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Screening of yeasts for production of xylitol from D-xylose and some factors which affect xylitol yield in *Candida guilliermondii**

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SUMMARY

The ability to convert D-xylose to xylitol was screened in 44 yeasts from five genera. All but two of the strains produced some xylitol with varying rates and yields. The best xylitol producers were localized largely in the species *Candida guilliermondii* and *C. tropicalis*. Factors affecting xylitol production by a selected *C. guilliermondii* strain, FTI-20037, were investigated. The results showed that xylitol yield by this strain was affected by the nitrogen source. Yield was highest at 30–35°C, and could be increased with decreasing aeration rate. Using high cell density and a defined medium under aerobic conditions, xylitol yield by *C. guilliermondii* FTI-20037 from 104 g/l D-xylose was found to be 77.2 g/l. This represented a yield of 81% of the theoretical value, which was computed to be 0.9 mol xylitol per mol D-xylose.

INTRODUCTION

The production of xylitol, a naturally occurring five-carbon polyalcohol, is of interest because of its potential use as a natural food sweetener, as a dental caries reducer and as a sugar substitute for treatment of diabetics [7]. While not common in North America, xylitol has been used in Germany, Switzerland and the Scandinavian countries as a sweetener in chewing gum, chocolate, bakery and other food products [19].

Xylitol is a normal intermediary product of carbohydrate metabolism in humans and animals [8]. It is also widely distributed in the plant kingdom, especially in certain fruits and vegetables [8,19]. However, the small amounts present in these sources render its quantitative extraction difficult and uneconomical. Currently, large-scale production is achieved by chemical reduction of D-xylose derived from hydrolysates of birchwood or other hemicellulose-rich materials [8].

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Xylitol can also be produced by yeasts from D-xylose [16], D-xylulose or D-glucose [17]. Onishi and Suzuki [16] found that certain *Candida* yeasts can accumulate polyol from D-xylose in the culture with about 40% yield in 7–10 days. Several *Candida* strains were reported to convert D-xylose to xylitol in high yield [5,9]. However, factors that control the rate and yield of this conversion are unknown.

To achieve efficient xylitol production from D-xylose, rapid production rates and high yields are desirable. The present study identified a *C. tropicalis* and a *C. guilliermondii* strain as efficient xylitol producers over a 48-h period. In addition, some of the factors which influence the yield by the selected *C. guilliermondii* strain are reported.

MATERIALS AND METHODS

Microorganisms

Forty-four yeast strains from the following five genera were screened: *Candida, Hansenula, Kluyveromyces, Pichia* and *Pachysolen*. They were obtained from a number of sources in Brazil: the Agronomic Institute (UNICAMP, Campinas), Foundation for Industrial Technology (FTI, São Paulo), Microbiology Institute (UFRJ, Rio de Janeiro) and Zymotechnic Institute (ESALQ, Piracicaba). All are now deposited at the culture collection of the Foundation for Industrial Technology. Strains were maintained at 4°C on agar slants or plates containing 10 g/l yeast extract, 20 g/l peptone and 20 g/l D-glucose.

Quantitative screening methodology

The inoculum was prepared by growing a loopful of cells from slants in 15 ml of medium containing 6.7 g/l yeast nitrogen base (YNB, Difco), 10 g/l yeast extract and 30 g/l D-xylose. The culture was kept at 30°C in a 50-ml glass test tube which was shaken at an angle 45° from horizontal at 150 rpm for 48 h using a New Brunswick reciprocal shaker. Inoculum was then seeded at an initial density of 10^7 cells/ml into 50 ml of the medium used to grow the inoculum. The culture was kept in a cotton-plugged 125-ml Erlenmeyer flask and agitated as above. At 0, 24 and 48 h, samples were removed and then centrifuged to obtain the supernatants and cell pellets.

Characterization of xylitol production by C. guilliermondii FTI 20037

The basal medium contained 5 g/l urea and 1.7 g/l YNB without amino acid or ammonium sulfate, to which D-xylose was added to the desired concentrations. All experiments employed this medium except when comparing the effect of nutrient sources. In these experiments, the use of ammonium sulfate on xylitol productivity was compared with that of urea. In addition, the effect of supplementation of the basal medium with various concentrations of yeast extract and casamino acids was tested.

