CHEMICAL ENGINEERING TRANSACTIONS
VOL. 37, 2014
A publication of AIDIC
The Italian Association of Chemical Engineering
www.aidic.it/cet
Guest Editors: Eliseo Ranzi, Katharina Kohse- Höinghaus

ISBN 978-88-95608-28-0; **ISSN** 2283-9216
DOI: 10.3303/CET1437132

Hydrothermal Pretreatment for Enhancing Enzymatic Hydrolysis of Seeds of Açaí (*Euterpe oleracea*) and Sugar Recovery

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The açaí seeds are an abundant byproduct of the chain of açaí and represent a significant source of lignocellulosic material for bioethanol production in the northern region of Brazil. In the present paper seeds of açaí were hydrothermally treated to overcome biomass recalcitrance for enzymatic hydrolysis. The effect of the hydrothermal pretreatment carried out with high solid loadings in the rates of sugars released was evaluated by subsequent enzymatic hydrolysis. The pretreatment used acid concentrations ranging from (0.5-1.5 %), solids loadings from 5 to 15 % (w/v) and time from 30 to 90 min at 120 °C under a pressure of 1.7 MPa. The enzymatic hydrolysis was carried out at a solid loading of 10 % DM. Hydrolysis was performed at 52 °C and pH 5.0 with an enzyme loading of 30 FPU of celullase and 50 CBU of β-glycosidase for 72 h. The results showed that hydrothermal treatment can remove significantly hemicelluloses from seeds of açaí (80 %) and improve of yield monosaccharides released by hydrolysis enzymatic. Pre-treatment with 1.5 % sulphuric acid and 5 % solid content for 90 min at 120 °C was found to be the best condition for pre-treatment and conversion.

1. Introduction

The oil crisis associated with the global trends of reducing emissions of greenhouse gases have been the main factor encouraging the development of new technologies in the fuel, based on renewable feedstocks. This need for eco-efficient processes has motivated extensive researches aiming to convert industrial and agricultural wastes into valued products (Christos et al., 2013).
Among the main products that can be obtained from the lignocellulosic material is the second generation ethanol (Sánchez and Cardona, 2007). The bioethanol or lignocellulosic ethanol or ethanol second generation is obtained by hydrolysis of polysaccharides present in the cell wall of biomass (Sims et al. 2010).
For the production of second generation ethanol is essential to apply pretreatments in material to increase the accessibility of the enzyme and consequent increase ethanol yield (Mosier et al., 2005). One of the pretreatments that presented good results for the effective increase of the enzymatic hydrolysis is hydrothermal treatment, which according to the latest research has shown better results when combined with addition of different concentrations of acids (Christos et al., 2013). Initially was used for the direct conversion of polysaccharides into their monomers, which occurs in drastic conditions called thermal liquefaction (Negro et al., 2003). For this purpose temperatures ranging between 280-370 °C are used and pressures of 10 and 25 MPa which require special material and high energy consume (Negro et al., 2003). This is the base of the hydrothermal pretreatment and this process when catalysed with acid needs less drastic operational conditions with a good performance (Hendriks and Zeeman, 2009).
Hydrothermal pretreatment has been studied as part of the biochemical conversion of lignocellulose into ethanol for a variety of feedstocks, including agricultural wastes, such as wheat straw, (Thompsem et al, 2006) rice straw (Chandra et al., 2012), corn stover (Saha et al., 2013) shorghum baggase, (Heredia-Olea et al., 2012) and sugarcane baggase (Boussarsar et al., 2009) and others. The acid hydrothermal acid pretreatment promotes the hemicellulose removal and this fact is one of the strands evaluated to improve the attack of the lignocellulosic material by hydrolytic enzymes (Christos et al., 2013). The presence of this component leads to an inefficient adsorption of the cellulase which promotes a reduction in the rate of

