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Biotechnological Production of Xylitol: Evaluation of Detoxification Process with Residual Lignin Using Response Surface Methodology

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The agroindustrial residues are among the greatest sources of biomass in the world which can be used in bioprocesses, because these lignocellulosic materials represent an abundant source of sugars. In northeastern Brazil one of the lignocellulosic residues in abundance, which has most of the potential wasted is the cashew apple bagasse; that through pretreatment with dilute acids makes possible the removal in hemicellulosic fraction (xylose, arabinose, glucose, mannose and galactose) which represent substrates that can be utilized for the production of xylitol by fermentation. However this process, besides the released sugars are also compounds fermentation inhibitors. In this study the main aim was to evaluate through response surface methodology detoxification treatment using residual lignin (cashew aplle bagasse which precipitates in the pretreatment liquor). The cashew apple bagasse was submitted to acid prehydrolysis H_2S0_4 using 3% (w/w), was then carried out analyzes of sugars and inhibitory compounds present in the prehydrolyzate liquor in high efficiency liquid chromatography (HPLC). The prehydrolyzate was concentrated and then treated under various conditions of mass residual lignin, pH and contact time to reduce concentration toxic compounds and improve efficiency process to subsequent fermentation (production of xylitol). The results indicate that to obtain in the prehydrolyzate liquor, a higher percentage removal from HMF (Hydroxymetylfurfural) and furfural, it should operate under conditions of pH 4, 0.65 g mass of lignin for 20 min, removing approximately 100%.

1. Introducion

The lignocellulosic agroindustrial residues are constituted by organic compounds which can be employed in bioprocesses, due to these materials represent a rich source of sugars. The specific characteristics of the techniques for using these materials are related to their constitution, composed in greater amount of sugars contained in cellulose fractions (glucose) and hemicellulose (xylose, arabinose, glucose, mannose and galactose) being considered one promising alternative, attracting research that can use them as substrates in the production of xylitol and ethanol by fermentative way. So are generated opportunities for the development of an industry based on renewable raw materials (biorefineries). Besides biofuels already known, a flow of innovations in development may be laying the foundation for an integrated industry of exploitation of biomass (Coutinho and Bontempo, 2011).
According to Pinho (2011), among the different biomasses that compose the lignocellulosic materials cashew apple bagasse stands out for being a byproduct from the Brazilian industry, with an estimated

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production around 2 million tonnes/year and total utilization of only 20%, with a waste of 80%. To that these residues be converted, it's necessary the use of pretreatments, which liberate fermentable sugars that exist in the composition of these materials (Lima et al., 2012). However, the biotechnological obtainment the xylitol presents restrictions regarding the efficacy during the fermentation process, because of the presence of inhibiting compounds to the microbial metabolism present in the hemicellulosic hydrolysates. The concentrations of these inhibitors (furfural, HMF and acetic acid) present in the liquor after the pretreatment, deserve attention, because they depend on the nature of the lignocellulosic material used and may limit the consumption of the carbon source, reducing the speed of growth (inhibitor) or even prevent the bioprocess (toxic agent). In this context, the detoxification aims to remove toxic compounds (furfural, hydroxymethylfurfural, acetic acid, formic acid, levulinic acid and compounds from the degradation of lignin, vanillin and p-hydroxybenzoic acid) present in hydrolysates obtained by pre-hydrolysis or hydrolysis (Saha and Cotta, 2006).

A new method currently employed for detoxification of lignocellulose hydrolysates, is to use the residual lignin from the hydrolysis as an adsorbent in the extraction, taking advantage of their hydrophobic properties. The advantage of using the residual lignin as detoxification agent front of the chromatographic resins is mainly economic. Lignin since it is a byproduct of hydrolysis after it has been used for detoxification, can also be used as a primary fuel (Parazzi, 2008). In this study the main objective was to evaluate through the response surface methodology the detoxification treatment using residual lignin (residue of the cashew apple bagasse which precipitates in the liquor pretreatment).

2. Material and Methods

This work was developed in the Laboratory of Biochemical Engineering at the CCT/UFCG/PB. It was used the bagasse from the cashew *in natura*, that was acquired from juice production industry, FRUTNAT, located in the city of Campina Grande/PB.

