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XYLITOL PRODUCTION FROM D-XYLOSE BY *CANDIDA GUILLERMONDII*: FERMENTATION BEHAVIOUR

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SUMMARY The ability of *Candida guillermondii* to produce xylitol from xylose and to ferment individual non xylose hemicellulosic derived sugars was investigated in microaerobic conditions. Xylose was converted into xylitol with a yield of 0,63 g/g and ethanol was produced in negligible amounts. The strain did not convert glucose, mannose and galactose into their corresponding polyols but only into ethanol and cell mass. By contrast, fermentation of arabinose lead to the formation of arabitol. On D-xylose medium, *Candida guillermondii* exhibited high yield and rate of xylitol production when the initial sugar concentration exceeded 110 g/l. A final xylitol concentration of 221 g/l was obtained from 300 g/l D-xylose with a yield of 82,6 % of theoretical and an average specific rate of 0,19 g/g.h.

INTRODUCTION

Xylitol, a naturally occuring five carbon polyalcohol, finds increased use as a sweetener in foods in relation with its higher sweetening power, its greater negative heat of solution and its anticariogenic property than common polyols (Pepper and Olinger, 1988). Furthermore, it may be used clinically as a sugar substitute for diabetes or of glucose 6 phosphate dehydrogenase deficient population (Ylikahri, 1979).

The small amounts of xylitol present in certain fruits and vegetables render its extraction uneconomical. In industrial scale, xylitol is consequently produced through chemical reduction of xylose derived from hemicellulosic hydrolysates of birchwood or other xylose rich materials (Hyvönen and Koivistoinen, 1983). As the hemicellulosic fraction of these raw materials contains amounts of polymers of other sugars, the process includes extensive purification and separation steps to remove these by-products from xylose or xylitol (Melaja and Hämälänen, 1977). Therefore, the recovered yield of xylans as xylitol is about 50-60 % and xylitol production is relatively expensive.

Xylitol can also be produced from D-xylose by many yeast species (Onishi and Suzuki, 1966). Xylitol can be secreted extracellularly as a metabolic by product of ethanol (Prior et al, 1989) or as the major product from D-xylose (Barbosa et al, 1988). The objective of the paper

was to investigate if xylitol production by yeasts could provide an alternative process for production of xylitol from xylose rich materials. Thus, the specificity of reduction in regard to xylose by *Candida guillermondii*, a high xylitol producing yeast was first studied. The substrate tolerance of the strain was also characterized, the level of produced xylitol during fermentation, as well as the yield of xylitol formation, being of importance for the step of isolation of pure crystalline product.

MATERIAL AND METHODS

Organism and medium : culture of *Candida guillermondii* NRC 5578 was maintained on slants of YM agar (Wickerham, 1951). The basal medium was composed of 6,7 g/l of Yeast Nitrogen Base (Difco), 10 g/g of Yeast Extract (Difco) and 20 g/l of sugar. Sugar solution was autoclaved separately.

Inocula and fermentation conditions : inocula were grown aerobically in Erlenmeyer flasks containing the above medium at 30°C on a rotary shaker at 150 rpm for 26 h. Fermentations were conducted in microaerobic conditions, using 500 ml flasks with 400 ml medium and equipped with cotton wool plugs. Flasks were inoculated at 10 % (v/v) and incubated at 30°C on a rotary shaker at 150 rpm. Initial pH of cultures was 6.

Analytical methods : Growth was monitored gravimetrically : 10 ml of fermentation samples were filtered, washed and dried to constant mass at 104°C. Sugars and polyols were determined by HPLC using a Browlee column and refractive index detector (Waters). Bidistilled and filtered water was used as the mobile phase at a rate of 0,3 ml/min at 86°C.

RESULTS AND DISCUSSION

Fermentations of hemicellulosic derived sugars

Kinetics of xylose fermentation

When *Candida guillermondii* was cultivated in the medium containing D xylose as the carbon source, xylitol production started without lag period and was correlated with growth (fig. 1).

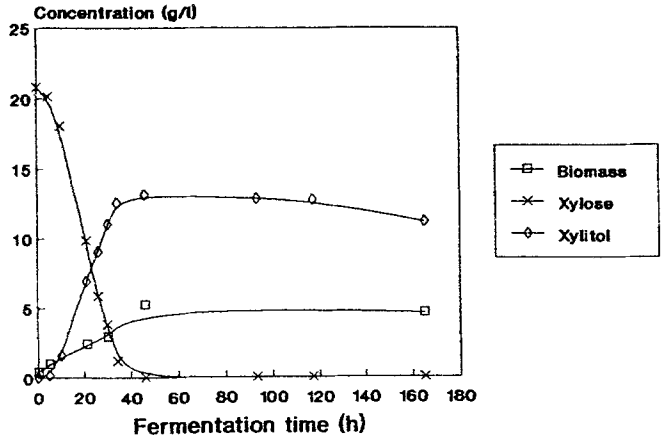

Figure 1. Xylose fermentation by *Candida guillermondii* grown in microaerobic conditions.

