al., 2008; Santos et al., 2010; Silva et al., 2010).

The structural and compositional modifications to sugarcane bagasse (50% humidity) with absorbed doses from 5 to 150 kGy, have been demonstrated elsewhere. Almost all the cellulose and hemicelluloses are converted to oligosaccharides with 70 kGy. The main byproduct identified was acetic acid, which originated from the de-acetylation of hemicelluloses (Khan et al., 2006; Ribeiro et al., 2010).

Hydrothermal hydrolysis for 40 min at 180 °C after irradiation at 50 kGy showed a total reduction in oligosaccharides, liberating mainly xylose. However, the presence of formic acid and furfural, after 40 min of thermal treatment, meant that xylose and glucose were being decomposed just after their liberation from hemicelluloses and cellulose. With the addition of diluted acid, the same amount of xylose can be liberated, in this case, the time was reduced from 40 to 10 min; and the absorbed dose was reduced from 50 to 10 kGy (Duarte et al., 2010).

The primary enzymes used in the hydrolysis of the lignocelulosic materials are cellulases that break down cellulose in cello-oligomers and cellobiose (two glucose molecules). Generally, the enzymatic load is supplemented with β-glycosidase, which is responsible for the conversion of cellobiose into two molecules of glucose. The enzyme dose is based on the calculation of total solids in the dry mass. This dose can vary significantly, due to the

* Corresponding author. Tel.: +55 11 31339820.
E-mail address: clduarte@ipen.br (C.L. Duarte).

0969-806X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.radphyschem.2011.11.008

composition of the lignocelluloses material and the nature of chemical and physical pretreatments. Sometimes, surfactants are added to reduce the adsorption of enzymes by lignin. (Hassuani, 2005; Sanchez and Cardona, 2008; Santos et al., 2010).

Radiation processing is widely used for medical product sterilization, polymeric materials irradiation, and wastewater, flue gases, and solid waste materials decontamination. For radiation processing, accelerators are available, supplying electron beams in the energy range up to 10 MeV, as well as, radionuclide sources Co-60, which emit 1.17/1.33 MeV gamma rays. Electron beam is characterized by limited penetration and the entire energy of high-energy electrons is deposited in relatively thin layers of material. In the case of gamma rays, the radiation is able to penetrate deeper into the materials but the dose rates are a few orders of magnitude lower in comparison to electron beam. The reactive species generated by the interaction of ionizing radiation with water (OH radicals, e-aq, and H) have been successfully applied for organic pollutants removal in environmental samples and industrial effluents (Duarte et al., 2007; Haji-Saeid et al., 2007).

The free radicals produced by interaction of high-energy radiation in water react with the polysaccharides decreasing the degree of polymerization and increasing the carbonyl content due to the chain scission reaction within the cellulose and hemicelluloses molecules (Khan et al., 2006).

The combination of pretreatment technologies is meant to decrease the severity of the process and to avoid excessive sugar degradation and the formation of toxic byproducts during the saccharification of lignocelluloses.

The main goal of this study was to evaluate the effect of three kinds of pretreatment on the enzymatic hydrolysis (EH) of cellulose and hemicellulose of sugarcane bagasse (SCB) using Celluclast 1.5 L and beta-glycosidase: electron beam processing (EBP) with absorbed doses from 10 to 150 kGy, EBP followed by hydrothermal treatment (EBP-TH) for 40 and 60 min at 180 °C, and EBP followed by TH with diluted acid (0.1% sulfuric acid) (EBP-AH).

2. Experimental

Sugarcane bagasse samples were obtained from a sugar and ethanol factory in Piracicaba, SP. The SCB used for enzymatic hydrolysis was natural (about 50% moisture content), and for dosimetry and sugar free evaluation, the sample was dried in a convection oven at 105 °C for 12 h, 24 h, 36 h, and 48 h.

2.1. Radiation processing

The electron beam processing of SCB was carried out with 1.5 MeV of electrons energy, provided by the Electron Beam Facility (Dynamitron type from Radiation Dynamics Inc., USA). The irradiation parameters were 112 cm (94.1%) scan and 6.72 m/min conveyor stream velocity. According to the density the width of the SCB samples for irradiation was calculated. The samples were placed in a Pyrex tray, the width varied from 3.0 cm (50% moisture) to 5.2 cm (5% moisture), and it was irradiated about 300 g each absorbed dose. The applied doses were in the range of 10–100 kGy.

