EVALUATION OF TREATMENT BY VACUUM EVAPORATION AND THE LIQUOR BIOSORPTION PREHYDROLYZATE FROM BAGASSE CASHEW FOR PRODUCTION OF XYLITOL

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ABSTRACT – The cashew apple bagasse is a promising agroindustrial residue for biotechnological processes. Thus, by prehydrolysis of the hemicellulose fraction, are released both sugars compounds as inhibitors of fermentation. The aim of the present study was to evaluate the characterization liquor resulting from the acid prehydrolysis before and after the vacuum concentration and treatment with residual lignin. The bagasse was submitted acid prehydrolysis using H_2SO_4 3% (weight/weight). The prehydrolyzate was concentrated and then treated with lignin residual, for improve the efficiency of the process for subsequent fermentation (production of xylitol). The liquor showed maximum concentrations of xylose, glucose and arabinose (4.5 g/L, 2.9 g/L and 18.52 g/L) and minimum acetic acid, 5-HMF and furfural (0.02 mg/L, 0.09 mg/L and 0.06 mg/L) in the liquor treated. In the fermentation process, after 72 hours (preliminary study) was produced xylitol 2296.31 mg/L (Q_p = 0.032 g/L h, $Y_{P/S}$ = 89.06%).

1. INTRODUCTION

The lignocellulosic biomass, which is constituted of renewable organic compounds, presents with great potential of use as raw material in bioprocesses. These materials are the major constituents of vegetable biomass such as, for example, the residues of: rice straw, eucalyptus, sucarcane bagasse and corn cob, which are accumulated in the environment, causing problems of pollution and representing the loss of valuable resources (Lynd *et al*., 2005).

The potential and the specific characteristics of the techniques for the use of these materials are related to their constitution. Its composition, in greater amount of sugars contained in cellulose fractions (glucose) and hemicellulose (xylose, arabinose, glucose, mannose and galactose) has been considered a promising alternative, attracting research that might use them as substrates in the production of xylitol and ethanol by fermentative pathway. Thus, are opening up opportunities for the development of an industry based on renewable raw materials (biorefineries). Besides the biofuels already known, a flow of innovations in development might be laying the foundation of an integrated industry of biomass exploration (Coutinho and Bontempo, 2010).

According to Pinho (2011), among the different biomasses that compose the lignocellulosic materials, bagasse from the cashew stalk stands out for being a byproduct of the Brazilian industry, with an estimated production of around 2 million tonnes/year and total use of only 20%, with a waste of 80%. Thereby making them, a promising source for bioconversion since it constitutes in abundant residue, renewable and low cost. In this way, the bagasse from the cashew stalk is an abundant agroindustrial residue, which presents in its composition, on average, 24.6% of cellulose, 15.1% hemicellulose and 24.6% lignin (Silva Neto *et al*., 2011).

In order that the lignocellulosic residues can be bioconverted, it is necessary the use of pretreatment that release the fermentable sugars existing in the composition of these materials (Lima et al., 2011). This procedure consists in using dilute acids so that occur the partial hydrolysis of the hemicellulose fraction most susceptible to the acid treatment (Aguilar et al., 2002). Also called prehydrolysis it is a process that employs less severe conditions, reaching high yields of conversion of xylans to xylose, besides presenting advantages for being less corrosive, toxic and dangerous compared to the use of concentrated acid (Sun and Cheng, 2002). The liquor obtained by this reaction contains constituent sugars from hemicellulose (xylose, glucose, arabinose, etc.), as also it is common to occur the formation of toxic compounds to the fermentation process (acetic acid, HMF and furfural). In this manner, it is necessary a suitable treatment to minimize these effects, in the liquor resulting from the prehydrolysis, which will be the raw material in the fermentation process (production of xylitol). The aims of this study were to evaluate the characterization (composition of formed sugars and inhibitors) of the liquor resulting from the acid prehydrolysis before and after concentration by evaporation and the treatment by adsorption with residual lignin.

2. METHOD AND MATERIALS

This work was developed in the Laboratory of Biochemical Engineering (LBE) and Laboratory of Porous Media and Particulate Systems (LPMPS) from the Academic Unit of Chemical Engineering in the Center of Science and Technology at the Federal University of Campina Grande - PB. It was used the cashew bagasse *in natura*, acquired from the juice production industry, FRUTNAT located in city of Campina Grande, Paraíba.