In microaerobic conditions, xylose was entirely consumed after an incubation time of 46 hours (fig. 1). An average specific xylitol productivity of 0,17 g/g/h and a final xylitol concentration of 14,1 g/l were obtained in these conditions. Throughout xylose fermentation xylitol yield was constant and reached a value of 0,63 g/g, corresponding to 69 % of the theoretical. Ethanol was produced in negligible amounts (0,05 g/l after 46 h).
For the tested initial substrate concentration, kinetic parameters of xylitol production by *Candida guillermondii* NRC 5578 compared favourably with values reported with *Candida tropicalis* 1004 grown on 30 g/l xylose a xylitol yield of 0,57 g/g. At initial xylose concentrations of 10 g/l and 50 g/l, a mutant strain of *Candida tropicalis* (HXP2) was found to exhibit calculated xylitol yields of 0,35 g/g and 0,77 g/g respectively (Gong et al, 1981).

Fermentations of individual hemicellulosic sugars others than xylose

The specificity of reduction in regard to xylose by *Candida guillermondii* was evaluated in studying the ability of this strain to ferment non xylose sugars commonly found in hemicellulosic hydrolysates. End products of glucose, mannose, galactose and arabinose fermentations by *Candida guillermondii* are presented in table 1.

Table 1. End products of glucose, mannose, galactose and arabinose fermentations by *Candida guillermondii* grown in microaerobic conditions.

carbon source	glucose	mannose	galactose	arabinose
fermentation time(h)	10	13	20	90
substrate used (%)	100	100	100	23.6
End products				
biomass (g/l)	3.8	3.0	3.5	3.3
polyol (g/l)	0	0	0	1.1
ethanol (g/l)	9.5	9.4	8.8	0

Glucose, mannose and galactose were rapidly fermented by *Candida guillermondii*, their specific uptake rates beeing 2,2 ; 1,8 and 1,5 times higher than for xylose. These hexoses were utilized by the strain only for growth and ethanol production : their corresponding polyols were not detected in the culture medium (table 1). Ethanol yields ranged from 0,44 to 0,47 g/g for the three studied carbon sources, whereas the volumetric rate of ethanol production from mannose and galactose were respectively about 25 % and 55 % lower than the corresponding value for glucose.
The specific rate of arabinose uptake (0,02 g/g.h) was very low, being one twelfth of xylose. By contrast to hexoses, the strain produced arabitol at the expense of arabinose. Accumulation of arabitol from arabinose in oxygen limited conditions has been observed with xylose fermenting yeasts (Chen and Gong, 1985). Bolen and Detroy (1985) reported with cell free extracts of *Pachysolen tannophilus*, that L-arabinose can serve as inducer and substrate for $NADPH_2$ linked xylose reductase. Arabitol formation could consequently result from action of a single aldose reductase with differing substrate specificities, although the existence of a separate enzyme could be possible (Bolen and Detroy, 1985; Wang and Letourneau, 1973; Suzuki and Onishi, 1975).

Reported results show that the fermentative process with *Candida guillermondii* could provide an alternative route for production of xylitol from xylose rich materials. First, the strain grown on xylose, rapidly excreted extracellulary xylitol. Furthermore, the yield of xylitol formation is very attractive in comparison with that reported for the chemical process (Melaja and Hämäläien, 1977).
Furthermore, the fundamental point is that the specificity of reduction in regard to xylose by *Candida guillermondii* is high. This physiological property of the strain is of prime importance as xylitol production is obligately achieved by reduction of non isolated xylose derived from hemicellulosic hydrolysates. In oxygen limited conditions which lead to high yield of xylitol from xylose, *Candida guillermondii* did not convert glucose, mannose and galactose into their corresponding polyols. Furthermore, the strain metabolizes these sugars to ethanol. Nevertheless, *Candida guillermondii* converts arabinose into arabitol, but at a low rate and yield of arabitol production.
It results that the biological process could alleviate or counteract the limiting operations which characterized the chemical process, as chromatographic fractionation with recycle steps to remove non xylitol polyols or non xylose sugars. Indeed, the fermentation of a hemicellulosic sugars mixture by *Candida guillermondii* could result in the formation of xylitol with only ethanol and arabitol as by products. In these conditions, ethanol could be easily separated from xylitol, without alteration of xylitol yield. Furthermore, *Candida guillermondii* has the ability to utilize ethanol as carbon source for growth. On the other hand, separation of arabitol from xylitol can be achieved through chromatographic fractionation which less alters xylitol yield than those used in the chemical process of xylitol production (Melaja and Hamalaïnen, 1975).

Substrate tolerance of *Candida guillermondii*

Global parameters for the fermentation of xylose with *Candida guillermondii* at initial xylose concentrations varying from 10 g/l to 300 g/l are presented in table 2.

Table 2. Effect of the initial substrate concentration (So) on the fermentation parameters of *Candida guillermondii* grown in microaerobic conditions.

