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Batch xylitol production from wheat straw hemicellulosic hydrolysate using *Candida guilliermondii* in a stirred tank reactor

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Abstract

Batch production of xylitol from the hydrolysate of wheat straw hemicellulose using *Candida guilliermondii* was carried out in a stirred tank reactor (agitation speed of 300 rpm, aeration rate of 0.6 vvm and initial cell concentration of 0.5 g l^{-1}). After 54 h, xylitol production from 30.5 g xylose l^{-1} reached 27.5 g l^{-1}, resulting in a xylose-to-xylitol bioconversion yield of 0.9 g g^{-1} and a productivity of 0.5 g l^{-1} h^{-1}.

Introduction

Biotechnological production of xylitol, a sweetener with outstanding organoleptic and anticariogenic properties, could be a cheaper alternative to the present industrial chemical reduction (Winkelhausen & Kuzmanova 1998). For this reason, our research group has been pursuing the development of a feasible technique for xylitol bioproduction from different lignocellulosic residues, like sugarcane bagasse (Silva *et al.* 1997), *Eucalyptus* wood chips (Canettieri *et al.* 2001) and rice straw (Mussato & Roberto 2001). These lignocellulosic residues, mainly composed of cellulose, hemicellulose and lignin, represent a renewable, widespread and cheap source of xylose that can be used as a substrate for xylitol bioproduction. Another residue generated in large amounts worldwide is wheat straw (Rosa *et al.* 1999). This material contains about 30–35% hemicellulose (Garde *et al.* 2002) and so it could also be profitably used as a raw material for the biotechnological production of xylitol.

The present study deals with xylitol production from wheat straw hemicellulosic hydrolysate in a stirred tank reactor. The results are compared with literature data regarding other lignocellulosic residues used as raw materials for xylitol bioproduction.

Materials and methods

Preparation and treatment of the wheat straw hemicellulosic hydrolysate

Wheat straw was hydrolysed at 145 °C for 30 min using a 2.5% (w/v) sulfuric acid solution. The solid/liquid ratio was 1:17.5 (w/v). After filtration, the hydrolysate was concentrated four-fold at 70 °C to increase the xylose concentration. The composition of the concentrated hydrolysate (g l^{-1}), determined by HPLC, was: glucose (8.4), xylose (39.6), arabinose (6.4), acetic acid (1.6), furfural (0.05) and hydroxymethylfurfural (0.12).

To minimize the concentrations of the main fermentation inhibitors, the concentrated hydrolysate was treated with calcium oxide to raise the pH to 7 and then with phosphoric acid to decrease the pH to 5.5. Next, active charcoal (10% w/v) was added to the hydrolysate, which was then agitated at 200 rpm and 30 °C for 1 h. In all the steps, the precipitates resulting from the pH adjustment and from the addition of active charcoal were removed by vacuum filtration. Afterwards, the hydrolysate was sterilised at 111 °C for 15 min to ensure aseptic conditions during the fermentation. Sugar

Table 1. Data on xylose-to-xylitol bioconversion by *Candida guilliermondii* using hydrolysates prepared from different raw materials.

Raw material	Operation mode	Reactor	X_0 ($g\ l^{-1}$)	S_0 ($g\ l^{-1}$)	P_F ($g\ l^{-1}$)	Q_P ($g\ l^{-1}\ h^{-1}$)	$Y_{P/S}$ ($g\ g^{-1}$)	Reference
Wheat straw	Batch	STR	0.5	30.5	27.5	0.5	0.9	Our work
Sugarcane bagasse	Batch	STR	0.5	62.1	41.8	0.9	0.7	Silva *et al.* (1997)
Sugarcane bagasse	Semi-continuous	STR	1	62.1	34	0.7	0.8	Rodrigues *et al.* (1998)
Sugarcane bagasse	Continuous	STR	0.5	51	18	0.7	0.7	Martinez *et al.* (2003)
Rice straw	Batch	EF	3	82	59	0.6	0.7	Mussato & Roberto (2001)
Eucalyptus	Batch	EF	3	60	10	0.1	0.2	Canettieri *et al.* (2001)

X_0: Initial cell concentration; S_0: initial xylose concentration; P_F: final xylitol concentration; Q_P: xylitol productivity; $Y_{P/S}$: xylose-to-xylitol bioconversion yield; STR: stirred tank reactor; EF: Erlenmeyer flasks.

degradation was not observed during the hydrolysate sterilisation.