The bagasse was washed in running water until ^{0}Brix near 0. After washing, the bagasse was taken on trays to dry in oven with air circulation at temperature of 55°C for 48 hours. Shortly

after it was milled in cutting mills (of the Tecnal brand) to reduce the size and sieved in a 48 mesh sieve and stored in polypropylene bags for later use

The yeast used in the research was *Candida guilliermondii* CCT 3544 (xylitol production) obtained at the Foundation André Tosselo - FAT Tropical Culture Collection.

2.1 Acid prehydrolysis of cashew apple bagasse

For the pretreatment, it was used the cashew residue on dry basis, which was treated with a dilute acid solution (sulfuric acid 95% purity of the VETEC/PA brand). The liquor from the pre-hydrolysis was obtained at 105°C for 1 h in a stainless steel pressure reactor with capacity of 700ml, using a weight ratio of 1:6 (100 g of sample/600 g H_2SO_4 at 3%v/v).

2.2 Prehydrolyzate Concentration

The prehydrolyzed liquor was subjected to the process of concentration in a rotary evaporator (of the Quimis brand) connected to a vacuum pump. The working temperature of the rotary evaporator was 70 ± 5 °C in order to increase the content of sugars, especially xylose. The original prehydrolyzate was concentrated by reduction of 1/2.5 (FC 2.5) of its initial volume.

2.3 Tratament of the prehydrolyzate

Then the liquor obtained from the acid prehydrolysis was treated using the chemical detoxification. This procedure involved the elevation of the initial pH (0.98) to final pH of 5.0 with addition of 17.5% NaOH. The liquor was then submitted to the adsorption process using as adsorbent the residual lignin according to the methodology described by Pivetta, (2008). The operating conditions were: 1:100 weight ratio (1g of sample/100g of the pre-hydrolyzed liquor), keeping the mixture under stirring in a SHAKE type incubator under the conditions: 150 rpm at 30°C for 1 hour, and vacuum filtered to eliminate the precipitate.

2.4 Microorganism and inoculum preparation

Candida guilliermondii CCT 3544 cells were maintained in the laboratory (LBE) on the malt extract Agar medium in 4°C. For the inoculum preparation, the cells were transferred under aseptic conditions, with the aid of a platinum handle, to test tubes containing about 5 ml of sterilized distilled water. 1 mL of aliquots of this suspension were then transferred to 125 mL Erlenmeyer flasks, containing 50 ml of the medium constituted of 30 g/L of xylose, 0.1 g/L of calcium chloride, 3 g/L of ammonium sulfate and 20 g/L of rice bran extract. Maintained in a rotary incubator at 200 rpm, at 28 °C for 24 hours. The cells were then separated by centrifugation at 2000 X g for 15 minutes and then resuspended in sterile distilled water. Suitable volumes of this suspension were used in the fermentation, so that the initial cell concentration was 3 g/L.

2.5 Medium and fermentation conditions

The detoxified and prehydrolyzed liquors and were sterilized in autoclave (111°C for 15 minutes) and supplemented with calcium chloride (0.5 g/L), ammonium sulfate (1.0 g/L) and rice bran extract (20.0 g/L). All the fermentations were carried in an orbital shaker at 200 rpm, 28 ° C in 125 mL Erlenmeyer flasks containing 50 mL medium. The flasks were inoculated with an initial cell concentration of mass of 3.0 g/L. The medium pH was adjusted to 4.0 at the beginning of fermentation.

2.6 Analytical methods

The prehydrolyzed liquor without being concentrate (original), concentrated, treated and fermented was characterized regarding to the concentrations of sugars (glucose, xylose and arabinose), acetic acid, hydroxymethylfurfural, furfural and xylitol. By means of HPLC equipped with a pump ProStar Model 210 (Varian); Manual injector with 20μL loop; Refractive index detector, ProStar model 356 (Varian) and UV/visible 284 nm (aldehydes); Hi-Plex H stainless steel analytical column (300mm x 7.7 mm, Varian). The conditions of the operations were as follows: Column temperature of the 40°C; Mobile phase: MilliQ water with an outflow 0.6 mL/min; Analysis time: 15 and 60 minutes to sugar contents and aldehydes, respectively. Internal standard solutions of sugars: glucose, xylose, arabinose and sucrose (Sigma 99.99% HPLC grade), HMF congeners (Aldrich 99.98%) and furfural (Vetec 99.9 UV/HPLC) were used in the quantification of the liquor components.