Please cite this article as: Oliveira J., Komesu A., Maciel Filho R., 2014, Hydrothermal pretreatment for enhancing enzymatic hydrolysis of seeds of açaí (euterpe oleracea) and sugar recovery, Chemical Engineering Transactions, 37, 787-792
DOI: 10.3303/CET1437132

bioconversion of cellulose to monomers glucose. The hydrothermal pre-treatment with hot water has been shown to be more effective when associate with mineral acids, for example sulfuric acid (Hendriks and Zeeman, 2009).
In this process hemicellulose can be converted to weak acids, furans and phenolic derivatives (Saha et al., 2013). Such compounds can inhibit the subsequent fermentation (Saha et al., 2013).
In recent years have been increasing the use of different biomass for the production of second generation ethanol, which requires adaptation or development of processes to achieve the desired performance (Christos et al., 2013). This is the case of açaí seeds. Açaí seeds are considered municipal waste and a huge inconvenience to the welfare and hygiene of cities in northern Brazil as Belém. The possibility of converting the material released by the processing for obtaining the açaí pulp into ethanol would be interesting from the point of view trade and environment. According to the literature this material contain large amounts of cellulose and hemicellulose (45%), which justifies its use for the production of second generation ethanol (Kim et al., 2005).Some studies have been conducted on the use of lump açaí from burning (Padilha et al, 2005). However this burning process is characterized as a low value and relatively low efficiency process so that there is an incentive to use such biomass as possible source for biofuels.
The aim of this paper was to study through an experimental design and response surface technique, the effect of hydrothermal treatment with Hot Water (LHW) and sulfuric acid at high concentrations of solids in chemical composition of resulting material e their effects for subsequent enzymatic hydrolysis.

2. Material and methods

2.1 Raw material

The açaí seeds were obtained in Belém - Pará - Brazil (01º 27' 21" S, 48º 30' 16" W). The material was dried for 48h at 50°C in an air circulating oven and left for 48 h at room temperature. The material was ground in a Wiley mill brand (Willye model TE-650 - Tecnal - Brazil) and sieved. The material used was the one who passed on 9 mesh sieve (2.0 mm) and was retained in the sieve of 35 mesh (0.43 mm). The material was stored in plastic bags at room temperature until use for pre-treatment. The biomass composition was analysed before pre-treatment.

2.2 Analysis method

2.2.1. Chemical Composition

Seeds of açaí untreated were milled to pass through a 0.75-mm screen. Approximately 8 g of milled sample was extracted by the method TAPPI T 264 cm-97 (TAPPI, 1997). Ash content was determined after burning of the samples in a muffle at 550 °C for 4 h (Ferraz et al., 2000). The untreated and pretreated seeds of açaí were analyzed for carbohydrate and lignin (acid-soluble and insoluble) according to the method presented by Sluiter et al., (2008) where Extracted samples were treated with 3 mL of 72% (w/w) H2SO4, stirring for 1h at 30°C. The reaction was interrupted by adding 84 mL of distilled water, transferred to a 250 mL Erlenmeyer flask and then heated at 121°C/1 atm for 1 h. The residual material was cooled and filtered through porous glass filter number 3. The solids were dried to constant weight at 105 °C and determined as insoluble lignin. The soluble lignin concentration in the filtrate was determined by measuring absorbance at 205 nm and using the value of 105 l g−1 cm−1 as the absorptivity of soluble lignin. The concentrations of monomeric sugars in the soluble fraction were determined by high performance liquid chromatography (HPLC) according item 2.2.4.

2.2.2. Hydrothermal treatment of açaí seeds

The quantity of raw material established in set experimental (5, 10 and 15% masse/volume of solution) were weighed in flasks of 250 mL which were added of a solution of H_2SO_4 with the concentrations (0.5, 1 and 1.5 % w/v) proposed. The flasks were placed in an autoclave at 121 °C (heating rate 15 and 20 °C min^{-1}). When the temperature of the autoclave reached the temperature study the treatment time count started (30, 60 and 90 min). The flasks were cooled in a water bath at 20 °C. The liquid phase was separated from the solid phase through filter paper. The solid phase was washed until neutral pH and dried for 24 h at 25 °C. Solid phase was analyzed for yields of carbohydrates and liquid phase to inhibitors both by HPLC. Pretreatment experiment was performed in triplicate. The severity factor (SF) was evaluated by following equation (1) (Garrote et al., 1999). Where t is the reaction time in min and T is the temperature of pretreatment in ºC. R or R_o is commonly used to represent SF. In practice, $\log_{10}R$ or $\log_{10}R_0$ is often used instead of R or R_0.

$$SF = \log [\mathrm{R}] = \log_{10}\left(\mathrm{t}\ \mathrm{x}\ \exp\left[\frac{T-100}{14.75}\right]\right) \quad (1)$$

