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Optimization of Acid Treatment of Cashew Peduncle for Ethanol and Xylitol Production

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Due to the large amount of waste generated by industries and commitments for environment preservation, there has been a growing interest in the use of alternative energy sources for the production of bioproducts of added value such as ethanol and xylitol. In this context, cashew has been considered a promising alternative to meet the global demand in a more sustainable way. Thus, the aim of this study was to optimize the acid treatment of cashew peduncle for biotechnological production of ethanol and xylitol. Physicochemical analyses were held for bagasse characterization, and sugars were assessed by HPLC (glucose, xylose and arabinose) and fermentation inhibitors (acetic acid, furfural and hydroxymethylfurfural) in the hydrolyzed liquor. The 2^3+3 factorial experimental design was applied with a total of 11 experiments to investigate the influence of variables temperature, bagasse/acid diluted and acid concentration to evaluate the release of pentoses (xylose and arabinose) and hexose (glucose) in the hydrolyzed liquor. The liquor from the acid treatment should operate under ratio conditions temperature at level +1 (160 °C), acid concentration and bagasse:diluted acid at level -1 (1% and 1:6), obtaining pentose yields (xylose and arabinose). It was observed that acid treatment liquor is very effective in providing high-susceptibility substrates for ethanol and xylitol production.

1. Introduction

Annually, million tons of agro-industrial wastes are produced and most of them are discarded, causing an excessive accumulation of organic matter available in nature. Among these wastes, cashew deserves special attention due to its socio-economic importance in the country. It is estimated that about 80 % of cashew pulp, i.e. cashew peduncle is not used. Therefore, 1.9 Mt of this food rich in nutritional value are wasted (Alcântara et al., 2010).

Cashew tree (*Anacardium occidentale* L.) is a tropical plant originated in Brazil, scattered in almost all Brazilian territory. The Northeastern region accounts for over 95 % of the national cashew production and its processing provides approximately 250 t of nuts and 2 Mt of cashew peduncle per year. Thus, cashew crop has great potential for technological development of its industrial waste that in general are reused in a small-scale or discarded due to the lack of encouragement of its use in human nutrition (Lima et al., 2014).

Lignocellulosic materials require treatment to facilitate the separation of cellulosic from hemicellulosic constituents and lignin (Lima et al., 2012). Among these, acid treatment aims to solubilize the hemicellulose fraction from biomass and make cellulose more accessible to enzymes, since there is an increase in pore size of the substrate. Treatment with dilute acid appears to be the most favorable method for industrial applications and has been studied in a variety of lignocellulosic biomasses. The main reaction that occurs during acid treatment is the hydrolysis of hemicellulose to produce oligomers and monomers. The dehydration of monomers, in turn, produces furfural, HMF and other volatiles (Alvira et al., 2010).

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Biotechnological production can be performed using some constituents present in the cashew peduncle bagasse waste, among them the most suitable for xylitol production is hemicellulose (constituent of lignocellulosic materials rich in pentoses such as xylose and arabinose). This material is an abundant source of sugars that through biotechnological processes can be converted into products of industrial interest such as ethanol and xylitol, meeting the challenges of sustainability (Lima et al., 2012).

Xylitol is an alcohol sugar of five carbons (polyalcohol) which can be found in nature in minor amounts. This sugar has attracted the global attention due to its sweetness similar to sucrose, but provides very few calories. Xylitol is also known for being metabolized through pathways independent of insulin in the body and therefore can be used as a sugar substitute for diabetics. In addition, xylitol has anticariogenic property, which can help promote oral health and also helps in preventing dental caries (Prakasham et al., 2009).

Ethanol is a renewable fuel, is clean burning fuel produced from agricultural products, such as agro-industrial waste (cashew peduncle bagasse) (Lima et al., 2012).

Thus, the aim of this study was to optimize the acid treatment of cashew peduncle bagasse to obtain the highest sugar concentration in the liquor for biotechnological production of ethanol and xylitol.

2. Material and Methods

2.1 Raw material

The raw material used was cashew peduncle bagasse (*Anacardium occidentale* L.) and the hydrolyzate of this waste (liquor from liquid treatment). Cashew peduncle bagasse was acquired from IDEAL pulp production industry located in João Pessoa - PB.

