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Biotechnological Production of Xylitol from Agroindustrial Residues

Evaluation of Bioprocesses

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ABSTRACT

Batch, fed-batch, and semicontinuous fermentation processes were used for the production of xylitol from sugarcane bagasse hemicellulosic hydrolysate. The best results were achieved by the semicontinuous fermentation process: a xylitol yield of 0.79 g/g with an efficiency of 86% and a volumetric productivity of 0.66 g/L/h.

Index Entries: *Candida guilliermondii*, sugarcane bagasse hemicellulosic hydrolysate, xylitol, batch, fed-batch, semicontinuous.

INTRODUCTION

Xylitol, a five-carbon sugar alcohol, has attracted much attention as a food sweetener because of its anticariogenic and cariostatic properties *(1,2)*. It can be used for the treatment of diabetes and disorders in lipid metabolism *(3,4)*. Although it is a constituent of many fruits and vegetables, its concentration levels are low, making its extraction very uneconomical *(5)*. Xylitol is currently obtained by catalytic hydrogenation of xylose with Raney-Nickel-catalysts *(6)*. This chemical process is very costly, since it demands a very pure xylose solution. An alternative method is the microbial conversion of the xylose present in agroindustrial residues.

A good and available source of D-xylose is sugarcane bagasse *(7,8)*. This residue is abundant in Brazil and represents a low-cost raw material for fermentation processes. It can be hydrolyzed with dilute acid to obtain a mixture of fermentable sugars, xylose being the major component *(9)*. In this hydrolysis, some byproducts are generated, such as acetic acid,

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furfural, phenolic compounds, and lignin-degradation products, which are potential inhibitors of microbial metabolism *(10,11)*. For the hydrolysate to become a suitable substrate for fermentation, these substances have to be removed by treatment with activated charcoal or cation-exchange resins *(12,13)*.

The yeast *Candida guilliermondii* FTI 20037, selected in our laboratory by Barbosa et al. *(14)*, is able to convert xylose to xylitol with high efficiency (81% of the theoretical value). The maximum theoretical yield of xylitol from D-xylose is 0.917 g/g *(14)*.

For most studies on batchwise production of xylitol by fermentation, Erlenmeyer flasks or bioreactors and synthetic medium were employed. Few reports describe the utilization of agroindustrial residues for xylitol production by biotechnological processes. This work evaluates some of these processes for obtaining xylitol from sugarcane bagasse hemicellulosic hydrolysate with a view to large-scale production.

MATERIALS AND METHODS

Hemicellulosic Hydrolysate

The hemicellulosic hydrolysate was obtained by acid hydrolysis of sugarcane bagasse, in a 250-L steel reactor under the following conditions: 121°C, 10 min reaction time and 100 mg sulfuric acid/g sugarcane bagasse (dry weight). After hydrolysis, the liquid was concentrated by heating at 70°C under vacuum, to obtain xylose at a concentration of 50–60 g/L. The hydrolysate was treated with CaO and aluminium sulfate *(15)* to minimize the inhibition of microbial metabolism.

Microorganism and Inoculum Preparation

A culture of *Candida guilliermondii* FTI 20037 was used. The cells were previously grown in a medium composed of hydrolysate supplemented with 20 g/L of rice bran extract, 0.1 g/L $CaCl_2/2H_2O$ and 5 g/L $(NH_4)_2SO_4$, in 125 mL Erlenmeyer flasks (50 mL of medium) placed on a rotatory shaker set at 200 revolutions/min at 30°C for 48 h. The initial cell concentration in all fermentations was 1.0 g/L (dry weight).

Fermentation Conditions

The fermentation medium used for obtaining the initial culture was the same described for the inoculum cultivation. The experiments were carried out in a 5-L fermenter (BIOFLO III, New Brunswick Scientific, New Brunswick, NJ) at 30°C, 300 revolutions/min, aeration of 0.4 vvm (volume of air per volume of medium per min), initial pH of 5.5.

The processes employed were batch, fed-batch, and semicontinuous. For the fed-batch fermentation a medium containing 79 g/L of xylose (supplemented with the aforementioned nutrients) was continuously fed at a

rate of 28 mL/h, using a peristaltic pump (Watson Marlow 505 S). The feeding was initiated after 65 h of batch cultivation. In the semicontinuous process, 67% of the fermented medium was removed after 63 h of batch fermentation. In all cases, samples were collected at different times to analyze cell concentration, xylose, xylitol, and pH.

Analytical Methods

Xylose, glucose, and xylitol were analyzed with a Shimadzu (Kyoto, Japan) high-performance liquid chromatograph (HPLC) using a refractive index (RI) detector and a BioRad (Hercules, CA) Aminex HPX-87H (300 × 7.8 mm) column at 45°C and 0.01 *N* H_2SO_4 as the eluant at a flow rate of 0.6 mL/min.