2.2.3. Enzymatic hydrolysis

The cellulase (Sigma) activity was determined by the method described by Adney et al., (1996) and Ghose (1987) and the activity of β-glucosidase (Sigma) was determined according to the methodology of Wood

and Bhat (1988). Enzymatic hydrolysis of dried material was performed in Erlenmeyer flasks (250 mL) with 10 % (w/v) raw or pretreated material with sodium citrate buffer 0.05 M (pH 4.8) and 0.7 % of sodium azide/ g of dry material. The enzymatic loading used was 15 FPU/g dry material for Celluclast 1.5 (Sigma-Aldrich) and 25 CBU/mL material for β-glucosidase (Sigma-Aldrich) at 50 ºC, 150 rpm and 72 h. The enzymatic activity were 55 FPU/mL and 298 CBU/mL for celullase and β-glucosidase respectively.

2.2.4. HPLC analysis

The released sugar monomers in the hydrolysate were determined by HPLC (Agilent) using a column (BioRad Aminex HPX-87H, 300x7.8 mm) at 35 °C and 4mM H2SO4 as eluent at a flow rate of 0.6 mL min-1 injected sample volume 25 µm through of the detector RI (refractive index). The concentration of furfural and HMF were also measured following conditions: column Hewlett-Packard RP 18 (200mm), Column temperature: 27°C UV detector SPD- 10A UV-VIS; eluent solution acetronitrila/water (1:8) with 1 % acetic acid, injected sample volume of 20µm.

2.2.5. Experimental design

A 2^3 central composite design was employed to reduce the total number of experiments needed to determine the best combination of parameters for optimization of the process of hydrothermal treatment. The software Statistica 7.0 was used for the central composite design and to analyze the experimental data obtained. The conditions for each experiment are shown in Table 1.

Table 1. Coded factor levels and real values for independent variables

	Levels				
Factors	-α	-1	0	+1	+α
Acid concentration (% W/V)	0.2	0.5	1	1,5	1.84
Treatment time (min)	9.5	30	60	90	111
Solid content (%)	1.6	5	10	15	18

3. Results and discussion

The seeds of açaí used in this study mainly contained glucan (44.14 %), xylan (18.70 %), and lignin (18.37 %) values that approximate a total of 60 % (Table 2) which would justify the use of this biomass to ethanol production (Kim et al., 2005), since associated to methods that optimize enzymatic attack such as the removal of hemicellulose and lignin and modification of cellulose structure (Rabelo et al., 2011), could lead to suitable process. The lignin content of seeds of açaí biomass is in the range of that reported for other agricultural residues such as corn stover (17–19 %) (Kim et al., 2005). Ash and extractives were present in concentrations higher (1.26 % and 8.15 %) than that reported to others biomass like sugarcane bagasse (Gomez et al., 2010) that were 1.79 % and 3.25 % respectively. Açaí seeds also contains a relatively low amount of protein (4.20 %) when was compared to soybean straw (Cassales, 2010).

Table 2. Chemical composition of açaí seeds

Chemical composition of açaí seeds	
Compounds	**(%)***
Ash	1.26 ±0.1
Extractives	8.15 ± 0.2
Glucan	38.14 ± 1.3
Xylan	24.70 ± 0.8
Acetyl groups	0.9 ± 0.8
Protein	4.20± 1.1
total Lignin	18.37 ± 0.3

After the pre-treatment two fractions (liquid and solid) were obtained and both were characterized. But more important in this study were the changes occurred in the solid phase resulting from the hydrothermal pre-treatment acid. In order to evaluate the efficiency of the pretreatment process the solids recovery in the solid phase was determined as percentage of the dry weight of solid remaining after pretreatment, relative to the dry weight of the raw material.

The recoveries achieved are shown in Table 3 and ranged from 56.87 % to 93.87 %, depending on the pretreatment conditions. The lowest recovery was obtained at 1 % acid concentration and 1.6 % solid loading for 60 min which was probably to decomposition of the resulting cellulose.

The SF of the pretreatment for 9.5, 30, 60, 90 and 111 min were 1.56, 2.0, 2.3, 2.5 and 2.6, respectively and are considered low when are compared with the intervals studied by Saha et al. (2013) which obtained values of Furfural+HMF (3.0 to 18 mg/g approximately) with SF of 3.6 to 4.8. The values obtained for the total concentration of furfural and HMF in this work ranged of 0.22 g/L to 1.182 g/L (not shown in table 1) which was lower than the concentration considered to inhibit fermentation (Nichols et al., 2008).