2.2. Dosimeter system

Dosimetric control was carried out using two commercial dosimeter films, cellulose triacetate and, CTA FTR125®, previously calibrated with an alanine primary dosimeter. Ten calibrated dosimeters of each type were distributed on the top and on the bottom of the SCB, placed on the corners and at the center of the Pyrex. The coefficient of variation of the absorbed doses was obtained considering the average of these results.

2.3. Thermal and diluted acid hydrolysis

The system for thermal and diluted acid treatment of the irradiated sugarcane bagasse has been described elsewhere (Duarte et al., 2010). In the present study, 4.0 g of irradiated and dried sugarcane bagasse was mixed with 50 mL of distilled water and the system was heated at 180 °C for 40 or 60 min (TH). In the second step, the thermal process was conducted under the same operational conditions as the hydrothermal treatment, but sulfuric acid was added at a concentration of 0.1% (m/m) (35 mg/g of dry mass); this step was called acid hydrolysis (AH).

2.4. Chemical analysis

The hot extraction and total solubility rate were determined using 2.0 g (initial weight, *Wi*) of dried sample added to 100 mL of distilled water, the mixture was refluxed for 3 h at 100 °C. The sample was filtered and the solid residue was recovered, dried at 60 °C for 24 h, and then weighed (final weight, *Wf*). The solubility rate, SR, was calculated as the ratio of the weight of the residue to the initial weight of the untreated bagasse by Eq. (1).

$$SR = \frac{Wi - Wf}{Wi} \quad (1)$$

The filtered samples were analyzed for the determination of sugars and byproducts, as described elsewhere (Duarte et al., 2010).

The compositional analysis of the SCB was carried out for the determination of mass balance for hydrolysis assays, and were performed according to National Renewable Energy Laboratory procedures (NREL, 2010), which are based on the analysis of monosaccharide by liquid chromatography after acid hydrolysis in an autoclave (121 °C for 60 min). Using these results, the proportion of cellulose and hemicellulose were calculated.

2.5. Enzymatic hydrolysis

The enzymatic hydrolyzes of SCB samples were performed using 100 g of total mass in the reactor with 8% solids (dry basis) and a commercial *Trichoderma reesei* cellulose preparation (Celluclast 1.5 L), kindly supplied by Novozymes (Bagsvaerd, Denmark), with 5 FPU/g of cellulose and beta-glycosidase 0.5% (p/p), 0.08 mL EDTA as a surfactant, and 11 mL of sodium citrate (pH 4.5). The incubation conditions were 50 °C for 48 h at 175 rpm.

The SCB samples submitted for enzymatic hydrolysis were those pretreated only by irradiation (EBP) at absorbed doses of 5 kGy, 10 kGy, 20 kGy, 30 kGy, 50 kGy, 70 kGy, and 150 kGy, and also those samples pretreated by EBP (20 kGy, 30 kGy, and 50 kGy) followed by thermal hydrolysis (TH) and diluted acid hydrolysis (AH) for 40 and 60 min. The maximum time of enzymatic hydrolysis was 48 h; sample aliquots were collected every 24 h and were submitted to sugar analysis.

Conversion yields on the basis of glucose release were calculated according to Eq. 2.

$$\text{Yield} = \frac{\text{Glucose Final Concentration} - \text{Glucose Initial Concentration}}{CF(M_{\text{bagasse}}/V)(M_{\text{glucose}}/M_{\text{cellulose}})} \quad (2)$$

where *CF* is the fraction of cellulose in bagasse (0.420); M_{bagasse} is the mass of bagasse; *V* is the volume of the reaction; M_{glucose} is the molar mass of glucose (180 g mol^{-1}) and $M_{\text{cellulose}}$ is the molar mass of cellulose (162 g mol^{-1}).

Hemicellulose conversion was calculated by taking into account the mass of xylose, arabinose, furfural, and acetic acid, and using the conversion factors (Rodrigues et al., 2010) of hemicelluloses (0.88 × xylose mass, 0.88 × arabinose mass, 0.72 × acid acetic mass and 1.37 × furfural mass).

3. Results and discussion

In Table 1 are presented the average and standard deviation of the measurements carried out for dosimetric control purpose on applied doses to natural SCB (50% moisture content) and also to those samples dried for different periods of time. The delivered average of absorbed doses was calculated on the top and on the bottom of the samples. The doses received on the top were a little higher than those on the bottom; nevertheless these results were a guarantee that the energy of the electron was homogeneously absorbed by the mass of SCB. The coefficient of variation for CTA film on natural SCB presented a variation from 9.3% for 10 kGy to 12.5% for 100 kGy. For natural SCB, the accuracy was 94.8% and for dried samples this was 92.08%.

