Upstream Parameters Affecting the Cell Growth and Xylitol Production by *Candida guilliermondii* FTI 20037

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The effects of yeast extract (0–10 g/l), methanol (0–10% v/v), acetic acid (0–1.0 g/l), furfural (0–0.5 g/l), glucose (0–30 g/l), inoculum age (15–70 h) and product concentration (18–230 g/l) on the xylose-xylitol conversion by *Candida guilliermondii* FTI 20037 were studied. The xylitol specific productivity increased about 35% at a yeast extract concentration of 1.0 g/l, whereas glucose showed a strong inhibitory effect on the xylitol production and a stimulating effect on the growth of *C. guilliermondii*. Methanol, acetic acid and furfural under the employed concentrations did not show any positive effect neither on the growth or on the xylose-xylitol conversion by the yeast. The inoculum age showed a strong influence on xylitol formation and the best fermentative parameters were attained using a 40-h inoculum age. A xylitol concentration in the fermentation medium higher than 80 g/l inhibited markedly the xylitol productivity by the yeast *C. guilliermondii*.

Introduction

Xylitol is a polyalcohol having great interest in food (chewing gums, tablets, diet food) and cosmetic (tooth paste) industries, due to its sweetening and anticariogenic properties. Besides, it does not need insulin for metabolism by the body which makes it interesting to diabetics (Hyvönen *et al.*, 1982; Bar, 1986; Wäler *et al.*, 1992; Aguirre-Zero *et al.*, 1993).

Xylitol is currently produced by catalytic hydrogenation of xylose obtained from hemicellulosic hydrolyzates (Jaffe *et al.*, 1974; Melaja and Hämäläinen, 1977). However, there are some yeast strains which can produce directly xylitol from xylose present in hemicellulosic hydrolyzates under milder conditions than those of the chemical process (Horitsu *et al.*, 1990; Felipe *et al.*, 1993; nolleau *et al.*, 1993; Silva *et al.*, 1994). In order to use microorganisms to produce xylitol utilizing hemicellulosic hydrolyzates from waste biomass, *Candida guilliermondii* FTI 20037 grown in a semisynthetic medium was used in order to evaluate the effect of some nutrients, such as yeast extract and, the effect of some compounds present in hemicellulosic hydrolyzates, such as acetic acid, furfural and glucose. The effects of the age of the inoculum and the xylitol concentration on the xylose/xylitol bioconversion were also evaluated. These are important upstream parameters that need to be evaluated in order to develop an efficient process for a large-scale xylitol fermentation.

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Materials and Methods

Microorganism

The experiments were carried out using the yeast *Candida guilliermondii* FTI 20037 described by Barbosa *et al.* (1988).

Inoculum preparation

Candida guilliermondii FTI 20037 cells obtained from a stock culture in malt extract agar at 4 °C were inoculated into fermentation medium containing the following nutrients: 5.0 g/l $(NH_4)_2SO_4$, 1.0 g/l yeast extract, 0.5 g/l $MgSO_4 \times 7H_2O$, 0.1 g/l $CaCl_2 \times 2H_2O$, 1.0 g/l KH_2PO_4 and 30.0 g/l xylose. The yeast was incubated in 500 ml Erlenmeyer flasks containing 200 ml medium, which was incu-

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bated at 30 °C in a rotatory shaker (200 min^{-1}) for 40 h. After that, the flask content was inoculated into the fermenter to give a cell concentration of 0.5 g/l.

Media and fermentation conditions

The tests were conduced in a semisynthetic medium consisting of the following nutrients: 5.0 g/l $(NH_4)_2SO_4$, 1.0 g/l yeast extract, 0.5 g/l $MgSO_4 \times 7H_2O$, 0.1 g/l $CaCl_2 \times 2H_2O$, 1.0 g/l KH_2PO_4 and 70 g/l xylose. The whole media composition was different depending on the test, as it will be explained in each case. All fermentations were carried out in a 2.5 l bench-fermenter containing 1.5 l of the medium at 30 °C, agitation of 300 r.p.m., aeration of 20 ml/min and pH 2.5.

Analytical Methods

The fermentation was monitored by taking samples for the measurement of xylose, xylitol and cell mass. The xylose and xylitol concentrations were measured by HPLC (Waters-Millipore-USA); with refractive index detector (differential refractometer – R401) and a sugar-PAK column, at 85 °C. Deionized water was used as eluent at a flow rate of 0.5 ml/min. The cell mass was estimated using a Pharmacia-Novaspec II spectrophotometer at 600 nm by the correlation of absorbance with cell dry weight (the washed cells were dried at 90 °C for 24 h).