Microorganism and inoculum cultivation

Candida guilliermondii FTI 20037, described by Barbosa *et al.* (1988), was used in the experiments. A loopful of cells was transferred to 125 ml Erlenmeyer flasks containing 50 ml medium consisting of ($g\ l^{-1}$): xylose (30), glucose (7), ammonium sulfate (2), calcium chloride (0.1) and rice bran extract (20), which was supplied by a local rice processing mill (Canas, São Paulo, Brazil). The cells were incubated for 24 h in a rotatory shaker (30 °C, 200 rpm), collected by a 15 min centrifugation at 2000 *g* and resuspended in sterile distilled water.

Medium and fermentation conditions

The sterilised hydrolysate was supplemented with ammonium sulfate ($1\ g\ l^{-1}$) and rice bran extract ($5\ g\ l^{-1}$) before being used as the fermentation medium. The xylose-to-xylitol bioconversion was carried out in a 5 l stirred tank reactor containing 3.5 l of fermentation medium with an initial cell concentration of $0.5\ g\ l^{-1}$ and operated at 30 °C for 60 h. The choice of agitation speed (300 rpm) and aeration rate (0.6 vvm) was based on previous works (Felipe *et al.* 1996, Silva *et al.* 1997, Rodrigues *et al.* 1998).

Analytical methods

Glucose, xylose, arabinose, xylitol and acetic acid concentrations were determined by HPLC using a refraction index detector and a Biorad Aminex HPX-87H column at 45 °C, 0.02 M H_2SO_4 as the eluent, flow rate of 0.6 ml min^{-1} and sample volume of 20 μl. Retention times (min) were as follows: glucose (9.1), xylose (9.8), arabinose (10.7), xylitol (11.4) and acetic acid (15.5). Furfural and hydroxymethylfurfural concentrations were determined by HPLC with detection at 276 nm and a RP18 column at 25 °C, acetonitrile/water (1:8 v/v) with 1% (v/v) acetic acid as the eluent, flow rate of 0.8 ml min^{-1} and sample volume of 20 μl. Retention times (min) were as follows: furfural (7.6) and hydroxymethylfurfural (5). Cell concentration for the inoculum was determined by its turbidity at 600 nm and correlated with the cell dry-weight through a corresponding calibration curve. Cell concentrations during the fermentations were determined by direct counting in a Neubauer chamber.

Results and discussion

Wheat straw hemicellulose was hydrolysed with sulfuric acid and the resulting hydrolysate was used for xylitol production by the yeast *Candida guilliermondii* in a stirred tank reactor after concentration and detoxification. Figure 1 shows the profiles of sugars and

