Downstream Processing for Xylitol Recovery from Fermented Sugar Cane Bagasse Hydrolysate Using Aluminium Polychloride

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Xylitol, a sweetener comparable to sucrose, is anticariogenic and can be consumed by diabetics. This sugar has been employed sucessfully in many foods and pharmaceutical products. The discovery of microorganisms capable of converting xylose present in lignocellulosic biomass into xylitol offers the opportunity of producing this poliol in a simple way. Xylitol production by biotechnological means using sugar cane bagasse is under study in our laboratories, and fermentation parameters have already been established. However, the downstream processing for xylitol recovery is still a bottleneck on which there is only a few data available in the literature. The present study deals with xylitol recovery from fermented sugar cane bagasse hydrolysate using 5.2 g/l of aluminium polychloride associated with activated charcoal. The experiments were performed at pH 9, 50 °C for 50 min. The results showed that aluminium polychloride and activated charcoal promoted a 93.5% reduction in phenolic compounds and a 9.7% loss of xylitol from the fermented medium, which became more discoloured, facilitating the xylitol separation.

Introduction

The use of lignocellulosic residues as energy sources in biotechnological processes helps to reduce the environmental pollution caused by the accumulation of such residues on the soil and contributes to the generation of valuable products (Molwitz *et al.*, 1996; Silva *et al.,* 1997).

Sugar cane bagasse, one of these residues, is generated by the Brazilian sugar-alcohol industry in large amounts. The hydrolysis of the hemicellulosic fraction of this biomass results in a hydrolysate containing hexoses and pentoses, but also various degradation products (Felipe *et al.,* 1977; Felipe *et al.,* 1997). Several physico-chemical treatments are necessary for removing these toxic substances from the hydrolysate before it can be used in bioprocesses. One of these bioprocesses, the conversion of xylose contained in sugar cane hemicellulosic hydrolysate into xylitol, is under study in our laboratories (Molwitz *et al.,* 1996; Felipe *et al.,* 1997).

Xylitol, a sugar with a sweetening power and chemical properties comparable with those of sucrose , is anticariogenic and can be consumed by diabetics. In Europe, as well as in Brazil, this sugar has been employed in chewing gums, toothpastes and tablets. Xylitol is usually produced by a chemical process consisting in catalytic xylose hydrogenation, which originates an impure solution containing other sugars and polyols. The purification of this solution is difficult. However, since the xylitol yield and quality depend on its purity, the purification process is indispensable. The discovery of microorganisms capable of converting xylose into xylitol offers an opportunity of replacing the chemical process by the microbiological one, which dispenses with the need for a pure xylose solution and prevents the formation of toxic residues coming from catalysis (Silva *et al.,* 1998).

Most of the fermentation parameters for xylitol production have already been established (Silva *et al.,* 1997; Felipe *et al.,* 1977; Felipe *et al.,* 1997; Silva and Afschar, 1994; Silva *et al.,* 1997). However, the ideal treatment of the hydrolysate and the downstream processing for xylitol recovery are still a bottleneck on which there is only a few data in the literature. Most papers on downstream pro-

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cessing techniques are concerned with enzyme separation and purification, but the conditions employed in these techniques can not be applied to xylitol recovery.

The present study deals with a simple and effective way of treating fermented sugar cane bagasse hydrolysate for xylitol recovery using aluminium polychloride associated with activated charcoal.

Materials and Methods

Hemicellulosic hydrolysate

The hemicellulosic hydrolysate was obtained by acid hydrolysis of sugar cane bagasse. The hydrolysis was carried out in a 350 l steel reactor under the following conditions: temperature of 121 °C, reaction time of 10 min, 10 l water/kg sugar cane bagasse (dry weight) and 100 mg sulfuric acid/g sugar cane bagasse (dry weight). After hydrolysis, the liquid was concentrated by heating at 70 °C under vacuum, in order to obtain a xylose concentration of 50–60 g/l.

Evaluation of different conditions for treating the hemicellulosic hydrolysate

Some degradation compounds, such as furfural, 5-hydroxymethylfurfural, acetic acid and phenolic compounds, all of them present in the hydrolysate, act as inhibitors of the microbial metabolism. In order to minimize this problem and consequently facilitate the xylitol recovery, the hydrolysate was previuosly treated. Analyses were carried out considering the factors pH (pH), temperature (T), aluminium sulfate concentration (AS) and aluminium polychloride concentration (AP) (Table I).