Results and Discussion

Effect of the yeast extract

The data presented in Table I, show the effect of yeast extract (at concentrations of 0, 1.0, 5.0 and 10.0 g/l) on the growth and the xylitol production by *C. guilliermondii* FTI 20037. The yield factor for the xylose-biomass conversion increased about 65% when the yeast extract concentration varied from 0 to 10.0 g/l. Such a result is reasonable, due to the high nutrient content suitable for cell grow present in yeast extract. Nevertheless, the best yeast extract concentration for xylitol production was 1.0 g/l, indicatives that the best growing condition is not necessarily the same for xylitol biosynthesis. As pointed out by Silva *et al.* (1996) the conversion of xylose into biomass and/or xylitol depends on the intracellular balance between xylose reductase and xylitol dehydrogenase activities. This result is not in accordance with Furlan (1991), who found a significant increase on xylitol production by *Candida parapsilosis* grown in a medium containing 10.0 g/l of yeast extract. Probably, each strain of yeast has a particular response to this nutrient.

Effect of methanol

There is evidence that methanol increases the production of xylitol in some yeasts and fungi (Vongsuvanlert and Tani, 1989; Dahiya, 1991). Thus, its effect on the xylitol production by *C. guilliermondii* FTI 20037 was studied. The methanol concentrations employed were 0, 0.5, 1.0, 3.0, 6.0 and 10% (v/v).

From Table I, it is clear that methanol concentrations above 3.0% strongly inhibited growth and the capability of the yeast to produce xylitol. Furthermore, the final cell concentration decreased about 24% in the presence of 6.0% of methanol. In spite of that reduction, the yield factor for the xylose-biomass conversion was not affected by this methanol concentration. By a methanol concentration of 10% the growth was fully inhibited.

Vongruvanlest and Tani (1989) observed that methanol at a concentration of 2% had a positive effect on the xylitol production by *Candida boidinii*. According to these authors, the methanol oxidation by this yeast lead to the increase of NADH, which, in turn, acting as a coenzyme for xylose reductase, enhanced the xylose-xylitol conversion. In the case of *C. guilliermondii* FTI 20037, in which methanol concentrations between 1% and 3% were not significantly inhibitory to the cell (Table I), a similar explanation could not be assumed, because the xylose reductase of *C. guilliermondii* is not NADH, but NADPH – dependent (Silva *et al.*, 1996).

Effect of acetic acid

The data published until now indicate that, on one hand, the acetic acid is a strong inhibitor of xylose metabolism (van Zyl *et al.*, 1991; Ferrari *et al.*, 1992), on the other hand, at concentration of about 1.0 g/l can enhance the growth (Lee and McCaskey, 1983). Moreover, acetic acid occurs in low concentration in hemicellulosic hydrolyzates

Table I. Effect of upstream parameters on the xylitol production rates by *Candida guilliermondii* growing on semi-synthetic medium.

Upstream parameters	Concentration [g/l]	X [g/l]	P [g/l]	$Y_{P/S}$ [g/g]	$Y_{X/S}$ [g/g]	δ [g/l h]	Q_p [g/g h]
Yeast extract	0.0	1.5	17.3	0.35	0.03	0.18	0.12
	1.0	2.5	43.2	0.72	0.04	0.45	0.18
	5.0	4.0	28.8	0.66	0.08	0.30	0.07
	10.0	6.5	24.0	0.58	0.09	0.25	0.04
Methanol	0.0	3.19	44.0	0.68	0.05	0.37	0.12
	5.0	3.22	43.8	0.65	0.04	0.37	0.12
	10.0	3.16	44.2	0.64	0.04	0.37	0.12
	30.0	3.53	41.1	0.62	0.05	0.33	0.09
	60.0	2.70	18.4	0.35	0.05	0.15	0.05
Acetic acid	0.0	2.95	50.0	0.70	0.04	0.52	0.18
	0.5	3.37	50.0	0.68	0.04	0.50	0.15
	1.0	3.27	43.0	0.66	0.04	0.46	0.13
Furfural	0.0	2.6	48.0	0.69	0.04	0.50	0.19
	0.3	3.0	47.0	0.71	0.05	0.49	0.16
	0.5	2.5	45.0	0.70	0.04	0.48	0.19
Glucose	0.0	2.65	48.6	0.72	0.04	0.52	0.20
	10.0	3.47	25.4	0.62	0.07	0.21	0.07
	20.0	4.14	12.6	0.35	0.08	0.11	0.03
	30.0	3.97	8.7	0.32	0.07	0.07	0.02
Xylitol	0	2.80	53.4	0.76	0.04	0.56	0.19
	18	2.80	51.1	0.74	0.04	0.53	0.19
	55	2.70	50.3	0.75	0.04	0.54	0.20
	80	2.10	39.4	0.56	0.03	0.42	0.19
	160	2.10	24.4	0.35	0.03	0.25	0.12
	230	2.20	18.5	0.33	0.03	0.17	0.22
Inoculum age	15 h	2.78	40.0	0.61	0.04	0.47	0.27
	26 h	2.80	50.1	0.70	0.04	0.56	0.28
	40 h	2.53	46.0	0.69	0.04	0.52	0.29
	70 h	3.23	46.5	0.69	0.04	0.52	0.23