In experiments where cultures were inoculated with low cell density, the inoculum was prepared by growing cells for 24 h in the basal medium containing 20 g/l D-xylose and 5 g/l urea or ammonium sulfate. A 0.1 ml sample of inoculum culture was transferred to 100 ml of test medium to start the experiment. For high-cell-density experiments, the inoculum consisted of cells grown for 24 h in 100 ml of basal medium containing 20 g/l D-xylose. The inoculum culture was washed twice with sterile distilled water before being resuspended in the test medium. For most experiments, 100-ml volumes of culture were kept in 250-ml loosely capped Erlenmeyer flasks which were shaken at 200 rpm at 30°C [14]. For growth-rate experiments, the flasks used were fitted with side arms through which absorbance at 600 nm was measured using a Coleman Model 295 spectrophotometer. When the effect of aeration rate was studied, 500-ml Erlenmeyer flasks were used with culture volumes ranging from 50 to 300 ml. The effect of temperature was investigated by incubating the cultures at temperatures ranging from 25 to 40°C. At intervals, samples were withdrawn and centrifuged to obtain the supernatants and cell pellets.

Sterilization

All solid media and liquid media used for screening were sterilized by autoclaving. In the latter, D-xylose and the other medium components were au-

toclaved separately. All other liquid media were sterilized by filtration.

Analytical

Absorbance was determined at 600 nm (1 cm path length) using a Beckman Model 35 spectrophotometer, after dilution to obtain values less than 0.6. D-Xylose and xylitol were determined by HPLC [13] and ethanol by GC [13,14] in supernatants. Cell dry weight was determined by washing the cells twice with distilled water and then drying to constant weight.

RESULTS

Utilization of D-xylose and production of xylitol

Of the 44 yeasts tested, three (*P. stipitis* 79-261, *C. tropicalis* 1004 and *C. guilliermondii* FTI-20037) consumed greater than 90% of D-xylose within the first 24 h (Table 1). Another 23 strains showed more than 90% substrate utilization by 48 h. The majority of these were *C. guilliermondii*, *C. tropicalis* or *K. marxianus* strains. D-Xylose was utilized very slowly (21–57% consumption at 48 h) by some strains of *C. utilis*, and all the *H. anomala* strains.

Almost all strains tested produced some xylitol, although the rates and yields varied. The exceptions were *P. stipitis* 79-261 and *K. marxianus* IZ-1339, where no xylitol was detected throughout the incubation period (Table 1). Seven *C. guilliermondii* or *C. tropicalis* strains accumulate xylitol at very rapid rates, yielding between 10 and 15 g/l within 24 h. By 48 h, 13 strains had accumulated more than 10 g/l xylitol. The two best producers were *C. tropicalis* 1004 and *C. guilliermondii* FTI-20037, which accumulated 17 and 16 g/l xylitol representing 0.57 and 0.53 g xylitol/g D-xylose consumed, respectively. Other members of these species in general showed very good yields of xylitol.

Among the more productive strains, three (*C. tropicalis* IZ-1958, *C. guilliermondii* IZ-803 and IZ-1739) showed the tendency to utilize xylitol between 24 and 48 h. *C. utilis* EQ2 and *K. marxianus* FTPTAT-106 produced 10–11 g/l xylitol at 48 h but the rest of these species showed very poor production. Strains of *Hansenula, Kluyveromyces, Pachysolen* and *Pichia* were in general poor xylitol producers over the 48-h test period.

Ethanol was produced by about 60% of the strains. The highest yield (11 g/l) was produced by *P. stipitis* 79-261 at 24 h, followed by *Pach. tannophilus* NRRL Y2460 and *C. tropicalis* IZ-1958 (5.2 g/l) at 48 h. Most of the *H. anomala* and *C. utilis* strains did not produce ethanol. Some strains of *C. tropicalis* and *C. guilliermondii* accumulated ethanol transiently in that ethanol was detected at 24 but not 48 h of culture.

The highest yield of dry cell weight (15 g/l) was produced by *C. utilis* EQ2. This was followed very closely at 13 g/l by two *C. guilliermondii* strains (IZ-1739 and IZ-1231) and *C. tropicalis* IZ-1958. The *Hansenula* strains showed the lowest accumulation of cell biomass. This may be attributed to poor substrate utilization during the incubation period.

Aspects of xylitol production by C. guilliermondii FTI-20037

Of the two best xylitol producers, *C. tropicalis* 1004 and *C. guilliermondii* FTI-20037, the latter was chosen for more detailed study. Xylitol yield by *C. guilliermondii* FTI-20037 was affected by the nitrogen source (Fig. 1). Substituting urea for ammonium sulphate increased yield, and further small increases were produced by the addition of casamino acids or yeast extract. The highest value was obtained using 5 g/l urea and 1 g/l yeast extract. The values shown are those when D-xylose was completely consumed (60–85 h) in cultures inoculated with a low density of cells. Increasing the concentrations of yeast extract led to a progressive decrease in xylitol yield. When incubations were prolonged beyond the point where all of the D-xylose was used, some of the xylitol was utilized.

