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A Preliminary Information About Continuous Fermentation Using Cell Recycling for Improving Microbial Xylitol Production Rates

Scientific Note

Silvio S. Silva,*[,1] Adolfo Q. Chanto,[2] Michele Vitolo,[3] Maria G. A. Felipe,[1] and Ismael M. Mancilha[1]

[1]Department of Biotechnology, Faculty of Chemical Engineering of Lorena, FAENQUIL/DEBIQ, Rod. Itajubá/Lorena km 74.5 Caixa Postal 116-12600-000, Lorena-São Paulo, Brazil, E-mail: silvio@debiq.faenquil.br;
[2]Biotechnological Development Center (CDB), Joinville, SC, Brazil;
[3]Department of Biochemical and Pharmaceutical Technology, University of São Paulo, SP, Brazil

Abstract

Xylitol is a sugar-alcohol with important technological properties, such as anticariogenicity, low caloric value, and negative dissolution heat. It can be used successfully in food formulations and pharmaceutical industries. Its production is therefore in great demand. Biotechnological xylitol production has several economic advantages in comparison with the conventional process based on the chemical reduction of xylose. The efficiency and the productivity of this fermentation chiefly depends on the microorganism and the process conditions employed. In this article a simple continuous culture with cell recycling was evaluated to enhance the capability of *Candida guilliermondii* FTI 20037 to produce xylitol. The fermentation was initiated batchwise by directly inoculating the grown seed culture in a 2-L bench-scale fermentor. Continuous feeding was begun at a dilution rate (D) of 0.060/h after the xylose concentration had completely consumed and the cell concentration was about 4.0 g/L. At a dilution rate of 0.060/h the xylitol concentration was about 15 g/L and increased by about 35%, whereas the dilution rate decreased by about 58%. Furthermore, the volumetric productivity, Q_p, markedly depended on the dilution rate, diminishing by about 37% as D was changed from 0.060 to 0.025/h. These preliminary results show us that the

*Author to whom all correspondence and reprint requests should be addressed.

continuous fermentation with cell recycling is a good way to study the xylitol production by xylose-fermenting yeasts.

Index Entries: Xylitol; yeast; continuous fermentation; recycling.

Introduction

Xylitol has valuable properties that have drawn the attention of the food and pharmaceutical industries. One of these properties is that xylitol is a sweetener with anticaries effects, since it can not be metabolized by mouth bacteria. In addition, its absorption by cells does not depend on insulin. For these reasons, xylitol is suitable for use both in toothpastes and in diabetic foods *(1)*. Currently, this polyalcohol is produced by catalytic hydrogenation of xylose extracted from hemicellulosic hydrolysates *(2)*. However, some yeast strains can convert xylose into xylitol under much milder conditions than those required by the chemical process and with no need of pure xylose *(3)*. Adequate temperature, pH, aeration rate, and cell concentration are very important for the success of this biotechnological process *(4–6)*. Because different strains have different demands, these parameters must be adapted to each particular strain. In this preliminary study, *Candida guilliermondii* FTI 20037 *(7)* was used to produce xylitol from xylose, and a continuous culture with cell recycling was evaluated mainly for improving cell and xylitol production rates.

Materials and Methods

Microorganism

C. guilliermondii FTI 20037, described by Barbosa et al. *(7)*, was employed in all fermentations. The culture was maintained on malt extract agar and stored at 4°C.

Inoculum Preparation

C. guilliermondii cells were inoculated in 500-mL Erlenmeyer flasks containing 200 mL of medium (5.0 g/L $(NH_4)_2SO_4$, 1.0 g/L yeast extract, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.1 g/L $CaCl_2 \cdot 2H_2O$, 1.0 g/L KH_2PO_4, and 30 g/L xylose). After 40 h of incubation at 30°C on a rotary shaker (200/min), this culture was used to inoculate the bioreactor.

Culture Conditions

All fermentations were carried out in a 2-L bench-scale fermentor containing the same medium used for the inoculum preparation, except that the xylose concentration was adjusted to 50 g/L. The fermentations were started as batch processes. After 60 h, when the initial xylose was completely consumed and the cell concentration was about 4.0 g/L, the medium was continuously poured into the fermentor. The continuous culture was carried out at pH 4.0, 30°C, agitation of 300 rpm, aeration of 20 mL/min, and dilution rate of 0.060 or 0.025/h.

Cell Recycling

Cell recycling was performed by continuously pumping the contents of the fermentor through an ultrafiltration module (UF Module, Fresenius, Germany) with an ultraporous polysulfone capillary, 500 μm in diameter, and 0.01 μm in pore diameter. The changes in optical density were measured on-line with a through-flow photometer and the data were recorded by the process controller coupled to the fermentor.

Analytical Methods

Cell mass was determined by measuring absorbance at 600 nm. The xylose and xylitol concentrations were measured through high-performance liquid chromatography (HPLC) with a Sugar Pack column, refractometer index (Waters, Division of Millipore, Milford, MA) and water as mobile phase.

Results and Discussion

Cell recycling in a continuous fermentative process can lead to an increase in the productivities of some biotechnological processes for the production of propionic acid and biomass, such as *Schizosaccharomyces pombe (8,9).* Therefore, this approach was employed to improve the xylitol production by *C. guilliermondii* FTI 20037 at dilution rates of 0.060/h or 0.025/h.