Furfural and hydroxymethylfurfural were measured by high-performance liquid chromatography using an ultraviolet (UV-VIS) detector and a Hewelett Packard RP18 (200 × 4.6 mm) column under the following conditions: acetonitrile (1:8) with 1% acetic acid as the eluant, 0.8 mL/min flow rate, column temperature 25°C, wavelength 276 nm, sample volume 20 μL.

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 (1 OD unit = 1.55 g dry weight cells/L).

RESULTS AND DISCUSSION

The basic composition of the sugarcane bagasse hydrolysate before and after concentration is shown in Table 1. Under the conditions used, a mixture of monosaccharides containing 78% xylose, 16% glucose, and 6% arabinose was obtained. The level of acetic acid (5.5 g/L) resulting from the decomposition of acetylated sugars was lower than the level normally found in wood hydrolysates (12 g/L) *(16,17)*. This difference was because of the lignocellulosic source and the hydrolysis conditions employed *(18)*. As reported by Felipe et al. *(19)*, acetic acid at concentrations up to 6 g/L is toxic for *Candida guilliermondii*. As the authors explained, the toxic effect of acetic acid is increased by a low pH value in the medium because of the acid's entry into the cell in its nondissociated form. Acetic acid inside the cell, especially under these conditions, may induce cytoplasm acidification *(20)*.

The progress of the fermentation runs is shown in Figs. 1–3. *Candida guilliermondii* was able to grow and accumulate xylitol in all processes (Table 2).

By means of the semicontinuous process, significant increases in the xylitol concentration and productivity were obtained both in relation to the batch process (84 and 128%, respectively) and in relation to the fed-batch process (191 and 275%, respectively). Also regarding the semicontinuous process, the maximum xylitol production was 34 g/L with a yield

Table 1
Basic Composition of the Sugarcane Bagasse Hemicellulosic Hydrolysate

	Hydrolysate (g/L)	
Components	Original	Concentrated
Glucose	5.5	8.0
Xylose	26.4	62.1
Arabinose	2.1	5.1
Acetic Acid	5.5	8.0
Furfural	<0.5	<0.1
Hydroxymethylfurfural	<0.1	<0.1

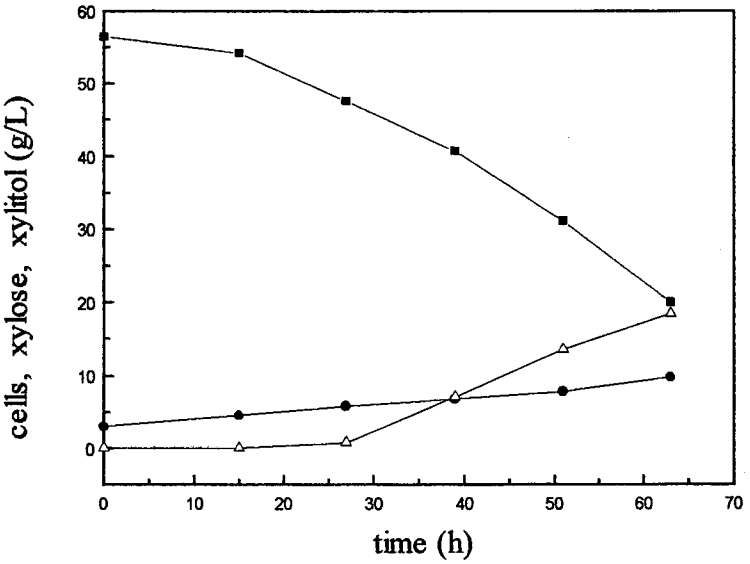

Fig. 1. Progress of the fermentation by batch process: (●) cell mass, (■) xylose, (Δ) xylitol.

of 0.79 g/g, a productivity of 0.66 g/L/h and an efficiency of 86%. Silva et al. *(21)*, using the same yeast in a batch cultivation in synthetic medium, had achieved a xylitol yield of 0.60 g/g and a productivity of 0.55 g/L/h.

Continuous and fed-batch culture techniques often provide better yields and productivities in the production of microbial metabolites than batch culture techniques *(22)*. In this study, however, the outcome of the fed-batch process was not any better, probably because of the fermentation conditions used, like the feeding rate.