A decrease in aeration rate increased xylitol yield, while the ethanol concentration and the rate of D-xylose consumption decreased (Fig. 2). The maximum concentrations of xylitol accumulated were the highest at 30 or 35°C (Table 2; Fig. 3). At these temperatures, the growth rates were higher and D-xylose was also consumed more rapidly.

Table 1

D-Xylose consumption and product formation by various yeasts

Yeasts	% Xylose consumed		[Xylitol] (g/l)		[Ethanol] (g/l)		Cell mass (g/l)	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
C. frareli FTI-20038	45	100	3.3	2.8	0.15	n.d.[a]	2.7	11
C. guilliermondii								
FTI-20037	95	100	13	16	0.75	n.d.	6.0	10
IZ-803	38	96	13	7	n.d.	n.d.	3.9	8.9
IZ-1739	57	100	11	8	n.d.	n.d.	7.1	13
IZ-1231	69	100	9.3	12	0.21	n.d.	4.9	13
IZ-1322	61	100	8.3	13	0.44	n.d.	3.5	10
IZ-1239	44	100	8.3	11	n.d.	n.d.	4.5	12
IZ-1422	52	100	7.4	14	0.12	n.d.	3.6	9.9
c-138	39	100	6.8	11	0.11	n.d.	3.9	12
17-07	50	97	5.0	10	0.43	0.6	6.7	12
IZ-1735	63	83	2.6	11	n.d.	n.d.	4.2	11
C. intermedia RJ-245	38	100	5.8	5.7	0.1	3.6	4.2	9.2
C. pseudotropicalis								
IZ-431	63	93	4.7	4.3	0.34	3.0	3.8	11
1006	43	88	2.4	2.1	0.61	2.4	3.5	12
C. tropicalis								
1004	95	100	15	17	1.1	n.d.	5.9	9.2
IZ-1824	80	100	15	15	1.5	n.d.	5.4	11
IZ-1958	85	100	14	7.8	2.0	5.2	5.5	13
53-51	80	100	13	13	1.2	n.d.	6.8	12
C. utilis								
FTI-20039	44	57	n.d.	1.9	n.d.	n.d.	7.2	7.9
74-64	70	100	7.8	9.7	4.8	4.1	5.0	6.6
1009	35	81	2.7	1.5	0.9	2.5	4.3	10
EQ2	53	100	2.7	11	n.d.	n.d.	7.3	15
IZ-1166	10	53	n.d.	1.3	n.d.	n.d.	1.9	7.6
IZ-1840	28	49	1.1	2.8	n.d.	n.d.	3.8	10
IZ-1841	21	34	n.d.	3.0	n.d.	n.d.	6.5	10
H. anomala								
IZ-1420	35	56	n.d.	6.1	n.d.	n.d.	2.3	6.0
IZ-229	25	51	n.d.	4.5	n.d.	n.d.	1.9	5.8
IZ-781	27	55	n.d.	4.1	n.d.	n.d.	2.4	7.7
IZ-1033	12	40	n.d.	2.2	n.d.	n.d.	2.9	6.4
RJ-510	12	21	n.d.	2.1	n.d.	n.d.	2.3	4.8
IZ-271	21	45	n.d.	1.7	n.d.	n.d.	2.3	7.3
IZ-1224	37	39	n.d.	1.22	0.1	0.2	2.9	4.9
IZ-1260	42	54	1.3	1.2	n.d.	n.d.	3.6	5.8
FTPTAT-106	27	42	n.d.	2.9	n.d.	n.d.	1.9	6.4
K. fragilis FTI-20066	46	97	5.0	4.6	0.7	3.5	4.2	10
K. marxianus								
IZ-1821	46	94	6.1	5.0	0.6	3.1	3.7	9.4
145	44	97	3.0	1.2	0.6	3.0	3.8	11
IZ-1339	36	91	n.d.	n.d.	1.2	2.6	5.3	11
276	46	97	3.7	2.7	n.d.	0.84	6.3	3.9

Table 1 contd.

Yeasts	% Xylose consumed		[Xylitol] (g/l)		[Ethanol] (g/l)		Cell mass (g/l)	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
IZ-619	37	67	1.5	n.d.	0.1	0.5	3.9	11
FTPTAT-106	54	99	4.2	11	0.2	2.0	4.3	8.8
Pach. tannophilus NRRL Y2460	38	100	0.69	2.2	0.8	5.2	3.5	9.5
P. butonii 68-1111	32	66	n.d.	2.5	n.d.	n.d.	4.9	10
P. stipitis 79-261	100	100	n.d.	n.d.	11	3.2	7.3	12

[a] n.d. = not detected.