The hydrolysate was initially treated by increasing the initial pH (0.5) to the desired pH value (Table I) by adding CaO. The precipitate formed was removed by filtration. The filtrate was than heated to the desired temperature and supplemented with aluminium sulfate or polychloride, under the conditions described in Table I. Finally, CaO was used to adjust the pH to 5.5. After 1 hour the treated hydrolysate was filtrated to remove the precipitates, supplemented with nutrients and used as a fermentation medium.

Microorganism and inoculum preparation

The yeast *Candida guilliermondii* FTI 20037 from the culture collection of the Department of Biotechnolgy of the Faculty of Chemical Engineering of Lorena, S.P, was used. The culture was maintained at 4 °C on malt extract agar slants.

Table I. Experimental matrix used to evaluate different conditions for treating the sugar cane bagasse hydrolysate.

Experiment #	pH	T (°C)	Concentration (g/l) AS	AP
1	6.7	40	2.5	2.0
2	9.3	40	2.5	2.0
3	6.7	60	2.5	2.0
4	9.3	60	2.5	2.0
5	6.7	40	7.5	5.0
6	9.3	40	7.5	5.0
7	6.7	60	7.5	5.0
8	9.3	60	7.5	5.0
9	8.0	50	5.0	3.5

T – temperature; AS – aluminium sulfate; AP – Aluminium polychloride.

The cells were previously grown in a medium composed of hydrolysate and the following nutrients (g/l): yeast extract 2.0, $CaCl_2.2H_2O$ 0.1 and $(NH_4)_2SO_4$ 5.0. The cell cultivation was carried out in 125 ml Erlenmeyer flasks (containing 50 ml of medium) on a rotatory shaker at 200 rpm, at 30 °C for 48 hours. The initial cell concentration in all fermentation runs was 10^7 cells./ml.

Fermentation of hemicellulosic hydrolysate to xylitol

The hydrolysate treated as described in Table I was supplemented with the same nutrients used for the inoculum preparation. The batch fermentation was carried out in a 2.0 l bioreactor (New Brunswick – Edison-N. J., USA) at 30 °C, 400 rpm under an aeration rate of 0.2 vvm (vol. air x vol. medium^{-1} x min^{-1}), corresponding to an oxygen volumetric transfer coefficient (K_La) of 25 h^{-1}. Samples were periodically collected from the fermented medium for evaluation of sugar consumption and xylitol production.

Assay for xylitol recovery from the fermented sugar cane bagasse hydrolysate

The fermented hydrolysate was purified with aluminium polychloride (Adesol Produtos Químicos Ltda P-887, Brazil) under selected conditions as shown in the statistical design. After treating the broth, activated charcoal (superficial area 500 to

600 m^2/g, density 0.26 to 0.30 g/cm^3, Synth 32603, Brazil) was added as described by Gurgel (1993). The effects of the active charcoal both on the clarification of the medium and on the loss of xylitol during its recovery were investigated.

Analytical methods

Quantitative determination of sugars, xylitol and acetic acid was made by high performance liquid chromatography (HPLC) using a Shimadzu chromatograph (Kyoto- Japan), a Bio-Rad Aminex HPX-87H column (300x7.8 mm) at 45 °C and 0.02 N H_2SO_4 as the eluent at a flow rate of 0.6 ml/min. The compounds were detected by a RID-6A Shimadzu detector and a Shimadzu CR7A integrator.

Furfural and 5-hydroxymethylfurfural were also determined by HPLC using a Hewlett Packard RP 18 HP column (200 x 4.6 mm) at 25 C and acetonitrile: water (1:8 v/v) with 1% acetic acid as the eluent at a flow rate of 0.8 ml/min. The compounds were detected by a U. V.-VS. Shimadzu SPD-10A detector and Shimadzu CR7A integrator.

Phenolic compounds were estimated by summing up the areas of different compounds that absorb ultraviolet light at 270 nm. The analyses were performed in a Shimadzu high performance liquid chromatograph, using a Hewlett Packard RP 18 HP column (200 × 4.6 mm) with a wavelength of 270 nm, at 25 °C and methanol:water (1:8 v/v) with 1% acetic acid as the eluent at a flow rate of 1.0 ml/min. The compounds were detected by using a U. V.-VS. Shimadzu SPD-10A detector and Shimadzu CR7A integrator.