3.1. Compositional analysis of sugarcane bagasse

The compositional analysis of untreated SCB and after EBP at different absorbed doses is presented in Fig. 1. The average value of untreated SCB was 41.9% cellulose, 31.3% hemicelluloses, 19.5% lignin, 6.3% soluble, and 1.0% ash. The structural and compositional changes in sugarcane bagasse with absorbed doses from 5 to 150 kGy have been demonstrated elsewhere (Ribeiro et al., 2010). The increase in the soluble portion is related to hemicellulose cleavage, forming water-soluble cello-oligosaccharides from xylanases.

The increase in solubility was proportional to the radiation dose and hydrolysis time, otherwise, radiation processing was more important when the samples were treated with thermal rather than acid hydrolysis. The main byproducts were acetic acid, furfural, and formic acid, formed by the degradation of hemicelluloses.

Considering only EBP, there was a conversion of 0.5% of the cellulose into glucose at 30 kGy. On the other hand, the SCB dried for 12 h, 24 h, 36 h, and 48 h presented conversions of 0.13%, 0.12%, 0.12%, and 0.10%, respectively. These results confirm the importance of the moisture content on the formation of OH radicals in addition to the radiation effect.

3.2. Enzymatic conversion of cellulose and hemicellulose

In Fig. 2 is shown the average of triplicate results of the enzymatic hydrolysis of SCB samples treated only by EBP in different absorbed doses. With 20 kGy, the conversion yield of cellulose to glucose increased from 8% to 12%, after 24 h of incubation and to 15% after 48 h.

In Table 2 is shown the results of enzymatic hydrolysis yield (24 and 48 h of incubation) of SCB after electron beam and thermal treatment. After TH for 40 and 60 min, the glucose conversion was 4% and 6%, respectively; irradiation increased this value by about 1%. However, after 48 h of EH, the conversion of SCB irradiated with 50 kGy reached 71.55% (TH for 60 min). When dilute sulfuric acid was added, an increase in cellulose conversion was observed; however, the time necessary for this process was reduced. The highest value (74.72%) was reached after 40 min of AH and 24 h of EH.

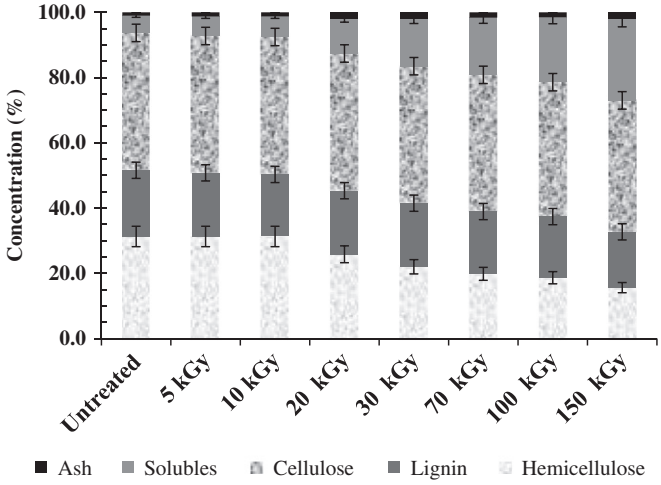

Fig. 1. Compositional analysis of SCB after EBP in different absorbed doses.

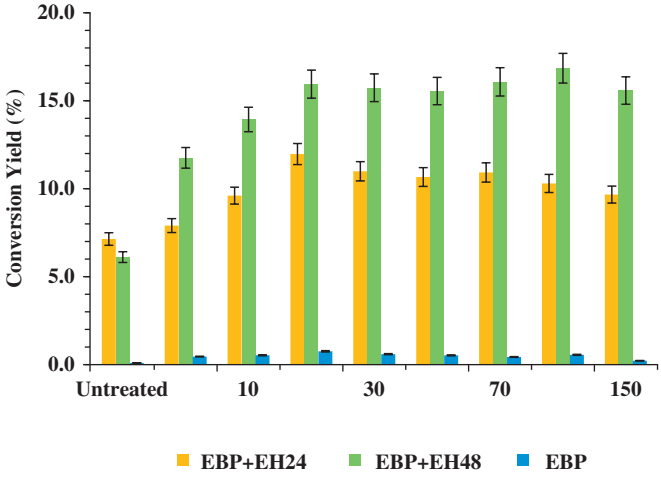

Fig. 2. Enzymatic conversion after 24 h and 48 h for SCB irradiated in different absorbed doses.

Table 1
Absorbed doses measured with CTA dosimeters in SCB samples for different moisture content and nominal absorbed doses.