At the dilution rate of 0.060/h a pseudosteady state was attained after 188 h of continuous feeding, and the cell, xylose and xylitol concentration values were respectively 13, 26, and 15 g/L (Fig. 1). The dilution rate was then reduced to 0.025/h, a new pseudosteady state occurring after 129 h, with cell, xylose, and xylitol concentration values of 13, 20, and 23 g/L, respectively (Fig. 1). As can be seen, the xylitol concentration increased by about 35%, whereas the dilution rate decreased by about 58%. The increase in xylitol concentration can be attributed to a diminution in xylitol dehydrogenase (XD) activity inside the cell, because of inhibitory effects on both biosynthesis and catalysis. Another possible explanation for these results is the reduction of the amount of XD intracellular cofactor (nicotinamide-adenine dinucleotide, NAD^+). Because the internal concentrations of cofactors, such as nicotinamide-adenine dinucleotide (NADH), nicotinamide-adenine dinucleotide phosphate (NADPH), NAD^+, and nicotinamide-adenine dinucleotide phosphate (NADP) (essential in the xylose metabolism), depend on the whole catabolic reduction charge *(10),* which, in turn, is connected with the mitochondrial activity *(11)*, the XD activity might ultimately depend on the dissolved oxygen availability. Perhaps this high cell concentration promotes a high oxygen-limited condition that favors the xylitol production and excretion in the broth.

Table 1 shows that when the dilution rate was reduced from 0.060/h to 0.025/h, the xylose-specific consumption rate (Q_S), xylitol specific pro-

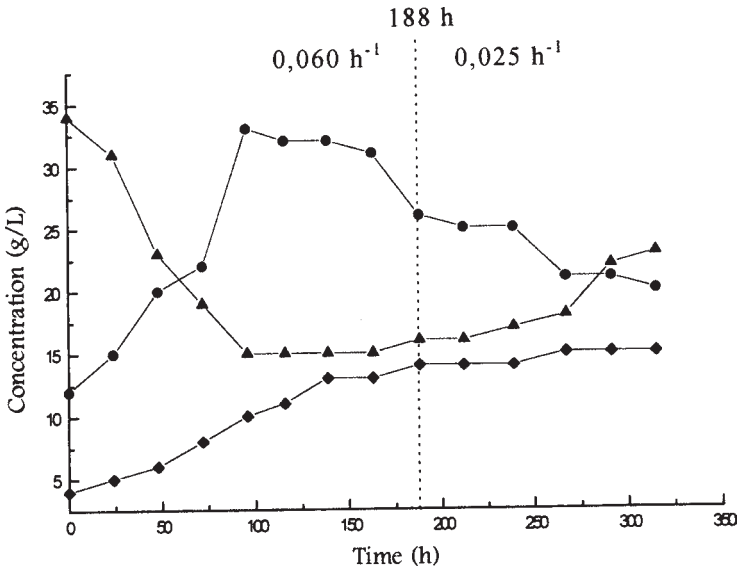

Fig. 1. Pseudo steady-state concentrations as a function of time for xylose/xylitol bioconversion by *C. guilliermondii* FTI 20037 in a continuous culture with cell recycling. —◆—, dry weight; —●—, xylose; —▲—, xylitol.

Table 1
Parameters Related to the Xylose/Xylitol Bioconversion Through a Continuous Culture with Cell Recycling

D, h^{-1}[a]	Xylose,[b] g/L	Q_S, g/g · h	q_P, g/g · h	Q_P, g/L · h	$Y_{P/S}$, g/g	$Y_{X/S}$, g/g
0.060	18.1	0.080	0.070	0.91	0.84	0.78
0.025	29.5	0.050	0.030	0.58	0.78	0.52

[a]D, dilution rate; Q_S, xylose uptake rate; q_P, specific xylitol production; Q_P, volumetric productivity; $Y_{P/S}$, xylitol produced/xylose consumed; $Y_{X/S}$, biomass produced/xylose consumed.

[b]Xylose, xylose in the fermented medium.

ductivity (q_P), volumetric productivity (Qp), xylitol/xylose yield ($Y_{P/S}$), and xylose/cell yield ($Y_{X/S}$) diminished by approx 38, 57, 36, 7, and 33%, respectively. However, the volumetric productivity of 0.91 g/L · h was at least 50% higher than those attained in batch culture (0.47 g/L · h) *(12)* and in continuous culture without cell recycling (0.51 g/L · h) (data not published). A similar result was observed in the continuous propionic acid production by Boyaval and Corre *(13).* According to these authors, such a result could derive from physiological perturbations suffered by the yeast resulting from nonspecific inhibition, substrate limitation, and/or change in the intracellular water activity. In addition, high cell concentration, as occurs in the cell recycling approach, can inhibit growth and/or the whole-cell metabolic capability *(14).* To overcome these problems, Groot et al. *(15)*

suggested working at high aeration rates; otherwise, the high amount of cell would promote a dissolved O_2 shortage, which should cause perturbation in the intracellular oxireduction power at the end.

These preliminary results encourage us to further explore this type of fermentation system; for example, using different dilution and aeration rates, mainly to improve the microbial xylitol production rates.

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