The clarification index of the hydrolysate was evaluated at 440 nm by using a Shimadzu UV 150–02 double-beam spectrophotometer, according to the method described by Gurgel (1993). The pH of the samples was initially adjusted to 5.5 and then the material was centrifugated and filtered through a Millipore Membrane GS (pore size = 0.45 nm).

Cell concentration was estimated by measuring absorbance at 600 nm. The relationship between absorbance and dry weight (g/l) was given by a standard curve (0.1 OD unit = 0.14 g dry weight cells/l hydrolysate).

Results and Discussion

Downstream processing of biotechnological products obtained by fermentation processes from lignocellulosic biomass is critical and difficult, mainly due to the complex nature of the hydrolysates. In this way, a partial characterization of the pretreated sugar cane bagasse hydrolysate obtained by dilute acid hydrolysis was initially carried out (Table II). Under the conditions used, a mixture of sugars (pentoses and hexoses) was obtained. Xylose was the major pentose present in this hydrolysate representing about 70% of the total monosaccharides. The pentoses and hexoses were partly degraded into furfural and 5 hydroxymethylfurfural, respectively. The presence of acetic acid was due to the de-o-acetylation of acetylated sugars from the hemicellulosic fraction. It was observed that a significant amount of phenolic compounds, derived from the lignin degradation, increased after the hydrolysate concentration step. The acetic acid concentration also increased from 3.68 to 6.86 g/l. A parallel increase in the xylose : glucose ratio was also observed. Hence, the concentration of the original hydrolysate corresponded to a second and convenient hydrolytic step (xylo-oligosaccharides to free xylose with simultaneous de-o-acetylation). Other compounds like furfural and hydroxymethyl furfural were also present in the hydrolysate at low concentrations (Table II). According to the literature (Felipe *et al.*, 1977; Felipe *et al.*, 1997; Silva *et al.*, 1998; Parajó *et al.*, 1996; Parajó *et al.*, 1998) acetic acid and phenolic compounds present in lignocellulosic hydrolysates are the most toxic compounds to the xylose-fermenting yeasts.

Table II. Partial characterization of the sugar cane bagasse hydrolysate.

	Hydrolysates	
Components (g/l)	Original	Concentrated
Glucose	1.21	5.45
Xylose	23.22	80.90
Arabinose	1.22	5.73
Acetic acid	3.68	6.86
Furfural	0.84	0.01
Hydroxymethylfurfural	0.07	0.10
Phenolic compounds	2.50	10.28
pH	1.14	0.49

Table III. Xylose and Acetic acid concentrations in the sugar cane bagasse hydrolysate treated with Al-salts according to the conditions described in the experimental design.

Experiment #	AL-SULFATE		AL-POLYCHLORIDE	
	Xylose (g/l)	Acetic acid (g/l)	Xylose (g/l)	Acetic acid (g/l)
1	69.1	5.9	67.0	5.8
2	68.9	5.8	71.1	5.3
3	72.1	5.8	72.1	5.3
4	69.1	5.8	73.0	5.4
5	67.3	5.7	69.2	5.3
6	70.4	5.5	70.6	5.3
7	72.2	5.5	70.8	5.2
8	72.8	5.5	67.4	5.2
9	71.1	5.5	70.5	5.2

The effect of aluminium salts on the removal or reduction of such compounds from the hydrolysate was verified before its use for the production of xylitol by *Candida guilliermondii*. According to Table III the acetic acid was partially removed from the hydrolysate using both Al-sulfate and Al-polychloride, the latter acting in a more effective manner as a flocullating agent. According to Ramos (Ramos, 1998) the acetic acid removal can be attributed to the fact this compound is present in the deprotonated form in the medium when the pH values are greater than its pKa (4.75) and it has a negative charge which interacts with the positive charge of the Al-gel. Thus, an electrostactic interaction occurs between the gel and the acetic acid, causing its removal from the precipitate formed. In the same way, the clarification of the hydrolysate and the removal of phenolic compounds were determined. It is clear from the results shown in Table IV that a clarification index around 70% and a removal of phenolic compounds around 30% were attained.