For an effective xylitol production, the first critical step is the rapid production of cell mass in the culture medium. This could be achieved by maintaining the medium at a high level of aeration throughout the

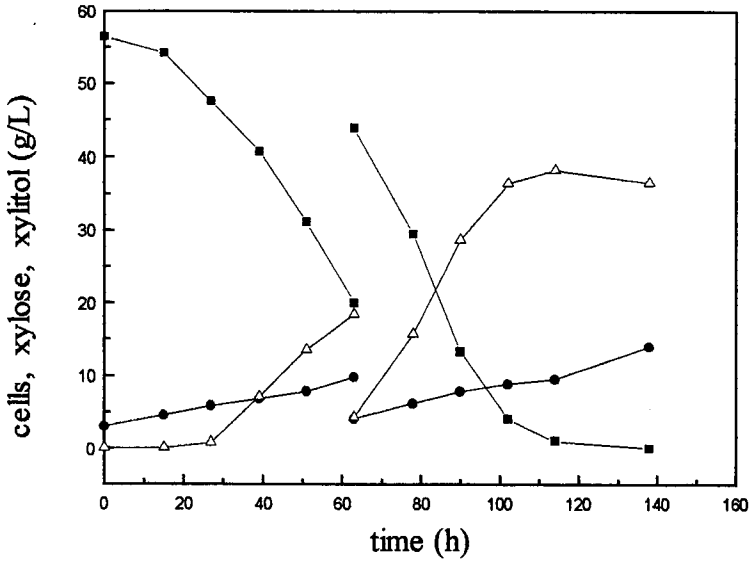

Fig. 2. Progress of the fermentation by semicontinuous process: (●) cell mass, (■) xylose, (Δ) xylitol (semicontinuous process started 63 h after batch).

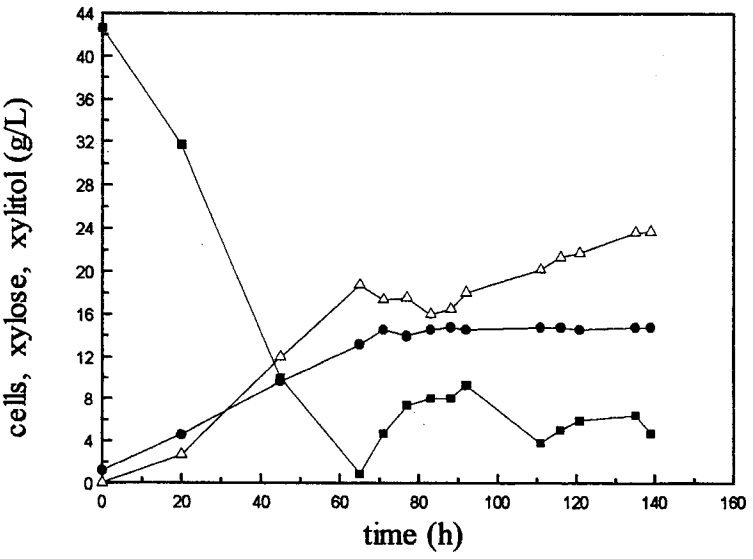

Fig. 3. Progress of the fermentation by fed-batch process: (●) cell mass, (■) xylose, (Δ) xylitol (feeding started 65 h and finished 92 h after batch).

fermentation, but in this case, cell mass would be produced instead of xylitol (23). The semicontinuous fermentation furthers cell adaptation to the fermented medium, thereby giving higher productivity rates than the batch process.

Table 2
Fermentations of the Sugarcane Bagasse Hemicellulosic Hydrolysate to Xylitol Using Different Fermentation Processes

Process	Maximum cell concentration (g/L)	Xylose consumption (%)	Acetic acid consumption (%)	Xylitol (g/L)	$Y_{P/S}$ (g/g)	Q_P (g/L/h)	Q_X (g/g/h)
Batch	9.8	65	58	18.4	0.50	0.29	0.04
Semi-continuous	5.5	98	65	34.0	0.79	0.65	0.12
Fed-batch	1.6	84	66	23.7	0.46	0.16	0.10

$Y_{P/S}$ product/substrate yield coefficient (g/g); Q_P: volumetric productivity (g/L/h); Q_X: specific rate of xylose consumption (g/g/h).

Felipe et al. *(19)*, using a synthetic medium containing xylose, studied the effect of acetic acid on xylitol production by *Candida guilliermondii*. For a medium containing 6 g/L of acetic acid, the xylitol yield was 0.66 g/g and the productivity 0.38 g/L/h. In this work, the concentration of acetic acid in the medium was 7.2–7.7 g/L. The results in Table 2 indicate a reduction in the inhibitory effect of the acetic acid during the semicontinuous process. Both the acetic acid and the xylose were consumed by the yeasts simultaneously, as previously observed by van Zyl *et al. (17)*.

The results lead to the conclusion that the hemicellulosic hydrolysate of sugarcane bagasse is a potential biomass for xylitol production by semicontinuous fermentation. Studies for the optimization and scale-up of this process shall be undertaken.

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