Improvement in Xylitol Production from Sugarcane Bagasse Hydrolysate Achieved by the Use of a Repeated-Batch Immobilized Cell System

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Candida guilliermondii cells were immobilized in Ca-alginate beads and used for xylitol production from concentrated sugarcane bagasse hydrolysate during five successive fermentation batches, each lasting 48 hours. The bioconversion efficiency of 53.2%, the productivity of 0.50 g/l × h and the final xylitol concentration of 23.8 g/l obtained in the first batch increased to 61.5%, 0.59 g/l × h and 28.4 g/l, respectively, in the other four batches (mean values), with variation coefficients of up to 2.3%.

Introduction

Xylitol, a sweetener widely used in food and pharmaceutical industries, can be obtained by biotechnological means from xylose contained in lignocellulosic residues, such as sugarcane bagasse. Before its biological utilization, the hemicellulosic fraction of these residues needs to be hydrolysed. However, the hydrolytic step leads to appearance of several undesirable degradation products that impair the fermentative process by inhibiting the microbial metabolism (Felipe *et al.*, 1997).

The use of a repeated-batch fermentation system for producing xylitol from hemicellulosic hydrolysates is an efficient way to overcome the toxic effects of these inhibitors, since it promotes a good adaptation of the cells (Sene *et al.*, 1998). Together with this fermentative system, it is recommendable to use cell immobilization not only to enhance cell stability, but also to maximize the fermented broth fraction discarded at the end of each batch, facilitate the reutilization of the biocatalysts and minimize time and separation costs (Gamarra *et al.*, 1986). One of the most effective immobilization methods is gel entrapment, Ca-alginate being the commonest support used for the immobilization of viable cells, since it does not require drastic conditions (Vitolo and Carreira, 1992).

There are few publications on the use of immobilized cells for xylitol production from lignocellulosic residues (Domínguez *et al.*, 1999; Carvalho *et al.*, 2000). The present study evaluates the xylitol production from a concentrated hemicellulosic hydrolysate by Ca-alginate entrapped yeast cells in a repeated-batch fermentation system.

Materials and Methods

Preparation of the sugarcane bagasse hydrolysate

The hemicellulosic hydrolysate was obtained by acid hydrolysis of sugarcane bagasse in a 250-liter steel reactor. The bagasse was treated with 100 mg of sulfuric acid per gram of bagasse (dry-weight) at 121 °C for 10 min. After hydrolysis, the xylose concentration was increased threefold at 70 °C under vacuum. The concentrated hydrolysate was treated with calcium oxide, phosphoric acid and active charcoal as described by Alves *et al.* (1998).

Microorganism and inoculum cultivation

Candida guilliermondii FTI 20037, described by Barbosa *et al.* (1988), was maintained on malt extract agar slant at 4 °C. A loopful of cells was transferred to 125-ml Erlenmeyer flasks containing 50 ml of medium consisting of xylose (30 g/l), ammonium sulfate (3 g/l), calcium chloride (0.1 g/l) and rice bran (20 g/l). The inoculum was cultivated in a rotatory shaker at 200 rpm and 30 °C for 24 h. Afterwards, the cells were harvested by

centrifugation (2000 x*g*, 15 min), rinsed thoroughly with sterile distilled water, centrifuged and resuspended in sterile distilled water.

Cell immobilization

The yeast cells were immobilized by entrapment in calcium alginate beads. An adequate volume of the cell suspension was added to a solution of sodium alginate (SG 800 – Sanofi do Brasil Indústria e Comércio Ltda., Brazil) previously heated at 121 °C for 15 min. The final concentration of sodium alginate was 20 g/l and the final concentration of cells was 3 g/l (dry-weight). Cell-gel beads (2.7 mm in diameter) were produced by dripping this suspension in a 11 g/l calcium chloride solution, using a 19-G needle (1 ½ inch) and a peristaltic pump. The beads were maintained in the calcium chloride solution at 4 °C for 24 h. Afterwards, they were washed with sterile distilled water and introduced into the fermentation flasks.