There was, however, no evident correlation between growth rate, cell dry weight and xylitol yield (Table 2).

When a high initial cell density and relatively high concentration of D-xylose, 104 g/l, were employed, the maximum xylitol concentration obtained was 77.2 g/l (Fig. 4). The fermentation was conducted at 30°C under aerobic conditions (100 ml of medium in 250-ml loosely capped flasks), using the basal medium containing 5 g/l urea as the nitrogen source. The xylitol produced corresponded to a yield of 0.74 g xylitol/g D-xylose consumed. Ethanol was produced in negligible amounts. The

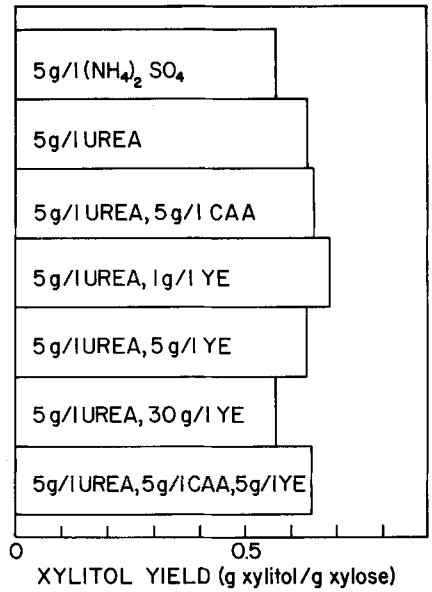

Fig. 1. Xylitol production from 40 g/l D-xylose by batch cultures of *C. guilliermondii* FTI-20037 in the presence of different nutrients. CAA = casamino acids; YE = yeast extract.

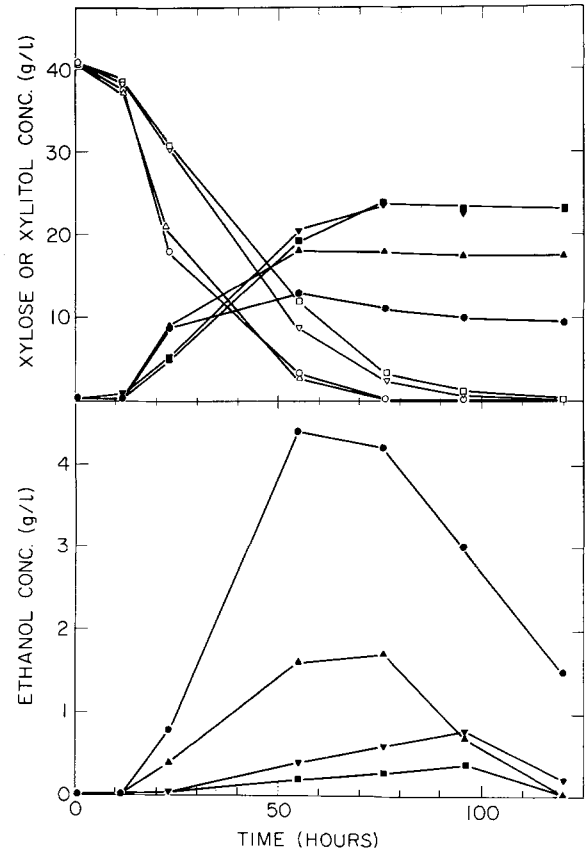

Fig. 2. Effect of aeration rate on D-xylose consumption (open symbols), and xylitol and ethanol production (closed symbols) in batch cultures of *C. guilliermondii* FTI-20037. Flask size was 500 ml and medium volumes were 50 ml (○,●), 100 ml (△,▲), 200 ml (▽,▼) and 300 ml (□,■).

Table 2

The effect of incubation temperature on the growth rate, cell and xylitol yield of *C. guilliermondii* FTI-20037 on 40 g/l D-xylose

Temperature (°C)	Growth rate u (h^{-1})	Dry cell weight at 100 h (g/l)	Maximum xylitol concentrations (g/l)	Xylitol yield at 76 h (g/g xylose consumed)
24.6	0.43	6.9	19	0.57
30	0.78	3.2	23	0.59
35.1	0.89	3.6	23	0.58
40.6	0.75	2.8	17	0.54

cells grew during the course of the experiment as evidenced by an increase in cell dry weight from 2.7 g/l at the time of inoculation to 6.5 g/l after 98 h.

Theoretical yield

Recent developments in our understanding of D-xylose metabolism in yeasts allow the computation of a theoretical yield for the conversion of D-xylose to xylitol. This yield is for situations where growth does not occur, hence is the maximum possible for the particular model considered.

