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Screening of Yeasts for Production of Xylitol from D-Xylose

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***Candida mogii* ATCC 18364 was selected for a xylitol producer ($Y_{P/S}$=0.62 g/g) from 11 strains of D-xylose utilizing yeasts. Systematic kinetic studies are presented for growth and xylitol formation in synthetic medium using D-xylose as the carbon and energy source. Xylitol is produced from D-xylose under aerobic as well as oxygen-limiting conditions, but not in the absence of oxygen. It can be seen from the experimental results that the concentrations of D-xylose and dissolved oxygen have a strong influence on the yield and rate for product formation. A maximum product yield was obtained when initial D-xylose concentration and specific oxygen uptake rate were 53 g/l and 0.5 mmol O_2/g/h, respectively. Kinetic studies of D-xylose uptake, D-xylose reductase and xylitol dehydrogenase were performed to explain the complex regulatory properties of *C. mogii* during aerobic and oxygen-limited xylitol formation.**

[**Key words:** xylitol production, *Candida mogii*, metabolic regulation, kinetic studies]

Wood or agricultural wastes, as renewable resources still represent an important source for organic chemicals and biotechnologically derived products for the future. The three well known major components of these residues are cellulose, hemicellulose and lignin. Among them, the importance of the hemicellulose fraction has obviously been considered as an inexpensive raw material for bioconversion to many useful products (1–5). The pentoses, mainly D-xylose, derived from hemicellulose carbohydrates can be used by bacteria and yeasts with different conversion pathways (2). Interestingly, the production of ethanol from D-xylose has been discussed for the past ten years by several groups of researchers. However, due to the difficulties in using microorganisms and high costs of the process, the bioconversion of D-xylose to ethanol was found to be no more attractive. Recently, xylitol production from D-xylose has been mentioned as an alternative utilization for hemicellulose (6–9). Xylitol is a good anticariogenic sweetener that can be metabolized by diabetic persons, because of its insulin-independent metabolic utilization (10, 11). The yeasts *Candida tropicalis* (8, 9), *Candida parapsilosis* (6) and *Candida guilliermondi* (7) have been reported to favorably produce xylitol from D-xylose.

In order to develop a biotechnological process for xylitol production using renewable hydrolysates as a substrate (12), the preliminary studies on xylitol production have been investigated in this paper. First, a strong xylitol producer was selected from D-xylose utilizing yeasts by screening in a complex medium. Next, some factors affecting xylitol formation were investigated using D-xylose as substrate. Finally, kinetic studies of the D-xylose reductase and the xylitol dehydrogenase were performed to explain the regulatory properties of xylitol formation during aerobic and oxygen limited growth of *Candida mogii*.

MATERIALS AND METHODS

Microorganisms The majority of D-xylose utilizing yeast strains listed in Table 1 were purchased from American Type Culture Collection (Rockville, MD, USA). The remaining strains were isolated by Hamad (Ph. D. thesis, Technische Universität Berlin, 1986) and made available for this study. These cultures were maintained on potato dextrose agar (Difco, Michigan, USA) slants at 4°C.

Culture media The YMP medium used for screening xylitol producers from D-xylose contained per liter: Bacto-yeast extract, 3 g; Bacto-malt extract, 3 g; Bacto-peptone, 5 g and D-xylose 10, 30 or 50 g. For kinetic studies, a synthetic medium (13) was used which contained per liter: 18.75 g KH_2PO_4, 6 g $(NH_4)_2HPO_4$, 1.13 g $MgSO_4 \cdot 7H_2O$, 0.1 g $CaCl_2$, 36.5 mg myo-inositol, 18.2 mg calcium pantothenate, 3.66 mg thiamine-HCl, 0.9 mg pyridoxal-HCl, 0.018 mg biotin, 9.1 mg $FeCl_3$, 6.4 mg $MnSO_4 \cdot H_2O$, 5.46 mg $ZnSO_4 \cdot 7H_2O$, 1.46 mg $CuSO_4 \cdot 5H_2O$ and carbon sources as indicated.