2.7 Statistical analysis

The ASSISTAT program was used (Silva and Azevedo, 2009), where was applied the Tukey's test, with 5% of probability, of sugars (xylose, arabinose and glucose) and fermentation inhibitors (acetic acid, HMF and furfural).

3. RESULTS AND DISCUSSION

The characterization results of the prehydrolyzed liquor, before and after concentration and treatment procedures, can be found in Table 1.

It can be seen in Table 1, the acid prehydrolysis of the bagasse from the cashew stalk has made possible the obtainment of liquor with predominant values for pentoses: xylose (1.43 g/L) and arabinose (7.12 g/L) corresponding to 92.64% compared to glucose (0.63 g/L). It should be noted that after the concentration the prehydrolyzate liquor occurred a significant increase in the content of these sugars, at around 2.5 times. Cunha (2006), Tamanini, also found similar values and Hauly (2004) and Sarrouh (2009) that verified the non-degradation of the sugars in the concentration step. According to these results, can be seen that the prehydrolysis process was able to liberate in the pre-treated liquor the sugars present in the hemicellulosic fraction of the

bagasse from the cashew stalk, also forming toxic components resulting, in part from the degradation of glucose (HMF) and xylose (Furfural).

Table 1 - Components of the hemicellulosic prehydrolyzate of the bagasse from the original cashew apple, concentrated and treated.

Concentration	Liquor prehydrolyzate		
	Original	Concentrated	Treated
Xylose (g/L)	1.43c	3.97b	4.54a
Glucose (g/L)	0.63c	2.62b	2.90a
Arabinose (g/L)	7.12c	17.76b	18.52a
Acetic acid (mg/L)	343.75a	69.00b	19.00c
5-HMF (mg/L)	0.02c	0.22a	0.09b
Furfural (mg/L)	0.09a	0.05a	0.06a

Means followed by the same letter in the row do not differ by Tukey test at 5% probability

It is also observed in Table 1 that the prehydrolyzed liquor presents several compounds considered toxic to yeasts, such as acetic acid (343.75 mg/L), furfural (0.02 mg/L) and hydroxymethylfurfural (0.09 mg/L), and being these generated from the degradation of pentoses and hexoses, as analyzed and reported by Silva *et al*. (2007), Carvalho *et al*. (2005). Duarte et al. (2005) reported that the presence of concentrations above 3 g/L of acetic acid, 1.5 g/L of HMF and 1 g/L furfural, has toxic effects, being sufficient to inhibit the action of microorganisms in the fermentation process. Depending on the concentrations of toxic (HMF, furfural and acetic acid) there may be the need for some kind of treatment for detoxification, because, even presenting in the pre-hydrolyzed liquor concentrations under the minimum concentration that is toxic can inhibit the fermentation process, due to the synergy of the inhibitory compounds. The toxic effect occasioned by the furanic compounds appears to be associated with the fact that, by being aldehydes, chemically reactive, can react with certain biological molecules such as lipids, proteins and nucleic acids or cause damage to the cell membrane (Palmqvist *et al*. 2000; Duarte *et al*. 2005; Rocha *et al*. 2011).

It also appears that in Table 1, after the concentration step the contents of sugar increasesed proportionally to the concentration factor employed (2.5 times). This is probably due to the non-degradation of sugars during the process. However, among the toxic compounds, despite having their values increased (0.02 to 0.22 mg/L) for HMF, this trend was not observed in the same way for acetic acid (345.75 – 69.00 mg/L) and furfural (0.09 – 0.05 mg/L) which decreased. Similar behavior was reported by Mussato *et al*. (2004) that found equal values to (1.24 g/L) acetic acid, (0.11 g/L) for HMF and furfural (nd) under the same conditions of this work (2.5 times). This probably occurred due to the conditions employed during concentration, provoking the partial volatilization of these compounds.