The computation is based on four conditions. The first is the nature of the pyridine nucleotide cofactors involved in the initial two steps of D-xylose catabolism. In the first reaction, xylose reductase reduces D-xylose to xylitol using largely $NADPH_2^+$ and, to a lesser extent, $NADH_2^+$ as the cofactor [2,18]. In the second reaction, xylitol dehydrogenase oxidizes xylitol to D-xylulose using primarily NAD^+ as the cofactor [18].

The second condition is the mechanism(s) by which these cofactors are regenerated. The operational model is that where, under aerobic conditions (Fig. 5), (a) all of the D-xylose is reduced to xylitol using $NADPH_2^+$ as the cofactor, (b) $NADPH_2^+$ is regenerated from $NADP^+$ via the pentose phosphate shunt, (c) all of the xylitol is oxidized to D-xylulose using NAD^+ as the cofactor, and (d) NAD^+ is regenerated from $NADH_2^+$ via the respiratory chain [11].

The third condition is that yeasts in general do not possess a mechanism, such as transhydrogenase

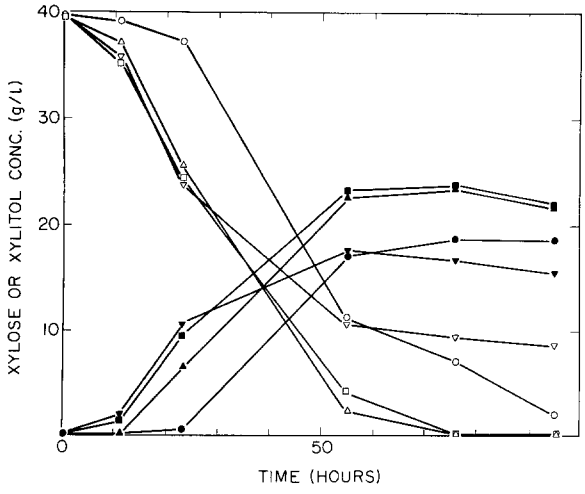

Fig. 3. Effect of incubation temperature on D-xylose consumption (open symbols) and xylitol production (closed symbols) in batch cultures of *C. guilliermondii* FTI-20037. The temperatures tested were 25°C (○,●), 30°C (△,▲), 35°C (□,■) and 40°C (▽,▼).

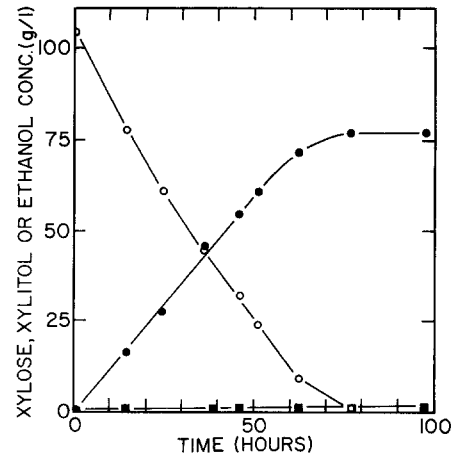

Fig. 4. Time course of production of xylitol (●) and ethanol (■) from 104 g/l D-xylose (○) in cultures of *C. guilliermondii* FTI-20037 employing high initial cell density.

activity, for the interconversion of $NADH_2^+$ and $NADPH_2^+$ [3]. The fourth condition is that, under non-growing conditions, xylitol will be oxidized only for the purpose of regenerating $NADPH_2^+$ and that excess xylitol will be excreted.

According to the above model, xylitol is produced under aerobic conditions in the following manner. Complete oxidation of 1 mol of glucose 6-phosphate to CO_2 and H_2O through the pentose phosphate shunt generates 12 mol of $NADPH_2^+$ from 12 mol of $NADP^+$ [4]. To maintain cofactor balance, the $NADPH_2^+$ produced is used to reduce D-xylose to xylitol. In the absence of growth, most of the xylitol formed is excreted. Xylitol is further catabolized only to produce eventually glucose 6-phosphate for the regeneration of sufficient $NADPH_2^+$ to maintain the cycle.

Based on this mode of operation where the substrate carbon and the pyridine nucleotide cofactors are balanced, the theoretical yield is 0.9 mol of xylitol per mol of D-xylose utilized. The relevant equations are shown below.