Then, samples were processed by two washes with distilled water at temperature of 50 °C for 20 min each wash; then, samples were submitted to two additional washes with distilled water at room temperature (about 25 °C) for leaching excess sugars.

After this process, the raw material was submitted to drying in a tray drier at 55 ºC for 1 h, being then removed and inserted in a Willey-type mill, and fractions that passed through a 48-mesh sieve and those that were retained in the 60-mesh sieve were used in the analysis, being vacuum packed in polypropylene bags for further use (Lima et al., 2012).

2.2 Obtaining hydrolyzate

Treatment was obtained from dried and ground cashew peduncle bagasse. Then, the material was submitted to acid treatment process performed in stainless steel pressurized reactor (MAINTEC FORNOS INTI) with temperature controller FE50RP (time, heating, internal/external temperature automation system) and 700 mL of capacity, filtered for the separation of solid constituents of cellulose and lignin.

2.3 Experimental design of acid treatment

A full 2^3 factorial design with three replications in central point was carried out to check the influence of variables temperature (105, 130 and 160 °C), acid concentration (1, 2 and 3 %) and bagasse/dilute acid ratio (1:6, 1:9 and 1:12) to treatment with sulfuric acid, totaling 11 experiments for each treatment. The thermal hydrolysis time was recorded from the time it reached the temperature established in the experimental design. After 60 min acid hydrolysis black fluid (liquor, liquid part) and bagasse (solid part) were removed from the reactor, being separated by filtration and only liquor was collected for subsequent analysis of sugars.

2.4 Biomass characterization

For the physicochemical characterization of dry cashew peduncle bagasse, analyses of moisture, pH, fixed mineral residue (ash), proteins and soluble solid were carried out according to *Association of Official Analytical Chemists* (AOAC, 2005).The concentration of reducing sugars was determined according to methodology described by Miller (1959).

The determination of extractives and analyses of lignocellulosic materials (lignin, cellulose and hemicellulose) were performed in triplicate according to methodology described by TAPPI T17 wd-70, TAPPI T 203 cm-99 and TAPPI T222 os-74 (2011).

2.5 Determination of carbohydrates and fermentation inhibitors

Carbohydrate contents were determined by High Performance Liquid Chromatography (HPLC), VARIAN, coupled with a refractive index detector (Varian 356 - LC), Hi-Plex Ca column (8 µm 300 x 7.7 mm). To determine inhibitors, Hi-Plex H column (300 mm x 7.7 mm) was used. Samples were investigated at temperature of 60 °C, mobile phase composed of ultra pure water for determination of sugars and H_2SO_4 0.0005 mol/L, flow rate of 0.6 mL/min, injecting 20 µL of sample and analysis time of 60 min. The chromatograms of samples were compared with standards of sugars analyzed, and quantification was

performed by the compound area in a calibration curve of each compound. Total contents of sugars (xylose, glucose and arabinose) and inhibitors (acetic acid, furfural and 5-hydroxymethylfurfural) were assessed.

2.6 Statistical Analysis

The results were statistically investigated by analysis of variance and regression analysis using the Response Surface Methodological (RSM) analysis to define the best acid treatment conditions for the production of pentoses (glucose, xylose and arabinose). Nonlinear regression at 95% confidence was carried out for each response using experimental data of the factorial experimental design.

3. Results and Discussion

3.1 Physicochemical characterization of dry cashew peduncle bagasse

Table 1 shows the parameters observed in the physicochemical analyses to characterize the dry cashew peduncle bagasse, as well as its standard deviation. It was observed that the moisture content measured on a dry basis resulted in 14.73 %, which greater value with values obtained by Alcântara et al. (2007), who studied the use of dry cashew peduncle bagasse for further use in a semisolid fermentation process and reported 11.69 % of moisture on a dry basis.

However, these contents presents greater than those found by Lima et al. (2012) who studied xylitol production using liquor from the acid hydrolysis of cashew peduncle bagasse and reported 9.29 % of moisture content, which can be justified by the drying method and types of dryers used during treatment of dry biomass.