Considering a high cellulose content in solid phase the optimal pretreatment condition was selected as 1.5 % of H_2SO_4 at 5 % solid content for 30 min.

Table 3. Results of central composite

Run	Solid recovery g/100 g untreated material	Central composite			Solid phase		Conversion (%)
		Acid concentration (% W/V)	Treatment time (min)	Solid content (%)	Xylan (%)	Cellulose (%)	
1	86.56	0.5	30	5	17.03	40.25	55.42
2	92.28	0.5	30	15	21.33	34.88	52.27
3	82.84	0.5	90	5	16.06	42.52	61.20
4	89.65	0.5	90	15	17.91	41.64	56.11
5	57.45	1.5	30	5	9.04	54.83	83.80
6	66.22	1.5	30	15	14.03	53.56	79.72
7	53.12	1.5	90	5	6.81	29.86	90.28
8	58.32	1.5	90	15	13.94	54.32	82.78
9	93.87	0.2	60	10	21.78	40.86	43.72
10	60.23	1.84	60	10	7.76	50.52	88.04
11	88.75	1	9.5	10	17.81	38.56	46.24
12	56.87	1	111	10	9.14	53.32	81.45
13	40.98	1	60	1.6	7.59	49.68	86.5
14	71.76	1	60	18	12.95	43.52	70.59
15	78.97	1	60	10	13.66	54.04	78.23
16	79.01	1	60	10	13.75	53.78	77.90
17	78.87	1	60	10	14.08	53.75	78.52

According to the experimental design an increase in the acid concentration of 0.5 % to 1.5 % under the same conditions of 90 min and 15 % solids led to an increase in the rates of cellulose 41.64 % to 54.32 % and a reduction in rates of xylan from 17.91 to 13.94 %. This behavior can be better observed by response surface obtained (Figure 1).The process performance, regarding the liquid stream was analyzed for monomeric and total sugars, degradation products, and solubilized lignin, as described in the item 2.2.4. Hemicellulose was the main biomass component removed by the hydrothermal treatment, monomeric xylose and xylose oligomers were the main sugars detected in liquid phase resulting (not shown in Table 3) and reductions can be observed in the levels of xylan in the solid phase. Rates of xylose recovered in the liquid phase reached maximum concentrations of 15.54 g/L (not shown in Table 3). The yields of acetic acid concentration ranging 0.46 to 3.07 g/L (not shown in Table 3).

In Figure 1 is possible to observe that increases in temperature and time provide increases in yields of cellulose. However severe conditions of the same parameters caused reductions on levels of cellulose recovered in the solid phase. In relation to results obtained for conversion after enzymatic hydrolysis is possible to identify the condition of 0.2 % sulfuric acid and 10 % of solids at 60 min as the one that best favored enzymatic hydrolysis with an overall yield of 84.72 %.

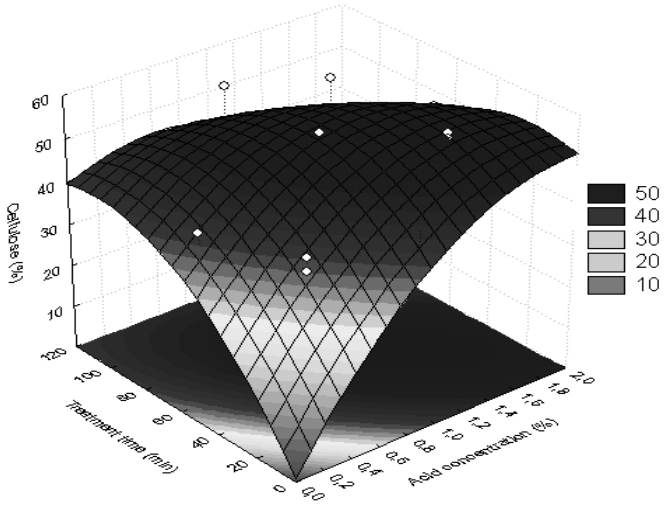

Figure 1. Response surface for cellulose content in relation to acid concentration and treatment time

4. Conclusion

Açaí seeds biomass can be an attractive feedstock for ethanol production due to its high carbohydrate content (above 60 %). This study showed that hemicelullose in açaí biomass can be efficiently removed by hydrothermal pretreatment acid (H_2SO_4) with a high concentration of solid (20 %). The best pretreatment condition that allowed the best conversion after enzymatic hydrolysis was: 1.5 % of sulfuric acid and 15 % of solids at 90 min which allowed a conversion of 90.28 %.