The sugar cane bagasse Al-salts treated hydrolysate was tested for xylitol production by *C. guilliermondii*. According to Table V, the xylitol production rates were not influenced by the Al-salt used in the pretreatment. However, an increase in xylitol concentration was observed after the fermentation of Al-polychloride treated hydrolysate, which can be due to the effectiveness of this salt in the removal or reduction of the microbial inhibitors present in the fermentation medium. When this salt was used, a xylitol concentration of 49.34 g/l was detected (Table V). This is very important, since a high xylitol concentration in the broth facilitates the downstream processing of this compound. Thus, these Al-salts, especially the Al-polychloride, can be successfully used in the treatment of the sugar cane bagasse hydrolysate for fermentation into xylitol. Their flocculent properties which are related to the overall charge and to the pH, are important for the removal of particles from aqueous solutions. Al-salts have also been used

Table IV. Clarification and removal of phenolic compounds from the sugar cane bagasse hydrolysate treated with Al-salts according to the conditions described in the experimental design.

Experiment #	AL-SULFATE		AL-POLYCHLORIDE	
	Clarification (%)	Reduction of Phenolics (%)	Clarification (%)	Reduction of Phenolics (%)
1	29.41	23.06	26.96	24.50
2	43.78	17.49	58.29	18.08
3	30.52	21.35	33.55	25.23
4	43.72	17.51	52.25	20.53
5	49.89	24.79	55.17	25.42
6	68.74	29.15	65.73	30.92
7	62.45	25.63	68.60	26.66
8	68.44	30.29	71.89	31.06
9	65.36	23.66	72.95	28.59

Table V. Microbial xylitol production rates attained from fermentation of sugar cane sugar bagasse hydrolysate treated with Al-salts.

Sugar cane bagasse hydrolysate treated with:	ΔS (%)	Xylitol (g/l)	Yp/s (g/g)	Qp (g/l.h)	E (%)
Al-sulfate	98.80	45.30	0.63	0.63	68.30
Al-polychloride	98.80	49.34	0.69	0.69	74.80

ΔS: consumption of xylose; Yp/s: xylitol yield; Qp: xylitol volumetric productivity; E: fermentation efficiency.

in other processes requiring removal of particles, such as water and wasterwater treatment.

The fermented sugar cane bagasse hydrolysate was submitted to tests for recovering the xylitol produced. At this stage, the hydrolysate was treated with 5.24 g/l of Al-polychloride and activated charcoal. The purification steps were performed at 50 °C, pH 9.04, for 50 minutes. According to Fig. 1 the increase in activated charcoal concentration promoted a reduction in the phenolic compounds still present in the broth. A 96.3% reduction was observed when using 25% of activated charcoal. The use of 15% of activated charcoal was sufficient to produce a significant reduction (95%) in the phenolic compounds. A loss of xylitol as a consequence of the treatment with activated charcoal was also observed. This loss was proportional to the increase in the activated charcoal concentration. The maximal xylitol loss occurred when 25% of activated charcoal was used. The adsorption property of the activated charcoal in adsorbing substances such as sugars, reduced-compounds and also xylitol is well known (Gurgel, 1993; Frazer, 1989; Rodrigues *et al.*, 1995) In our tests, a preferential adsorption of phenolic compounds present in the broth in relation to xylitol was observed.

The combination of two chemical properties, flocculation and adsorption, has been successfully

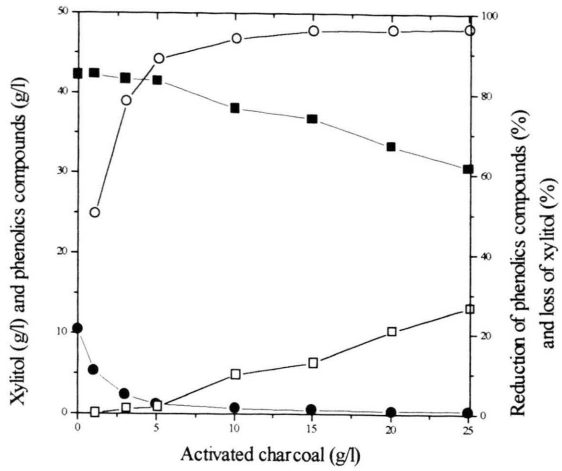

Fig. 1. Effect of activated charcoal concentration on xylitol (■) and phenolic compounds concentration (●) in the fermented sugar cane bagasse hydrolysate treated with Al-polycloride and on the loss of xylitol (□) and reduction of phenolic compounds (○) from this medium.