The pH value found (5.56) was higher than those found by Rocha et al. (2014a) who analyzed the protein enrichment of cashew bagasse and found values of 4.76. The pH value is a very important factor because its variation can cause enzyme inactivation in the case of xylitol enzymatically produced or inhibit the multiplication of *Candida guilliermondii* yeast if its value is below 4, which confirms that the material analyzed in this research is favorable to biotechnological ethanol and xylitol production.

The soluble solid content (SS) found in this study was 0.00; this is due to the washing treatment previously performed in the sample to remove the remaining sugars from cashew peduncle pulp, this value was also reported by Rocha et al., (2014a).

It was also observed that the ash values or fixed mineral residue was 1.35 g.100g^{-1}. This value is consistent with those reported by Lima et al. (2012), who reported 1.20 and 1.72 % ash in the dry cashew peduncle waste. The amount of crude protein found by Alcântara et al. (2007) was 11.54 ± 1.20 g.100 g^{-1}, a value close to that found in our study, which was 9.85 ± 0.10 g.100 g^{-1}.

For reducing sugars (% glucose), value of 0.11 g.100g^{-1} was found, confirming the removal of residual sugars remaining in the washed bagasse.

The values of extractives (9.51 %) were lower than those found by Rocha et al. (2014a) who found values of 15.13%.It was observed that levels found in this study were 21.45 % of cellulose, 10.96 % of hemicellulose and 35.39 % of lignin. These results are in agreement with those found by Rocha et al. (2014b), who studied treatment of cashew bagasse with dilute acid to produce ethanol, obtaining values of 20.9 ± 2.0 % of cellulose, 16.3 ± 3.0 % of hemicellulose and 33.6 ± 5.3 % of lignin.

Table 1: Mean values (± standard deviation) of physicochemical characterization of dry cashew peduncle bagasse

Physicochemical assessment	Dry waste	Standard Deviation
Moisture (g.100g^{-1}) db	14.73	0.37
Ash (g.100g^{-1}) db	1.35	0.00
pH	5.56	0.04
Total Soluble Solids (ºBrix)	0.00	0.00
Reducing Sugars (g.100g^{-1})	0.11	0.01
Proteins (g.100g^{-1})	9.85	0.10
Extractives (%) (w/*w)*	9.51	0.50
Cellulose (%)(w/*w)*	21.45	0.31
Hemicellulose (%)(w/*w)*	10.96	0.31
Lignin (%)(w/*w)*	35.39	0.97

Table 2 shows the actual and coded levels of treatments that were used in the experimental design, as well as the responses of sugars (glucose, xylose and arabinose) and inhibitors (acetic acid, furfural and 5-hydroxymethylfurfural).

Table 2: Concentrations of pentose, hexose and inhibitors in the liquor from acid treatment

Run	Independent variable (Real/coded levels)			Response					
	(T ºC)	Acid concentration (%)	B/DA ratio	G ($g.L^{-1}$)	X ($g.L^{-1}$)	A ($g.L^{-1}$)	HMF ($g.L^{-1}$)	Furfural ($g.L^{-1}$)	Acetic Acid ($g.L^{-1}$)
1	-1(105)	-1 (1)	-1(1:6)	0.06	0.31	3.33	0.00	0.00	0.44
2	1(160)	-1(1)	-1(1:6)	4.45	8.23	5.19	0.14	0.27	1.00
3	-1(105)	1(3)	-1(1:6)	0.45	1.06	5.29	0.00	0.00	0.39
4	1(160)	1(3)	-1(1:6)	4.20	7.25	3.69	0.21	0.41	0.83
5	-1(105)	-1(1)	1(1:12)	0.32	0.64	1.87	0.32	0.02	0.02
6	1(160)	-1(1)	1(1:12)	2.68	4.91	3.16	0.06	0.14	0.48
7	-1(105)	1(3)	1(1:12)	0.17	0.69	3.32	0.00	0.00	0.14
8	1(160)	1(3)	1(1:12)	3.76	6.38	3.34	0.18	0.47	0.49
9	0(130)	0(2)	0(1:9)	1.35	2.49	3.63	0.00	0.00	0.55
10	0(130)	0(2)	0(1:9)	1.86	3.48	3.30	0.01	0.03	0.14
11	0(130)	0(2)	0(1:9)	1.82	3.29	3.19	0.00	0.00	0.51

T - temperature, B/DA - bagasse:diluted acid, G - glucose; X-xylose; A - arabinose, HMF - 5-hydroxymethylfurfural.