The bagasse was washed in water, until reaching the ºBrix close to 0. And then taken to drying in a greenhouse on trays with air circulation at a temperature of 55°C for 48 hours. After was milled in a cutting mills, sieved in 48-mesh sieves and stored in polypropylene bags

2.1 Acid prehydrolysis of the bagasse from the cashew stalk

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

2.2 Concentration of Prehydrolysate

The prehydrolysate liquor was subjected to the process of concentration in a rotary evaporator (Quimmis brand). The working temperature of the rotary evaporator was 70 ± 5°C in order to increase the sugar content, mainly xylose. The original prehydrolysate was concentrated by the reduction of 1/2.5 (HR 2.5) of its initial volume.

2.3 Treatment of the prehydrolysate

Then the liquor obtained from the acid prehydrolysis was treated using chemical detoxification. This procedure involved the elevation of the initial pH (0.98) to final pH 5.0 with the addition of 17.5% NaOH. The liquor was then submitted to the adsorption process using as adsorbent residual lignin as a 2^3 factorial planning matrix, were conducted to verify the influence of factors (mass ratio of residual lignin, pH and contact time) on the variable responses (concentrations in the prehydrolysed liquor: glucose, xylose, arabinose, acetic acid, HMF and furfural). The levels of the factors used in the planning are shown in (Table 1), where (-1) and (+1) mean the lowest and highest levels, respectively, and (0) means the level of the center point, also the 23 factorial planning matrix with duplicate on the central point.

The actual levels shown in Table 1 were defined observing the studies by Mussato and Roberto (2004) by studying the optimization of the treatment of the hydrolysate from rice straw with active charcoal, aiming to evaluate the effect of variables such as pH, contact time, agitation and temperature in the process of detoxification of the hydrolysate.

Table 1: 2^3 Factorial design matrix, with 2 replications at the center point, with the levels in real and coded values for the evaluation of lignin adsorbent, pH and contact time on the pentoses

Trials	Real/coded levels		
	Lignin mass (g)	pH	Contact time (min)
1	-1 (0.25)	-1 (4.0)	-1 (20.0)
2	+1 (0.65)	-1(4.0)	-1 (20.0)
3	-1 (0.25)	+1(10.0)	-1 (20.0)
4	+1 (0.65)	+1(10.0)	-1 (20.0)
5	-1 (0.25)	-1 (4.0)	+1 (60.0)
6	+1 (0.65)	-1(4.0)	+1 (60.0)
7	-1 (0.25)	+1(10.0)	+1 (60.0)
8	+1 (0.65)	+1(10.0)	+1 (60.0)
9	0 (0.45)	0 (7.0)	0 (40.0)
10	0 (0.45)	0 (7.0)	0 (40.0)

2.4 Analytical methods

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

3. Results and discussion

Verify the values the percentage response of removing sugars and toxic compounds in Table 2. It is observed that the central points showed little variation for the responses indicating significant repeatability of the process, except for the reduction of arabinose.
It was found that the adsorption process promoted partial removal of acetic acid and practically 100% of the HMF and furfural. It is noted that there were no significant losses to the pentose (xylose) in the pre-hydrolysed liquor after the treatment, and this was expected, as it favors the production of xylitol.

Table 2: Values of the percentage response removing sugars and toxic compounds in the prehydrolysed liquor of the cashew apple bagasse

Trials	Percentage removal					
	G	X	A	Aa	HMF	F
1	7.85	0.72	3.40	44.79	22.22	94.34
2	10.15	1.38	6.34	44.41	98.89	92.45
3	13.52	11.99	9.94	44.45	83.33	5.66
4	69.92	42.15	34.76	67.43	33.33	66.04
5	9.21	10.49	9.46	28.68	72.22	98.11
6	15.33	5.16	9.32	46.02	98.89	92.45
7	66.66	33.33	27.72	45.06	77.78	98.11
8	11.67	13.12	4.86	36.54	66.67	20.75
9	6.82	5.53	2.04	51.57	92.00	83.02
10	5.41	4.19	0.73	51.30	94.44	84.91

G - glucose; X - xylose; A - arabinose; Aa - acetic acid; HMF - hydroxymethylfurfural; F - furfural

This stage is investigated through the methodology of experimental planning and the response surface analysis, the main effects (lignin mass, pH and contact time) and the interactions between the three factors that significantly influenced the concentrations of sugars, HMF, furfural and acetic acid, in the adsorption process.

The experimental data were subjected to a linear regression analysis, it was possible to construct empirical models that related the removal of sugars and fermentation inhibitors with the parameters studied. In Table 3 there are the linear regression models considering only the parameters statistically significant at 95% of confidence and the respective coefficients of determination (R^2) and values of the F test.