6 xylose + 6 $NADPH_2^+$	$\longrightarrow$	6 xylitol + 6 $NADP^+$ (1)
6 xylitol + 6 NAD^+	$\longrightarrow$	6 xylulose + 6 $NADH_2^+$ (2)
6 $NADH_2^+$ + 3 O_2 + 18 ADP + 18 P_i	$\longrightarrow$	6 NAD^+ + 18 ATP + 24 H_2O (3)
6 xylulose + 6 ATP	$\longrightarrow$	6 xylulose 5-phosphate + 6 ADP (4)
6 xylulose 5-phosphate + H_2O	$\longrightarrow$	5 glucose 6-phosphate + P_i (5)
5 glucose 6-phosphate + 60 $NADP^+$ + 35 H_2O	$\longrightarrow$	60 $NADPH_2^+$ + 30 CO_2 + 5 P_i (6)
Net (Eqns. 1–6):		
6 xylose + 54 $NADP^+$ + 12 ADP + 12 P_i + 12 H_2O + 3 O_2	$\longrightarrow$	54 $NADPH_2^+$ + 12 ATP + 30 CO_2 (7)
54 $NADPH_2^+$ + 54 xylose	$\longrightarrow$	54 xylitol + 54 $NADP^+$ (8)
Net (Eqns. 7 and 8):		
60 xylose + 12 ADP + 12 P_i + 12 H_2O + 3 O_2	$\longrightarrow$	54 xylitol + 12 ATP + 30 CO_2 (9)

Eqn. 7 represents the complete oxidation of D-xylose to CO_2 via glucose 6-phosphate. Eqn. 8 represents the reduction of D-xylose to xylitol by the $NADPH_2^+$ equivalents generated in Eqn. 7. The overall process, represented by Eqn. 9, generates some ATP which is potentially usable in other processes.

Some modifications to the above calculations based on different cofactor model in Fig. 5 are necessary if $NADH_2^+$ can serve, to some extent, as the cofactor for xylose reductase. Under such a scheme, cofactor recycling between the reactions catalyzed by xylose reductase and xylitol dehydrogenase can occur as follows [2]:

xylose + $NADH_2^+$	$\longrightarrow$	xylitol + NAD^+	(10)
xylitol + NAD	$\longrightarrow$	xylulose + $NADH_2^+$	(2)

Under aerobic conditions, ATP required to phosphorylate xylulose (Eqn. 4) can be obtained from oxidative phosphorylation (Eqn. 3). Subsequent metabolism of xylulose 5-phosphate through glucose 6-phosphate generates $NADPH_2^+$ for the reduction of D-xylose to xylitol (Eqn. 1). For the case where the substrate carbon and pyridine nucleotide cofactors are balanced, the yield is 0.905 mol of xylitol per mol of D-xylose consumed (Appendix 1).

Thus, under aerobic conditions, the theoretical yield is not significantly affected whether xylose reductase uses $NADPH_2^+$ solely or uses both $NADPH_2^+$ and $NADH_2^+$ as the cofactors. However, the two situations differ in that the former generates more ATP than the latter. Therefore, the different cofactor model, which generates more energy, might be favored under conditions where the maintenance energy requirements are higher, such as that when a defined medium is employed.

Under anaerobic conditions, $NADH_2^+$ produced cannot be regenerated through oxidative phosphorylation (Eqn. 3). As a result, an alternate