used in some technological processes, such as the purification of sucrose (Spencer and Meade, 1967; Kirk and Othmer, 1978). Thus, the utilization of Al-polychloride in combination with activated charcoal is a good approach for the treatment of the fermented hydrolysate, in order to recover the xylitol. To efficiently clarify the sugar cane bagasse hydrolysate and reduce the phenolic compounds with an insignificant loss of xylitol, Al-polychloride and 10% of activated charcoal were used. As a result, a 93.5% reduction in the phenolic compound was observed and xylitol was recovered from the broth with 9.7% of loss of this compound.

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Felipe M. G. A., Vitolo M., Mancilha I. M. and Silva S. S. (1997), Environmental parameters affecting xylitol production from sugar cane bagasse hemicellulosic hydrolysate by *Candida guilliermondii.* J. Ind. Microbiol. Biotechn. **18**, 251–4.

Felipe M. G. A., Vitolo M., Mancilha I. M. and Silva S. S. (1997), Fermentation of sugar cane bagasse hemicellulosic hydrolysate for xylitol production: effect of pH. Biomass and Bioenergy **13**, 11–14.

Frazer F. R. and McCaskey T. A (1989), Wood Hydrolysate treatments fermentation of sugar to 2,3-butanodiol. *Biomass* **18**, 31–42.

Gurgel P. V. (1993), Avaliação de métodos de recuperação de xilitol obtido por via fermentativa. M. Sc. Thesis, Universidade Federal de Viçosa, Viçosa, M. G., Brazil, 83p.

Kirk R. E. and Othmer D. F. (1978), Encyclopedia of Chemical Technology. The Interscience Encyclop. Inc. New York, John Wiley.

Molwitz M., Silva S. S., Ribeiro J. D., Felipe M. G. A., Prata A. M. R. and Mancilha I. M. (1996), Aspects of the cell growth of *Candida guilliermondii* in sugar cane bagasse hydrolysate. J. Bioscience **51C**, 404–8.

Parajó J. C., Domingues H. and Domingues J. M. (1996), Charcoal adsorption of wood hydrolysates for improving their fermentability: influence of the operational conditions Bioresource Technol. **57**, 179–185.

Parajó J. C., Domingues H. and Domingues J. M. (1998), Improved xylitol production with *Debaryomyces hansenii* Y-7426 from raw or detoxified wood hydrolysates. Enzyme M. Technol. **21**, 18–24.

Ramos R. M. (1998), Tratamento de hidrolisado hemicelulósico de bagaço de cana-de-açúcar com sais de alumínio para a obtenção de xilitol por via fermentativa. M. Sc. Thesis, Universidade Federal de Viçosa, Viçosa, M. G., Brazil, 116p.

Rodrigues R. C. L. B., Prata A. M. R. and Ramos R. M. (1995), Influência do pH e do carvão ativo na redução de impurezasdo hidrolisado hemicelulósico de eucalipto Congresso Brasileiro de Engenharia Química, 1, São Carlos, São Paulo, Brazil.

Silva S. S. and Afschar A. S. (1994), Microbial production of xylitol from D-xylose using Candida tropicallis. Bioprocess Eng. **11**, 129–134.

Silva S. S., Felipe M. G. A. and Mancilha I.M (1998), Factors that affect the byosynthesis of xylitol by xylose-fermenting yeasts: a review. Appl. Biochem. Biotechnol. **70/2**, 331–9.

Silva S. S., Quesada-Chanto A. and Vitolo M. (1997), Up stream parameters affecting the cell growth and xylitol production by *Candida guilliermondii. Z. Naturforsch.* **52 C**, 359–363.

Silva S. S., Ribeiro J. D., Vitolo M. and Felipe M. G. A. (1997), Maximizing the xylitol production from sugar cane bagasse hydrolysate by controlling the aeration rate. Appl. Biochem. Biotechnol. **63–65** : 557–63.

Spencer E. F. and Meade G. P. (1967), In: Manual de Azucar de cana. Barcelona: Mantaner y Simon (ed), 940 p.