X: cell dry weight; P: xylitol concentration; $Y_{P/S}$: product yield (xylitol produced/substrate consumed), $Y_{X/S}$: cell yield (biomass produced/substrate consumed), δ: volumetric productivity (xylitol produced/fermentation time), Q_p, specific productivity (xylitol produced/cell mass produced/fermentation time).

from woods. That is why, in this study, its effect on the fermentative capability of *Candida guilliermondii* FTI 20037 was analysed at concentrations of 0, 0.5, and 1.0 g/l. In the concentrations used in this work, this acid showed neither inhibitory nor stimulating effect on the growth of *C. guilliermondii* or on its xylose-xylitol conversion (Table 1). The effect of acetic acid depends much on the growth conditions, such as pH, temperature, aeration, being the inhibition at low pH values stronger. Many different effects have been reported in the literature in this respect, with different strains and conditions (Rodrigues-Alves *et al.*, 1992, Felipe *et al.*, 1995).

Effect of furfural

As furfural is always present in hemicellulosic hydrolyzates from woods, and considering the possible effect on cell metabolism (Azhar *et al.*, 1981), its effect on *C. guilliermondii* FTI 20037 was analysed using three different concentrations (0, 0.3 and 0.5 g/l). From Table I, it is evident that furfural did not influence the fermentative performance of this yeast under the used conditions.

Effect of glucose

Glucose is also always present in hemicellulosic hydrolyzates from wood, so its effect was studied

at concentrations of 0, 10, 20 and 30 g/l. The total sugar concentration was kept at 70 g/l, being modified only the ratio xylose:glucose (6:1, 5:2 and 4:3). It is clear from Table I that glucose inhibited strongly the xylitol production by *C. guilliermondii* FTI 20037. Similar result was found by Slininger *et al.* (1985) and du Preez *et al.* (1986) with *C. shehatae*. There are two hypothesis to explain such a result. The first of them considers the occurrence of a competition between glucose and xylose for the sugar transport system located into the plasmalemma of the cell (Webb, 1990). The other assumes the glucose repression on the xylose catabolic enzymes (Lee 1992). Anyway, it is very important to optimize the hydrolysate production, in order to minimize the amount of glucose produced during this process.

Effect of the inoculum age

The inoculum age clearly affected the xylitol production, the volumetric productivity and the yield of xylitol in relation to xylose consumed (Table I). For instance, with the 15 h-age inoculum, P, δ and $Y_{P/S}$ (see Table I for abbreviations) were respectively 20%, 16% and 13% lower than those observed for the 26 h-age inoculum. Thus, we can assume that the 15 h-age cells were not full metabolically active. It must be pointed out that P, δ and $Y_{P/S}$ were practically independent of the inoculum, with an age higher than 26 h. This means that even 70 h-age cells were still active, although a decrease around 21% in the specific xylose consumption (Q_S) was observed (Table I).

There is no information in the literature about the effect of the inoculum age on the xylose-xylitol conversion parameters for *C. guilliermondii* FTI 20037. Nevertheless, Silva *et al.* (1996) showed a possible correlation between inoculum age and xylose-reductase activity, a key intracellular enzyme in the convertion of xylose into xylitol. Accordingly, cells having high xylose-reductase activity (about 564 Units of enzymatic activity /mg of protein) were attained when a 40 h-age inoculum was used.

Effect of the product concentration

Table I, shows that initial xylitol concentrations higher than 80.0 g/l inhibited markedly the xylitol productivity by *C. guilliermondii* FTI 20037, indicative of a kind of product inhibition on the conversion of xylose-xylitol. To improve the xylitol production, a system can be developed, that allows to remove the product continuously, thus maintaining its concentration below 80.0 g/l.

Conclusions

The xylitol production has created a market for it large scale utilization by food and odontological industries. The biological synthesis of xylitol appears to be very interesting mainly due a low-cost technology. With this study we improved our understanding of the xylitol fermentation by *Candida guilliermondii* FTI 20037 by studying several important physiological and upstream parameters for large-scale xylitol production.

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