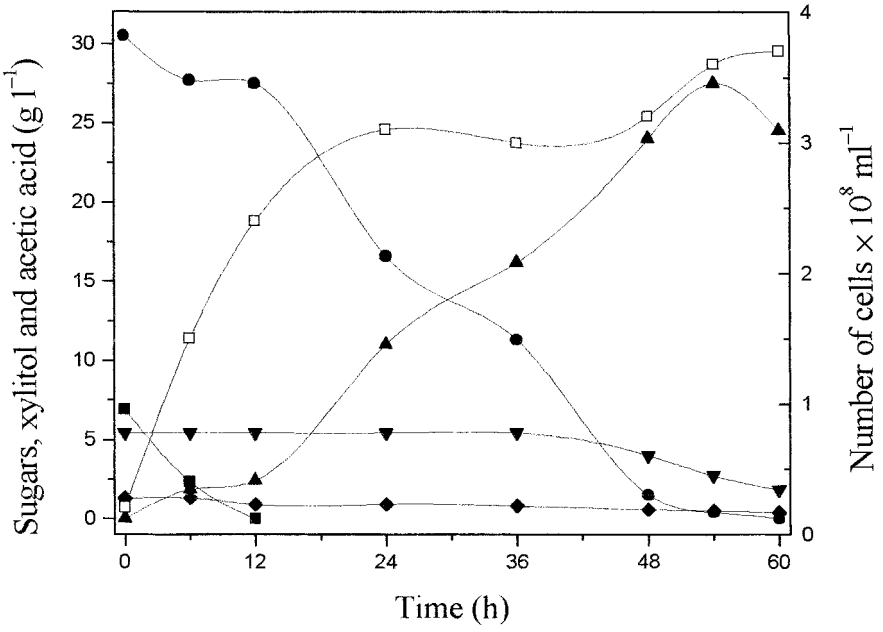

Fig. 1. Profiles of glucose (■), xylose (●), arabinose (▼) and acetic acid (♦) consumptions as well as of xylitol (▲) production and cell growth (□) during the xylose-to-xylitol bioconversion.

acetic acid consumption as well as of xylitol production and cell growth during the bioconversion. As can be seen, 27.5 g xylitol l^{-1} was produced after 54 h, the xylose-to-xylitol bioconversion yield being 0.9 g g^{-1} and the productivity 0.5 g l^{-1} h^{-1}. At the end of fermentation, all the xylose had been consumed and the cells started to consume the xylitol produced and excreted into the medium. A similar behaviour was previously observed by Felipe *et al.* (1996) using the same yeast in sugarcane bagasse hemicellulosic hydrolysate. While glucose was completely consumed within the first 12 h of fermentation, arabinose was not consumed before the almost complete exhaustion of xylose. Parajó *et al.* (1996) also observed a low arabinose consumption by *Debaromyces hansenii* in *Eucalyptus* hemicellulosic hydrolysate containing xylose. It is possible that the glucose present in the medium in the first 12 h of fermentation might have affected the xylose consumption by the yeast cells, leading to a lower consumption rate. Walther *et al.* (2001) have also reported a similar behaviour for *Candida tropicalis* cultivated in synthetic medium. Acetic acid, present in the medium at 1.3 g l^{-1}, which is lower than the threshold level of inhibition of 3 g l^{-1} previously determined by Felipe *et al.* (1997), was slowly consumed by the yeast cells during the fermentation.

As shown in Table 1, hemicellulosic hydrolysates prepared from sugarcane bagasse, rice straw and *Eucalyptus* wood chips have been used for xylitol production by *Candida guilliermondii* FTI 20037. The maximum xylitol concentration (59 g l^{-1}) was obtained from rice straw hydrolysate with a high xylose concentration (82 g l^{-1}), and the maximum xylitol productivity (0.9 g l^{-1} h^{-1}) was attained with sugarcane bagasse hydrolysate containing a relatively low initial cell concentration (0.5 g l^{-1}). From Table 1 it is also evident that the semi-continuous or continuous modes of operation used for fermentation of sugarcane bagasse hydrolysate did not lead to high xylitol productivities. The very low values of xylitol productivity and yield provided by *Eucalyptus* hydrolysate were attributed to high concentrations of inhibiting by-products of hydrolysis.

According to the data shown in Table 1, the best yield of xylose-to-xylitol bioconversion (0.9 g g^{-1}) was achieved in the present study, using wheat straw hemicellulosic hydrolysate and cultivating the cells under oxygen-limited conditions. This bioconversion yield was very close to the maximum theoretical yield (0.917 g g^{-1}) proposed for the yeast *Candida guilliermondii* FTI 20037 (Barbosa *et al.* 1988), evidencing the potentiality of wheat straw for xylitol bioproduction. Future work should focus on xylitol purification

and recovery from the fermented medium, thus allowing a better evaluation of the economic viability of the biotechnological production process.

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