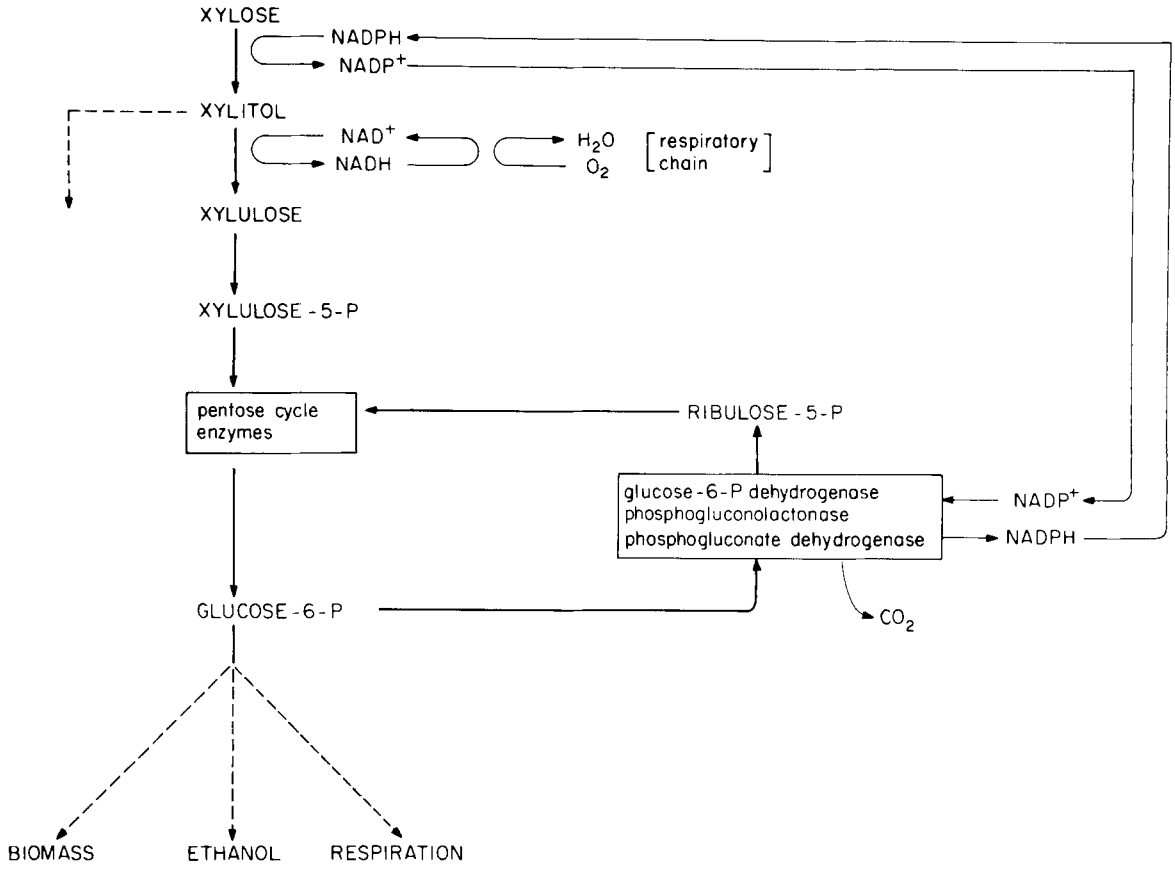

Fig. 5. Schematic diagram of cofactor regeneration in xylose-fermenting yeasts – the different cofactor model.

source of ATP has to be obtained. This is most likely achieved through metabolism of some of the glucose 6-phosphate to form glyceraldehyde 3-phosphate [2], which can be channelled through glycolysis to form ATP and ethanol [15]. The relevant equations are shown in Appendix 2. The maximum yield of xylitol is 0.875 mol/mol D-xylose consumed. In addition, some ethanol is co-produced. However, no ATP is generated to satisfy maintenance requirements. Thus, in practice, xylitol yield is expected to be even lower, with more glucose 6-phosphate being metabolized through glyceraldehyde 3-phosphate to ethanol (Eqns. 14 and 15).

The yield of 0.74 g xylitol per g D-xylose obtained in the present study under aerobic conditions, during which growth occurred and trace ethanol was formed, corresponds to 81% of theoretical for non-growing conditions.

DISCUSSION

The present study showed that 42 out of 44 yeast strains surveyed accumulated some xylitol in the culture media. This indicates that xylitol production is a relatively common feature among xylose-utilizing yeasts, as suggested also by other workers [10]. Furthermore, the screen identified *C. tropicalis* and *C. guilliermondii* as good producers of xylitol from D-xylose. Strains of these species can carry out this conversion rapidly and efficiently. In particular, *C. guilliermondii* possesses the advantage that yield of the by-product, ethanol, is very low. Onishi and Suzuki [16] have previously identified *H. anomala* strains as good polyol producers. However, the present study showed that their rates of production of xylitol are very slow compared to those of *C. tropicalis* or *C. guilliermondii*, which began to pro-

duce xylitol in the first 24 h. In contrast, xylitol was not accumulated by almost all the *H. anomola* strains until after 24 h of culture (Table 1).

A recent study has shown that xylitol yield from D-xylose by *C. guilliermondii* FTI-20037 is optimal under conditions where growth is not limited by biotin deficiency [12]. The present study showed that this conversion is sensitive to the nutrient source. The use of urea led to higher xylitol productivity than with ammonium sulfate, and supplementation of urea with casamino acids improved performance with urea only slightly. Yeast extract improved yields, but only at low concentrations. Increasing oxygen limitation led to increased xylitol productivity and decreased ethanol productivity with *C. guilliermondii*. This contrasts with *Pach. tannophilus* and *Candida* sp. XF217 where xylitol and ethanol productivities increased concurrently as aeration rate decreased [1,6,20]. This suggests that the regulation of metabolite formation in *C. guilliermondii* differs from that in other xylose-fermenting yeasts.

A notable aspect of the present study was that a relatively high yield could be obtained with a defined medium, specifically 81% of theoretical with a medium consisting of yeast nitrogen base without amino acids and ammonium sulfate, with urea as the nitrogen source. The use of defined media is important in technological applications, because the cost is lower than for complex media. A somewhat higher yield from 216 g/l pure D-xylose has been reported with a yeast designated as *Candida* sp. B-22, using a medium supplemented with yeast extract, malt extract and peptone [5]. The yield in that study corresponded to 94.4% of the theoretical yield computed for aerobic conditions in this study.