3.2 Acid treatment study

Table 3 shows the nonlinear regression models considering statistically significant parameters at 95% confidence ($p < 0.05$), determination coefficients (R^2) and Fcal / Ftab ratio values (F test).

Table 3: Regression model for Glucose, Xylose, arabinose, 5-HMF and furfural concentrations in the liquor from acid treatment of cashew peduncle bagasse

Coded variable	Equation	Fcal/Ftab Ratio	R^2
Glucose	**1.92 + 1.76 (T)** + 0.13 (C) – 0.28 (R) – 0.072 (TxC) – 0.27 (TxR) + 0.099 (CxR)	4.50	97 %
Xylose	**3.52 + 3.01 (T)** + 0.16 (C) – 0.53 (R) – 0.037 (TxC) – 0.52 (TxR) + 0.22 (CxR)	4.30	97 %
Arabinose	**3.57** + 0.20 (T) + 0.26 (C) – **0.73 (R) - 0.59 (TxC)** + 0.13 (TxR) + 0.15 (CxR)	1.38	90 %
5-HMF	**0.09 + 0.03(T) – 0.17 (C) + 0.03 (R) + 0.06 (TxC) – 0.05 (TxR) – 0.04 (CxR)**	0.3	65 %
Furfural	**0.12 + 0.16 (T) + 0.06 (C**) – 0.01 (R) + **0.06 (TxC)** – 0.01 (TxR) + 0.02 (CxR)	0.7	82 %
Acid acetic	**0.45 + 0.23 (T) –** 0.01 **(C) – 0.19 (R) –** 0.03 **(TxC)** – 0.02 (TxR) + 0.04 (CxR)	1.0	86 %

T - temperature, C - acid concentration, R - bagasse / diluted acid ratio and 5-HMF - 5-hydroxymethylfurfural

Coefficients in bold of the regression models in Table 3 (responses) are statistically significant at 95 % confidence. According to equations shown in Table 3, it is known that the first-order models are statistically significant for the glucose, xylose, arabinose and acetic acid concentrations in the hydrolyzate from the acid treatment of cashew peduncle bagasse because they show Fcal/Ftab ratio equal to or greater than 1 (Rodrigues and Iemma, 2014).

Statistically significant models were used to built up the response surfaces to seek to optimize the acid hydrolysis process.

Seeking to optimize the process, the objective function was defined as the highest concentration of hexose (glucose) and pentoses (sum of xylose and arabinose) in the hydrolyzate liquor, with a high ratio (to obtain higher amounts of liquor for the study for expanding the ethanol and xylitol production scale).

Figure 1 shows that setting the temperature at 160 ^{o}C (level +1) of the acid treatment process, operating with the ratio of 1:6 and initial acid concentration of 1 %, high concentration of hexose and pentoses was obtained in the hydrolyzate liquor (glucose, xylose and arabinose), of approximately, regression model, 7.8 gL^{-1} and 4.9 gL^{-1} (sum of approximately 13.0 gL^{-1}) and consequently 4.2 gL^{-1} glucose concentration and inhibitor

concentrations of 0.8 gL^{-1} of acetic acid, 0.2 gL^{-1} of HMF and 0.4 gL^{-1} of furfural. These values are approximate experimental values, run 2 (Table 2).

However, the maximum values of sugars obtained were reached at temperature and acid concentration at +1 (160 °C), acid concentration and ratio at level - 1 (1 %, 1:6), concentration of pentoses (sum of approximately 17.9 gL^{-1}).

Further studies aimed at expanding the ethanol and xylitol production scale should be carried out (4 L, 16 times), seeking to optimize ethanol and xylitol production using concentrations optimized in this work.