Sugarcane bagasse samples	Nominal absorbed dose (kGy)	Moisture content (%)	Density (g/cm^3)	Measured absorbed dose (kGy) (Bottom)	Measured absorbed dose (kGy) (Top)	Measured absorbed dose (kGy) (Total)	Accuracy (%)
Natural	10	49.6	0.150	10.36 ± 0.98	11.60 ± 0.64	10.98 ± 1.02	90.20
Natural	30	49.6	0.150	29.22 ± 0.37	31.05 ± 1.40	30.13 ± 2.38	99.57
Natural	50	49.6	0.150	47.94 ± 1.93	50.98 ± 0.78	49.46 ± 2.12	98.92
Natural	70	49.6	0.150	67.70 ± 1.10	71.72 ± 1.66	69.71 ± 7.50	99.59
Natural	100	49.6	0.150	86.34 ± 16.94	107.10 ± 1.93	96.72 ± 15.78	96.72
Dried 12 h	30	23.3	0.096	27.05 ± 3.44	27.38 ± 0.88	27.21 ± 2.38	90.37
Dried 24 h	30	9.5	0.081	30.18 ± 2.86	25.76 ± 0.82	27.97 ± 3.06	93.24
Dried 36 h	30	8.6	0.070	26.94 ± 0.25	27.62 ± 1.30	27.28 ± 0.95	90.94
Dried 48 h	30	5.0	0.060	30.12 ± 5.19	28.38 ± 1.14	29.25 ± 3.66	97.50

Table 2
Enzymatic conversion yield (%) by 24 h and 48 h of cellulose to glucose for SCB untreated and EBP with 20 kGy, 30 kGy and 50 kGy followed by thermal (TH) and acid (AH) treatment for 40 and 60 min.

Absorbed dose (kGy)	Thermal treatment					
	Initial time		EH 24 h		EH 48 h	
	40 min	60 min	40 min	60 min	40 min	60 min
Unirradiated	4.00	6.00	47.60	42.75	45.87	54.71
20	4.47	6.33	47.10	43.18	52.71	59.53
30	5.47	7.39	47.89	45.60	53.94	70.44
50	4.47	7.33	44.86	46.99	62.27	71.55
	Diluted acid treatment					
	Initial time		EH 24 h		EH 48 h	
	40 min	60 min	40 min	60 min	40 min	60 min
Unirradiated	7.81	11.45	49.83	43.31	37.36	37.42
20	12.00	17.47	58.84	55.04	58.03	61.06
30	14.83	16.77	58.15	50.05	62.12	56.41
50	13.08	13.48	60.69	55.45	74.72	71.89

Table 3
Enzymatic conversion yield (%) by 24 h and 48 h of hemicellulose to glucose for SCB untreated and EBP with 20 kGy, 30 kGy and 50 kGy followed by thermal (TH) and acid (AH) treatment for 40 and 60 min.

Absorbed dose (kGy)	Thermal treatment					
	Initial time		EH 24 h		EH 48 h	
	40 min	60 min	40 min	60 min	40 min	60 min
Unirradiated	12.40	18.20	15.20	19.20	12.40	20.20
20	38.18	36.30	38.18	36.10	39.50	38.10
30	35.40	42.56	40.00	40.20	45.20	43.60
50	42.00	56.20	41.00	57.20	44.50	56.20
	Diluted acid treatment					
	Initial time		EH 24 h		EH 48 h	
	40 min	60 min	40 min	60 min	40 min	60 min
Unirradiated	88.00	88.90	86.00	97.00	89.60	98.00
20	92.00	99.00	93.00	95.00	93.40	95.00
30	86.94	99.00	88.00	96.00	88.00	96.00
50	86.90	99.00	89.00	98.00	89.00	97.00

These results can be considered promising when compared with others reported in Ref. Silva et al. (2010) obtained 69.2% cellulose conversion, when SCB was treated at 195 °C for 10 min, following 72 h of EH, and 89.2% after delignification of these samples with 1.0% NaOH at 100 °C for 1 h.

The hemicellulose conversion results are shown in Table 3. EBP (50 kGy) combined with TH (60 min) converted approximately 60% of the hemicellulose to xylose, and almost the totality was hydrolyzed after diluted acid treatment. As expected EH showed no effect on these results, since these enzymes do not act on hemicelluloses, but rather only on cellulose.

These results are comparable to some studies of xylose yield in the literature, but under more severe conditions. Boussarsar et al. (2009) obtained 55% hemicellulose to xylose conversion, after 4 h at 170 °C, but after 2 h, the yield was 48.8%. The hightest value of xylose yield obtained by Rodrigues et al. (2010) was 73.4% at 130 °C for 20 min with 100 mg acid/g of dry bagasse.