So (g/l)	Tf (h)	xylose used (%)	xylitol (g/l)	$Y_{P/S}$ (g/g)	qp g/g.h	$Y_{X/S}$ (g/g)	μ_{max} (h^{-1})	$Y_{E/S}$ (g/g)
10	46	100	6.2	0.46	0.08	0.31	0.11	0.04
20	69.75	97.5	14.2	0.59	0.11	0.15	0.13	0.02
50	165	98.3	30.9	0.59	0.10	0.09	0.11	0.02
110	238	100	68.7	0.60	0.17	0.10	0.03	0.03
150	238	100	110.3	0.70	0.20	0.04	0.03	0.02
200	291.5	100	151.7	0.71	0.22	0.03	0.02	0.03
300	406	100	221	0.75	0.19	0.02	0.01	0.02

An increase in the initial sugar concentration from 10 g/l to 300 g/l led to activation of xylitol production. Indeed, the xylitol yield increased gradually with So increasing. The highest value in xylitol yield was obtained at So = 300 g/l and reached 0,75 g/g, corresponding to 82,6 % of

the theoretical. The low yield of xylitol obtained from low xylose concentrations were due to its use mainly for cell mass production (table 2). Ethanol, co product of xylitol in xylose metabolism, did not interact in the dependance of xylitol yield with the initial sugar level. Indeed, whatever the initial sugar level may be, ethanol yield was very low and was around 0,03 g/g (table 2).

As xylitol yield, the rate of xylitol production increased when the initial sugar concentration increased and did not exceed 200 g/l. At So = 200 g/l, the value in specific xylitol productivity was 2,4 times higher than the qp value observed at So = 10 g/l. Investigations related to the evolution of the specific xylitol productivity during fermentations showed that, when the initial sugar level did not exceed 200 g/l, the maximum values of qp were observed in the first 20 hours of culture. At So = 300 g/l, inhibition by the substrate concentration involved an increase of the fermentation time required to reach the maximum value of qp.

By contrast to the xylitol production process, growth process was gradually inhibited by an increase in the initial xylose concentration. Both the yield and the specific rate of cells production declined when the amount of xylose initially present in the culture increased (table 2). At the highest tested value in So, xylitol was produced in conditions which approximate to the conditions of resting cells (table 2). The changes in specific growth rate coefficient during fermentation demonstrated a typical inhibition of growth process by the substrate level. When the initial sugar concentration increased, a decrease in the maximum values of μ was noted, the highest value of 0,11 h^{-1} being observed at So = 20 g/l and 50 g/l. Furthermore the fermentation time at which the maximum values of μ occured, increased with the initial substrate level increasing. Compared to xylitol production process, the higher sensibility of growth process to any increase in the initial xylose concentration was also observed with *Candida sp* B22, a high xylitol producing yeast strain (Chen and Gong, 1985).

The highest fermentative performances of *Candida guillermondii* were obtained at So = 300 g/l. A final xylitol concentration of 221 g/l was obtained with a yield of 0,75 g/g (82,6 % of theoritical) and an average specific rate of 0,19 g/g.h, the last mentioned value being 13,6 % less than the maximal qp value obtained at So = 200 g/l (table 2). The fermentative performances exhibited by *Candida guillermondii* compare favourably with those reported for others xylitol producing microorganisms. Chen and Gong (1985), using *Candida sp.* B22 grown of xylose at So = 249 g/l, reported a q_{pmax} of 0,27 g/g.h and a xylitol yield corresponding to 84,5 % of theoritical. Two others tested xylitol producing yeast strains, *Candida tropicalis* HXP2 (Gong et al, 1981) and *Candida boidinii* (Vongsuvanlert and Tani, 1989) showed the highest amounts of produced xylitol (144 g/l and 39 g/l respectively) at respective values in So of 200 g/l and 100 g/l.

CONCLUSION

From the data presented, *Candida guillermondii* is characterized by a high potential for production of xylitol from xylose rich materials. First, the specificity of reduction in regard to xylose by the strain is high. Among the tested hemicellulosic derived sugars other than xylose, only arabinose was converted in its corresponding polyol. Furthermore *Candida guillermondii* exhibited high yield and rate of xylitol production, particularly at high initial xylose concentrations. Final concentrations of

xylitol up to 100 g/l can be expected to be attained, this being of prime importance for the step of isolation of pure crystalline xylitol. Compared to the chemical way for xylitol production, the high fermentative potential of the studied yeast strain permits to consider specific conditions of the biological way, particularly the mild conditions of pressure and temperature used during the reduction step.

NOMENCLATURE

Q_P : average volumetric productivity of xylitol (g xylitol/l per hour)
q_P : average specific productivity of xylitol (g xylitol/g of cells per hour)
So : initial xylose concentration (g/l)
tf : incubation time (hours)
$Y_{P/S}$: xylitol yield (g of xylitol produced/g of xylose utilized)
$Y_{E/S}$: ethanol yield (g of ethanol produced/g of substrate utilized)
$Y_{X/S}$: cells yield (g of cells/g of substrate utilized)
μ : specific growth rate coefficient (h^{-1})
μ_{max} : maximum specific growth rate coefficient (h^{-1})

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