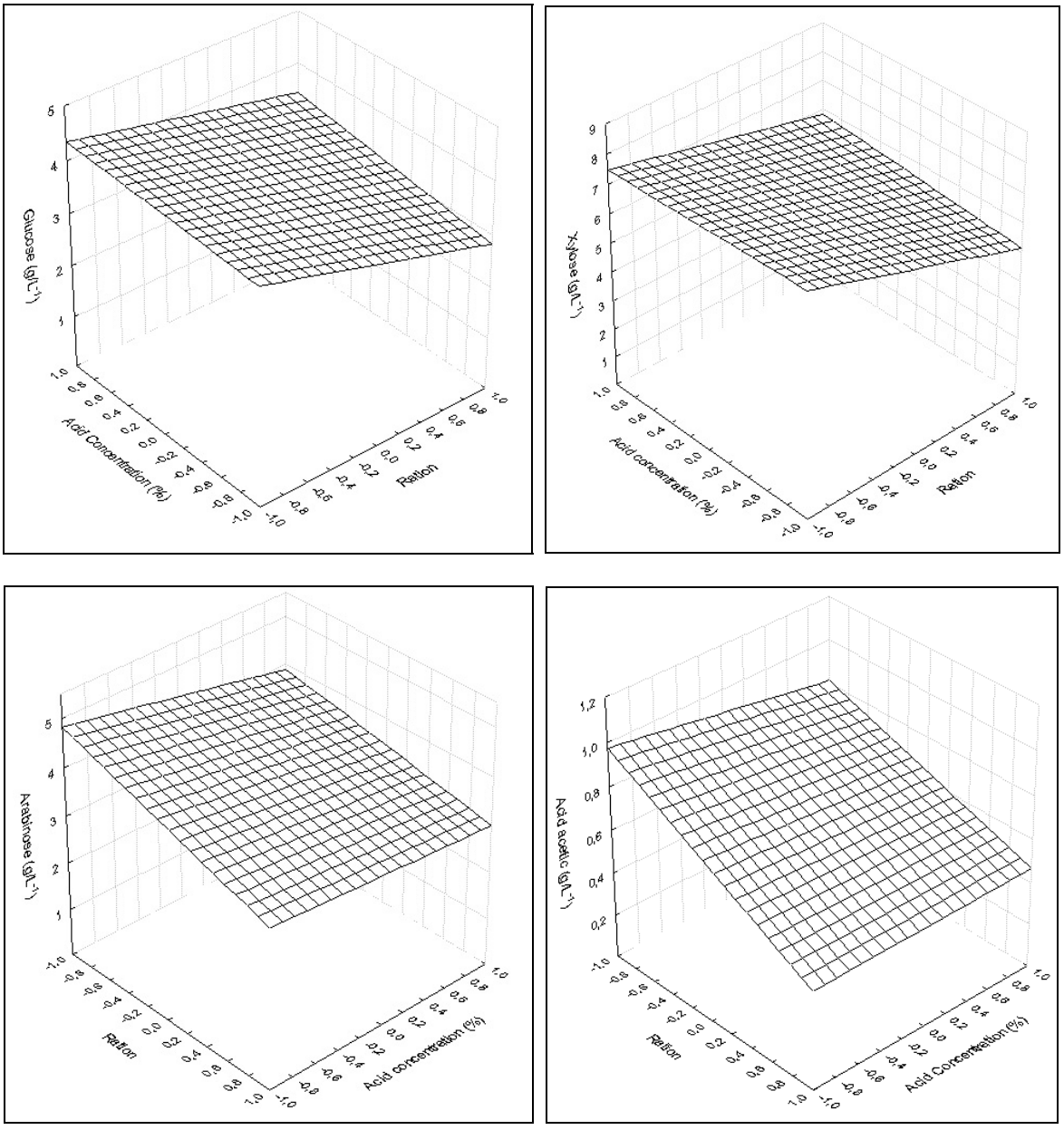

Figure 1: Response surface for the concentration (glucose, xylose, arabinose and acid acetic) fixing the temperature in the upper level +1 (160 ºC)

4. Conclusions

According to the results of this study, the variables used for obtaining liquor from the acid treatment should operate under ratio conditions temperature at level +1 (160 °C), acid concentration and bagasse: diluted acid at level -1 (1 % and 1:6), obtaining high hexose and pentose yields (glucose, xylose and arabinose).

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