References

Adney B, Baker J. 1996. Measurement of cellulase activities. NREL analytical procedure. National Renewable Energy Laboratory: Golden, CO, USA.

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

Cardona C.A. and O. J. Sanchez., 2007, Fuel ethanol production: Process design trends and integration opportunities, Bioresource Technology, 12, 2415-2457.

Cassales A.R., 2010, Optimization of hydrolysis of soybean hulls (Glycine max) and assessing the ability of xylitol and ethanol production by microorganisms on this hydrolyzate, MSc Dissertation, Rio Grande do Sul, Brazil.

Chandra R., Takeuchi H., Hasegawa T., 2012, Hydrothermal pretreatment of rice straw biomass: A potential and promising method for enhanced methane production, Applied Energy, 94, 129–140.

Christos K. N., Konstantinos A. M., Kostas S. T., 2013, Optimization of Hydrothermal Pretreatment of Lignocellulosic Biomass in the Bioethanol Production Process, ChemSusChem, 6, 110 – 122.

Ferraz A., Baeza J., Rodriguez J., Freer J., 2000, Estimating the chemical composition of biodegraded pine and eucalyptus wood by DRIFT spectroscopy and multivariate analysis. Biores. Technol, 74, 201-212.

Garrote G., Dominguez H., Parajo J.C., 1999, Hydrothermal processing of lignocellulosic materials, Holz Als Roh-und Werkst, 57, 191-202.

Gómez S.M.R., Andrade R.R., Santander C.G., Costa A.C., MacielFilho R., 2010, Pretreatment of sugar cane bagasse with phosphoric and sulfuric dilute acid for fermentable sugars production by enzymatic hydrolysis, Chemical engineering transactions, 20, 321-326.

Ghose T.K (1987). Measurement of cellulase activities. Pure Appl. Chem. 59: 257-268.

Hendriks A., Zeeman A., 2009, Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour Technol, 100, 10–18.

Heredia-Olea E., Pérez-Carrillo E., Serna-Saldívar S.O., 2013, Production of ethanol from sweet sorghum bagasse pretreated with different chemical and physical processes and saccharified with fiber degrading enzymes, Bioresour Technol. 134, 386-390.

Kim S., Holtzapple M.T., 2005, Lime pretreatment and enzymatic hydrolysis of corn stover, Bioresour. Technol, 96, 1994–2006.

Mosier N., Wyman C., Dale B., Elander R., Lee Y.Y., Holtzapple M., Ladisch M., 2005, Features of promising technologies for pretreatment of lignocellulosic biomass, Bioresour Technol,, 96, 673–86.

Nichols N.N., Sharma L.N., Mowery R.A., Chambliss C.K., Walsum G.P.V., Dien B.S., Iten L.B., 2008, Fungal metabolism of fermentation inhibitors present in corn stover dilute acid hydrolysate, Enzyme Microb. Technol., 42, 624–630.

Padilha J.L., Rendeiro, G., Brasil, A.C.M., Santos, R.E.J., Pinheiro, G., 2005, Potential of Electric Power Generation in the State of Pará Using Lumber Biomass, Revista Biomassa e Energia. 2, 267-284.

Rabelo S. C., Amezquita Fonseca N. A., Andrade R. R., Maciel Filho R., Costa A.C., 2011, Ethanol production from enzymatic hydrolysis of sugarcane bagasse pretreated with lime and alkaline hydrogen peroxide, Biomass and Bioenergy, 35, 2600-2607.

Saha B.C., Yoshida T., Cotta M.A., Sonomoto K., 2013, Hydrothermal pretreatment and enzymatic saccharification of corn stover for efficient ethanol production, Industrial Crops and Products, 44, 367–372.

Sims R.E.H., Mabee W., Saddler J.N., Taylor M., 2010, An overview of second generation biofuel technologies, Bioresource Technology, 101, 1570–1580.

TAPPI Test Methods, 2007, Standard Methods for Pulp and Paper, Technical Association of Pulp and Paper Ind, TAPPI Press, Technology Park, Atlanta, GA-330348-5113, USA, 2007.

Thomsen M.H, Thygesen A., Jørgensen H., Larsen J., Christensen B.H., Thomsen A.B., 2006, Preliminary results on optimization of pilot scale pretreatment of wheat straw used in coproduction of bioethanol and electricity, Applied Biochemistry and Biotechnology, 130, 448-460.