APPENDIX 1

Calculation of xylitol yield from D-*xylose where* $NADH_2^+$ *can serve as the cofactor for xylose reductase – aerobic conditions*

$$6\ \text{xylose} + 6\ NADH_2^+ \longrightarrow 6\ \text{xylitol} + 6\ NAD^+ \quad (10)$$
$$12\ NAD^+ + 12\ \text{xylitol} \longrightarrow 12\ \text{xylulose} + 12\ NADH_2^+ \quad (2)$$
$$6\ NADH_2^+ + 3\ O_2 + 18\ ADP + 18\ P_i \longrightarrow 6\ NAD^+ + 18\ ATP + 24\ H_2O \quad (3)$$

Net (Eqns. 2, 3 and 10):

$$6\ \text{xylose} + 6\ \text{xylitol} + 3\ O_2 + 18\ ADP + 18\ P_i \longrightarrow 12\ \text{xylulose} + 18\ ATP + 24\ H_2O \quad (11)$$
$$12\ \text{xylulose} + 12\ ATP \longrightarrow 12\ \text{xylulose 5-phosphate} + 12\ ADP \quad (4)$$
$$12\ \text{xylulose 5-phosphate} + 2\ H_2O \longrightarrow 10\ \text{glucose 6-phosphate} + 2\ P_i \quad (5)$$
$$10\ \text{glucose 6-phosphate} + 120\ NADP^+ + 70\ H_2O \longrightarrow 120\ NADPH_2^+ + 60\ CO_2 + 10\ P_i \quad (6)$$

Net (Eqns. 4–6 and 11):

$$6\ \text{xylose} + 6\ \text{xylitol} + 3\ O_2 + 6\ ADP + 6\ P_i + 48\ H_2O + 120\ NADP^+ \longrightarrow 120\ NADPH_2^+ + 6\ ATP + 60\ CO_2 \quad (12)$$
$$120\ NADPH_2^+ + 120\ \text{xylose} \longrightarrow 120\ \text{xylitol} + 120\ NADP^+ \quad (8)$$

Net (Eqns. 8 and 12):

$$126\ \text{xylose} + 3\ O_2 + 6\ ADP + 6\ P_i + 48\ H_2O \longrightarrow 114\ \text{xylitol} + 6\ ATP + 60\ CO_2 \quad (13)$$

Yield (xylitol/xylose) = 114/126 = 0.905 mol/mol

APPENDIX 2

Calculation of xylitol yield from D-*xylose where* $NADH_2^+$ *can serve as the cofactor for xylose reductase – anaerobic conditions*

$$6\ \text{xylose} + 6\ NADH_2^+ \longrightarrow 6\ \text{xylitol} + 6\ NAD^+ \quad (10)$$
$$6\ \text{xylitol} + 6\ NAD^+ \longrightarrow 6\ \text{xylulose} + NADH_2^+ \quad (2)$$
$$6\ \text{xylulose} + 6\ ATP \longrightarrow 6\ \text{xylulose 5-phosphate} + 6\ ADP \quad (4)$$
$$6\ \text{xylulose 5-phosphate} + H_2O \longrightarrow 5\ \text{glucose 6-phosphate} + P_i \quad (5)$$
$$2\ \text{glucose 6-phosphate} + 24\ NADP^+ + 14\ H_2O \longrightarrow 24\ NADPH_2^+ + 12\ CO_2 + 2\ P_i \quad (6)$$
$$3\ \text{glucose 6-phosphate} + 18\ NADP^+ + 3\ H_2O \longrightarrow NADPH_2^+ + 9\ CO_2 + 3\ \text{glyceraldehyde 3-phosphate} \quad (14)$$
$$3\ \text{glyceraldehyde 3-phosphate} + 3\ P_i + 6\ ADP \longrightarrow 6\ ATP + 3\ \text{ethanol} + 3\ CO_2 + 3\ H_2O \quad (15)$$
$$42\ NADPH_2^+ + 42\ \text{xylose} \longrightarrow 42\ \text{xylitol} + 42\ NADP^+ \quad (8)$$

Net (Eqns. 2,4,5,6,8,10,14 and 15):

$$48\ \text{xylose} + 15\ H_2O \longrightarrow 42\ \text{xylitol} + 24\ CO_2 + 3\ \text{ethanol} \quad (16)$$

Yield (xylitol/xylose) = 42/48 = 0.875 mol/mol
Yield (ethanol/xylose) = 3/48 = 0.0625 mol/mol

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