Table 3: Linear regression models for percentage response variable of removal

Percentage removal	Regression model	R^2	F Test
Glucose	$=21.65 + 14.90_{pH} - 13.44\ _{mLxTc}\ -14.40\ _{mLxpHxTc}$	0.88	3.20
Xylose	$=12.80 + 10.35_{pH} - 7.04\ _{mLxpH} - 5.55\ _{mLxpHxTc}$	0.85	2,53
HMF	$=73.98+9.73_{Tc}-20.56_{mLxpH}+11.11\ _{mLxpHxTc}$	0.80	1.74
Furfural	$=73.50 - 23.34_{pH} + 6.37_{Tc} - 17.69\ _{mLxTc} - 16.75\ _{mLxpHxTc}$	0.90	4.17

m_L - lignin mass; T_c - contact time

According to the equations presented in Table 3 it can be seen that the 1st order models are statistically significant for glucose, xylose, furfural and HMF, for presenting the ratio $F_{calculated}/F_{tabulated}$ more than 1 (F Test) (Rodrigues and Iemma, 2009). It is also verified that the pH variable exerted a significant influence on the percentage removal (glucose, xylose and furfural) either in an isolated way or by interaction with other variables. A positive sign of this coefficient indicates that to obtain a maximum percentage removal of glucose and xylose in the studied range, the pH of the medium should be high (+1), suggesting that to obtain the minimum percentage removal in the sugars which is desired, the pH must be at level (-1), whereas for furfural, the negative sign of the pH indicates maximum percentage removal.

There are, in Table 3 analysis of variance (ANOVA), the R2 and the F test used to verify the statistical significance of empirical models of the variables: % removals of glucose, xylose, furfural and HMF. These models present 88.41, 85.74, 80.46 and 90.85% of the variations obtained, which can be explained by the models and by the ratio $F_{calculated}/F_{tabulated}$ equal to 3.20, 2.53, 1.74 and 4.17 indicating that the same are statistically significant with 95% confidence (Rodrigues & Iemma, 2009). As the empirical regression model of the experimental data of percentage response of removing of glucose, xylose, furfural and HMF are statistically significant at the confidence level of 95%, we can construct the response surface for the analysis of the influences of the variables under study on the response.

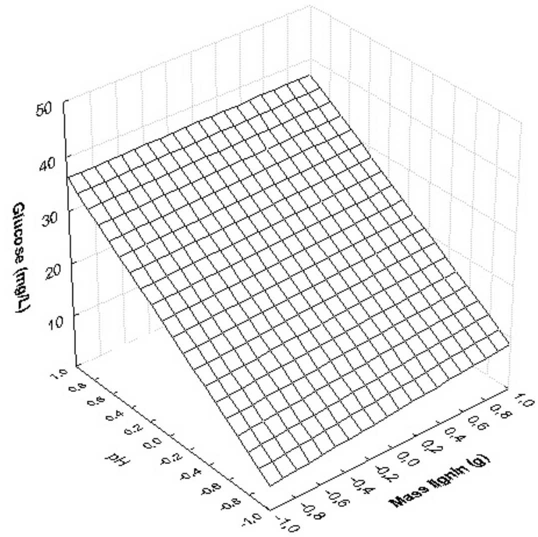

Figure 1: Response surface for the percentage removal of glucose

For glucose and xylose the responses were generated in relation to the effects of the input variables the lignin mass and the pH, fixing up contact time at the central point. It is noted that when operating with the pH level -1, independent of the concentration of lignin mass, we obtain the removing values below than 10% (Figures 1 and 2).

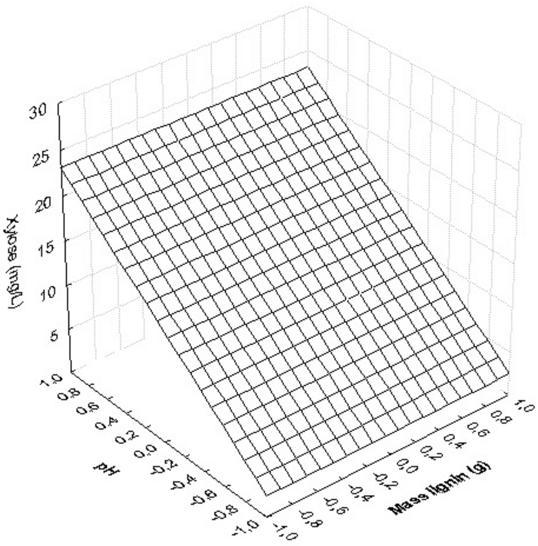

Figure 2: Response surface for the percentage removal of xylose

For the% removal of furfural and HMF (Figures 3 and 4), it can be observed that one obtains % removal of approximately 100% when the pH decreased, fixing the mass in the upper level (+1).