Medium and fermentation conditions

The treated hydrolysate was heated at 110 °C for 15 min and supplemented with ammonium sulfate (3 g/l), calcium chloride (0.1 g/l) and rice bran (20 g/l) before being used as a fermentation medium. Duplicate repeated-batch fermentation runs were carried out in 125-ml Erlenmeyer flasks containing 10 ml of immobilized biocatalysts (void volume neglected) and 40 ml of fermentation medium. The flasks were maintained in a rotatory shaker at 200 rpm and 30 °C for 48 h. At the end of each batch, the fermented medium was discarded and the immobilized biocatalysts were refed with fresh medium.

Analytical methods

The xylose and xylitol concentrations were determined by HPLC as described by Carvalho *et al.* (2000). The cell concentration used in the immobilization step was determined by absorbance at 600 nm and correlated with the dry-weight of the cells through a corresponding calibration curve. The suspended cell concentrations in the fermentation medium (number of suspended cells/ml medium) were determined by direct counts in a Neubauer chamber. The immobilized cell concentrations in the Ca-alginate beads (number of immobilized cells/ml beads) were measured in the same way, after dissolving the beads in a 20 g/l potassium citrate solution. Both immobilized and suspended cell concentrations were also related to the reactor working volume (50 ml) and expressed as number of immobilized cells/ml reactor working volume and number of suspended cells/ml reactor working volume, respectively.

Results and Discussion

As can be verified from Table I, the xylose-to-xylitol bioconversion efficiency increased after the first fermentation batch, leading to increases in the xylitol concentrations and productivities in the last four batches. However, Sene *et al.* (1998), using free cells of the same yeast strain under similar conditions, did not observe marked differences in the xylose-to-xylitol bioconversion efficiency throughout five successive batches.

It is well known that xylitol is formed as a metabolic intermediary of D-xylose fermentation by yeasts through the oxido-reductive pathway shown in Fig. 1. Under oxygen-limiting conditions the xylitol excretion is maximized, because the NADH

Table I. Fermentative performance of the immobilized cell system during the five successive fermentation batches (averages of two repetitions).

	Batch					VC*
	1st	2nd	3rd	4th	5th	(%)
Xylose consumed (%)	94.6	97.8	97.8	97.4	96.9	0.4
Xylitol produced (g/l)	23.8	28.3	28.6	28.9	27.6	2.1
Bioconversion efficiency (%)¶	53.2	59.5	62.6	62.4	61.4	2.3
Productivity (g/l × h)§	0.50	0.59	0.60	0.60	0.58	1.7

* Variation coefficient [(standard deviation/mean)x100] determined for the last four batches.
¶ [(xylitol produced/xylose consumed)/0.917]x100 (Barbosa *et al.,* 1988).
§ [(xylitol concentration/fermentation time)].

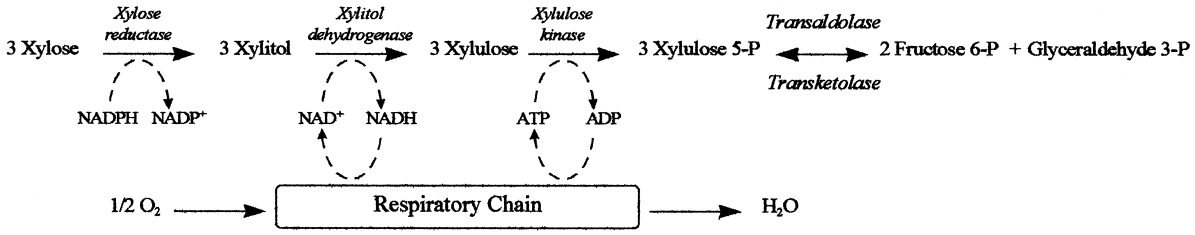

Fig. 1. Schematic diagram of the initial steps of D-xylose metabolism in *Candida guilliermondii.*

coenzyme can not be completely re-oxidized to NAD^+ by the respiratory chain. On the contrary, under aerobic conditions, mainly cell mass is produced (Hahn-Hägerdal *et al.*, 1994).