For the concentrations of acetic acid (19.00 mg/L), HMF (0.09 mg/L) and furfural (0.06 mg/L) after detoxification process, it is observed that the behavior was coherent to the reported by Silva and Felipe (2006), Carvalho *et al*. (2005) and Tamanini and Hauly (2004). It has been

found that the process of detoxification promoted partial removal of acetic acid, HMF and furfural, respectively. It's noted that didn't occur losses in the content of sugars (xylose, glucose and arabinose) in the prehydrolyzed liquor after detoxification treatment, fact that was desired, since the increase in the initial concentration of xylose favors the production of xylitol.

It is observed in Figure 1, variations on the concentrations of xylose, xylitol over time in the fermentations.

As shown in Figure 1, throughout the fermentation, insofar as the xylose is consumed, there is a linear decrease of its concentration and an increase of xylitol. In this step were analyzed the responses of xylitol yield factor ($Y_{P/S}$ = gram of xylitol formed per gram of xylose consumed) and volumetric productivity (Qp = gram of xylitol formed per liter of medium per hour) obtained at the end of fermentation (72 hours). In fact, the highest concentration of xylitol was 2296.31 mg/L after 72 hours of fermentation, with Qp = 0.032 g/L h and $Y_{P/S}$ = 89.06%.

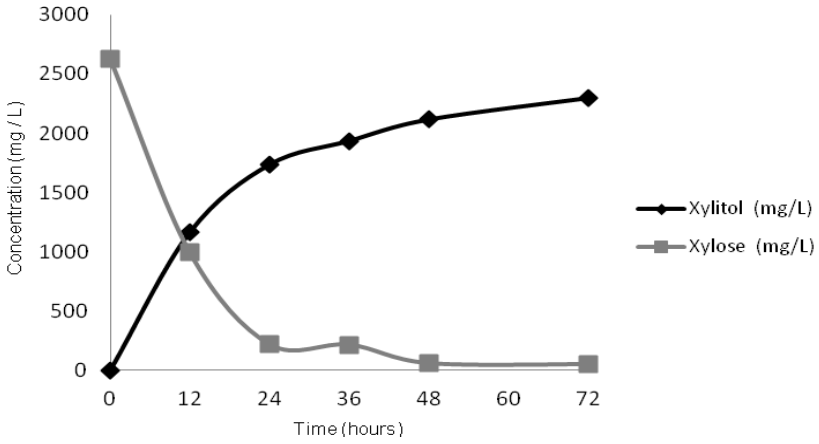

Figure 1 - Profile of consumption of xylose and xylitol production in function of the fermentation time.

Similar profile of xylose consumption and xylitol production is reported by other authors through the fermentation kinetic parameters such as Pivetta *et al*. (2008) who observed in the hydrolyzate of the sugarcane bagasse the productivity values (0.48 g/L h) and yield (0.79 g/g) of xylitol. Now Ferreira *et al*. (2005) verified that after 72 hours of fermentation a xylitol production of 35.63 g/L, with a conversion of 63% of the consumed xylose to xylitol, accompanied by a volumetric productivity of 0.50 g/L h and bioconversion efficiency of 69% regarding to the theoretical value of yield in xylitol. Barbosa *et al*. (2008) when studying the ability of *Candida guilliermondii* to ferment hemicellulosic hydrolyzate of barley straw, have obtained an efficiency of 60.4% of bioconversion of xylose to xylitol with 0.48 g/L.h and 0.55 g/g for volumetric productivity and conversion factor with 48h of fermentation. However, Gimenes et al. (2003) verified when studying the influence of the initial xylose concentration on xylitol production by

Candida guilliermondii that the maximum accumulation of xylitol coincided with the exhaustion of xylose, reaching the best factors in xylitol yield (YP/S) and volumetric productivities (QP) with average values of 0.794 g/g and 1.1 g/L h, respectively.

4. CONCLUSION

The results of this study allow to conclude that the prehydrolyzed liquor from the cashew apple bagasse presents great potential for use as fermentation medium, this is due the conditions employed in the acid prehydrolysis, but also, the concentration procedures and treatment, providing high concentrations of sugars and a bigger removal of inhibitors, becoming favorable the xylitol production.

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