4. Conclusion

This study has shown that the combination of pretreatment technologies can enhance the enzymatic hydrolysis of cellulose in SCB, and EBP may be promising, depending on these combinations.

The addition of diluted acid improved the thermal treatment, allowing for a shorter treatment time. EBP with 50 kGy increased the yield of the EH of cellulose by 20% after TH, reaching 71.55% and 30% after AH, reaching 74.72%.

Hemicelluloses were totally hydrolyzed after EBP and AH for 60 min and EH did not change these results.

Although the water solubilization of SCB was proportional to the severity of pretreatment, enzymatic hydrolysis showed different behavior due to the formation of byproducts, mainly furfural.

Acknowledgments

The authors gratefully acknowledge the Research Foundation of São Paulo State, FAPESP, (BIOEN project 08/056066-1), the International Atomic Energy Agency (research contract 4709) for the financial support, the Sugarcane Technology Center for the sugarcane bagasse samples, and Novozymes for furnishing the enzymes.

References

Boussarsar, H., Rogé, B., Mathlouthi, M., 2009. Optimization of sugarcane bagasse conversion by hydrothermal treatment for the recovery of xylose. Biores. Technol. 100, 6537–6542.

Duarte, C.L., Oikawa, H., Mori, M.N., Sampa, M.H.O., 2007. Decontamination of pesticide packaging using ionising radiation. Radiat. Phys. Chem. 76 (11–12), 1885–1889.

Duarte, C.L., Ribeiro, M.A., Mori, M.N., Oikawa, H., 2010. Study of thermal treatment combined with radiation on the decomposition of polysaccharides in sugarcane bagasse In: Proceedings of the International Symposium on Radiation Application of Polymers, IRAP2010, Maryland, USA, October 12–17.

Haji-Saeid, M., Sampa, M.H.O., Chimielewski, A.G., 2007. Radiation treatment for sterilization of packaging materials. Radiat. Phys. Chem. 76, 1535–1541.

Hassuani, S.J., 2005. Biomassa Power Generation: Sugarcane Bagasse and Trash. In: Hassuani, I.C., Leal, M.L.V., Macedo, I.C. (Eds.), (PNUD-CTC, Piracicaba), pp. 200–217.

Khan, F., Ahmada, S.R., Kronfli, E., 2006. γ Radiation induced changes in the physical and chemical properties of lignocellulose. Biomacromolecules 7, 2303–2309.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., Ladisch, M., 2005. Features of promising technologies for pretreatment of lignocelluloses biomass. Biores. Technol. 96, 673–686.

National Renewable Laboratory, 2010. Analytical Procedures for Standard Biomass Analysis. Available electronically 〈http://www.nrel.gov/biomass/analytical procedures〉.

Ribeiro, M.A., Mori, M.N., Oikawa, H. Duarte, C.L., 2010. Degradation mechanism of polysaccharides in irradiated sugarcane bagasse sugarcane bagasse In: Proceedings of the International Symposium on Radiation Application of Polymers, IRAP2010—Maryland, USA, October 12–17.

Rodrigues, R.C.I.B., Rocha, G.J.M., Rodrigues Jr, D., Filho, H.J.I., Felipe, M.G.A., Pessoa Jr, A.P., 2010. Scale-up diluted sulfuric acid hydrolysis for producing sugarcane bagasse hemicellulosic hydrolysate (SBHH). J. Chem. Technol. Biotechnol. 101, 1247–1253.

Sanchez, O.J., Cardona, C.A., 2008. Trends in biotechnological production of fuel ethanol from different feedstocks. Biores. Technol. 99, 5270–5295.

Santos, V.T.O., Esteves, P.J., Milagres, A.M.F., Carvalho, W., 2010. Characterization of commercial cellulases and their use in the saccharification of a sugarcane bagasse sample pretreated with dilute sulfuric acid. J. Ind. Microbiol. Biotechnol. 17 October 2010..

Silva, V.F.N., Arruda, P.V., Felipe, M.G.A., Gonçalves, A.R., Rocha, G.J.M., 2010. Fermentation of cellulosic hydrolysates obtained by enzymatic saccharification of sugarcane bagasse pretreated by hydrothermal processing. J. Ind. Microbiol. Biotechnol. 17 October 2010..

Yu, Y., Lou, X., Wu, H., 2008. Some recent advances in hydrolysis of biomass in hot-compressed water and its comparisons with other hydrolysis methods. Energy Fuels 22, 46–60.