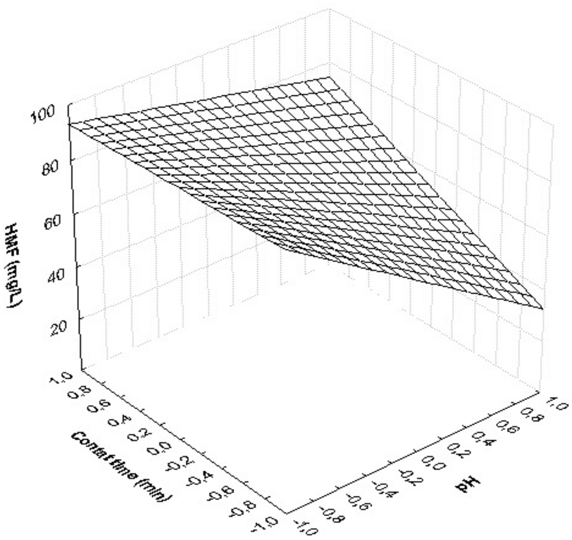

Figure 3: Response surface for the percentage removal of HMF

4. Conclusion

The results of this work indicate that to obtain the prehydrolysate liquor, one less percentage removal of glucose and xylose and removal of greater concentrations of furfural and HMF, should operate under the following conditions: pH level -1 and contact time at + 1 (4 and 60 min. respectively) and the mass of residual lignin of level +1 (0.65 g)

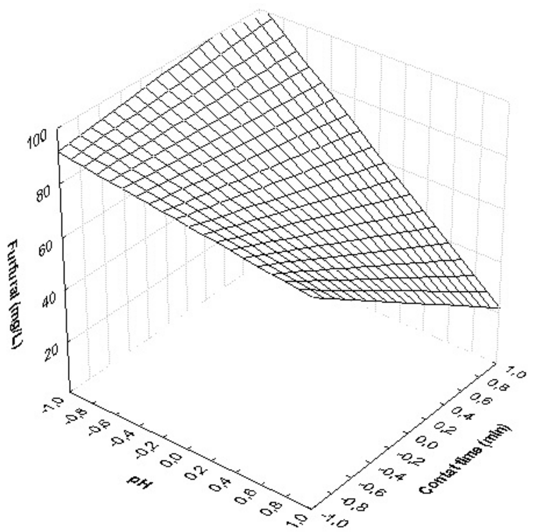

Figure 4: Response surface for the percentage removal of furfural

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References

Coutinho P.L.A., Bomtempo J.V., 2011, A technology roadmap in renewable raw materials: a basis for public policy and strategies in Brazil. Química Nova, 34, 5, 910-916. (in portuguese)

Lima F.C. S., SILVA F.L.H., GOMES J.P., SILVA NETO J.M., 2012, Chemical composition of the cashew apple bagasse and potential use for ethanol production. Advances in Chemical Engineering and Science, 2, 519-523

Mussato S.I, Roberto I.C., 2004, Optimal experimental condition for hemicellulosic hydrolyzate treatment with activated charcoal for xylitol production. Biotechnology Progress, 20, 1, 134-139.

Parazzi C., Arthur C., LOPES J.J.C., CORGES M.T.M.R., 2008, Evaluation and characterization of the main chemical compounds of sugar cane brandy aged in oak barrels (*Quercus* sp). Journal of Food Science and Technology, 28, 1,193-199. (in portuguese)

Pinho L.X., Afonso M.R.A., Carioca J.O.B., Costa J.M.C., Rybka A.C.P., 2011, Dehydration and utilization of waste cashew apple as adding fiber in the preparation burger, Jornal Alimento e Nutrição, 22, 571-576. (in portuguese)

Rodrigues M.I.; Iemma A.F., 2009, Design of experiments and optimization of processes, 2ª Ed. – Campinas, SP, 358.

Saha B.C., Cotta, M.A., 2006, Ethanol production from alkaline peroxide pretreated enzymatically saccharified, wheat straw. Biotechnology Progress, v.22, 449-453.