Fig. 2 shows the concentrations of suspended and immobilized cells determined during the first and fifth batches, since the other three batches did not differ significantly from the last one. Since the immobilized cell concentration determined at the end of the first batch was very close to the immobilized cell concentrations determined during the other four batches, the improvement in the bioconversion

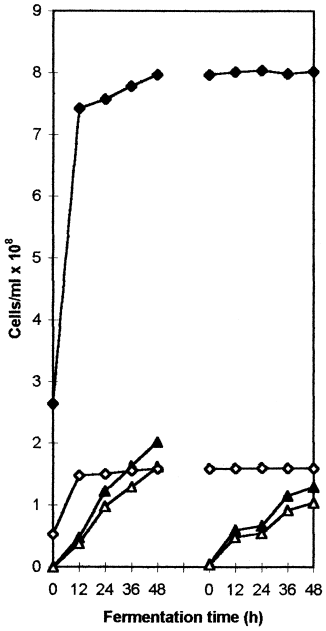

Fig. 2. Number of suspended cells / ml of fermentation medium (▲), number of suspended cells / ml of reactor working volume (△), number of immobilized cells / ml of beads (◆) and number of immobilized cells / ml of reactor working volume (◇) during the first and fifth fermentation batches (averages of two repetitions).

efficiency observed after the first fermentation batch may have resulted from limitations in the growth of the immobilized cells. These findings suggest that the oxygen transfer from the bulk fermentation medium to the alginate beads limited the cell growth after the first batch, maximizing the xylitol production by the immobilized cells.

In fact, the xylose-to-xylitol bioconversion efficiency of 19.3% observed during the first 12 h of fermentation in the first batch, when the rate of immobilized cell growth was the highest, increased significantly to 51.6, 54.2, 54.8 and 57.3%, in the same period of time, during the subsequent batches, when the immobilized cells stopped growing. Consequently, the xylitol production of 2.3 g/l observed during the first 12 h of fermentation in the first batch increased to 9.2, 8.7, 9.2 and 8.8 g/l, in the same period of time, during the other four batches. The results of the first and fifth batches are shown in Fig. 3, since the xylose consumption and xylitol production profiles observed during the other three batches did not differ significantly from the last one.

As can be seen in Fig. 2, free cells grew in the fermentation medium in all the batches as a consequence of cell release by the calcium alginate beads in the beginning of the fermentations. A similar behavior was also observed by Yahashi *et al.* (1996) in a synthetic xylose medium.

It is clear that the use of high cell concentrations can be ensured by cell immobilization with Ca-alginate beads, since the number of immobilized cells per ml of beads was significantly higher than the number of suspended cells per ml of medium in all the fermentation batches. According to Felipe *et al.* (1997), the use of high cell concentrations also helps to reduce the toxic effects of the hydrolysate on the activity of the microorganisms. Unfortunately, the small proportion of the volume of Ca-

alginate beads (10 ml) to the volume of fermentation medium (40 ml) used in this study brought about similar concentrations of suspended and immobilized cells (when both of them are related to the reactor working volume) in the reactor at the end of all the fermentation batches. Therefore, the present data apply more properly to a mixed immobilized/free yeast system than to an immobilized cell system. This pronounced amount of free cells in comparison with the amount of immobilized cells might have accounted for the low overall bioconversion efficiency at the end of the fermentation batches.

It should be noticed that the sodium alginate and the immobilization conditions used in this study allowed the reutilization of the immobilized cells during five successive fermentation batches without any bead solubilization.

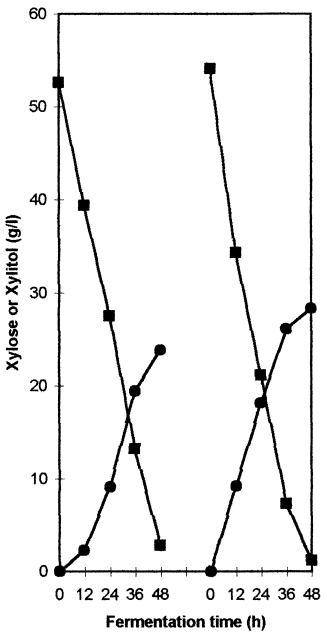

Fig. 3. Xylose consumption (■) and xylitol production (●) during the first and fifth fermentation batches (averages of two repetitions).

Acknowledgements

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