Culture conditions Screening of xylitol producing yeasts by shake-flask experiments was conducted in 100-ml Erlenmeyer flasks at 30°C in a water bath rotary shaker at 250 rpm (HT, Infors AG, CH-4103 Bottmingen). Cells were grown aerobically in 50 ml YMP medium containing 5% D-xylose until substrate was depleted.

Preliminary experiments were carried out in 1-*l* Erlenmeyer flasks containing 300 ml of synthetic medium and a mixture of 20 g/*l* glucose and 5 g/*l* D-xylose for biomass production (pH 5.0, 250 rpm, 30°C). After depletion of both monosaccharides, certain concentrations of D-xylose were added to D-xylose induced cells to study the formation of xylitol.

Fermentor experiments were carried out in a 3-*l* (PecTec, Mühlacker, FRG) or in a 30-*l* stirred bioreactor (Bioengineering, Wald, Switzerland) containing 1.5 or 15 *l* synthetic medium and specified concentrations of D-xylose. Two (3-*l* bioreactor) or ten shaking flasks (30-*l* bioreactor) containing D-xylose induced cells were used to inoculate the fermentor. Cultivations were performed at 30°C and pH 5.0 under controlled aeration (1 vvm) and agitation (500 rpm), unless mentioned otherwise.

For fed-batch experiments, the feeding medium had the same composition as the synthetic medium but contained 100 g D-xylose per liter (C_S^0).

Analytical methods The samples were taken at

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specified time intervals and centrifuged. After washing with water the residues were dried for cell mass determination at 105°C for 24 h. The supernatants were analyzed for monosaccharides and xylitol by high performance liquid chromatography (HPLC) using a HPX-87P column (Biorad, Richmond, USA). Protein concentrations were determined by the method of Bradford (14), using bovine serum albumin as a standard. Ethanol concentration was determined spectrophotometrically at 340 nm using a commercial test kit of Boehringer.

Optical densities of culture samples were measured at 620 nm. In the absorbance range of 0.05 to 0.45 the optical density was directly proportional to the dry cell mass concentration.

Enzyme assay Enzyme activities of D-xylose reductase (15) and xylitol dehydrogenase (16) were determined spectrophotometrically at 340 nm. Enzyme units were defined as μmol nicotinamide nucleotide reduced or oxidized per minute. Specific activities were expressed as units per milligram of protein.

Oxygen uptake and carbon dioxide production rates The difference in percentage of O_2 and percentage of CO_2 between inlet and outlet gases was determined by passing the gas through a paramagnetic oxygen analyzer (Maihak, FRG) and an infra-red carbon dioxide analyzer (Maihak). These data were used to calculate the oxygen uptake and carbon dioxide production rates. Constant oxygen transfer rates were obtained by varying the agitation rates. Dissolved oxygen tension was measured with a polarographic oxygen electrode (Ingold, FRG).

Kinetic analysis Rates of cellular growth, D-xylose uptake and xylitol formation were determined from the slope of the measured concentrations versus time. Specific rates were evaluated as the ratio of the above mentioned rates to the corresponding biomass concentration. Values of the yields for growth and xylitol formation were calculated from the slopes of biomass vs. D-xylose and xylitol vs. D-xylose plots, respectively.

RESULTS

Screening of high yield xylitol producers Among the 11 strains tested in shake flask cultures (250 rpm) regarding their ability to produce xylitol from D-xylose, *C. mogii* ATCC 18364 was found to be the one with the highest product yield, $Y_{P/S}=0.62$, in a complex medium (YMP). This result is in agreement with the data of Gong *et al.* (8), who found that the same strain of *C. mogii* produced xylitol with a yield of $Y_{P/S}=0.59$ in YMP medium during aerobic growth on D-xylose. Meyrial *et al.* (7) reported a yield of $Y_{P/S}=0.59$ for *Candida quilliermondii* NRC 5578 growing in a complex medium containing 10 g/*l* yeast extract and 6 g/*l* YNB which is in the range of product yields observed for *C. mogii*. The two strains tested of *Saccharomyces* sp. and *Pichia stipitis* ATCC 7124 produced negligible amounts of xylitol while consuming D-xylose as a carbon source. The remaining species in Table 1 produced xylitol with a yield ranging from 0.11 to 0.40 g/g. Therefore, *C. mogii* ATCC 18364 was used as a xylitol producer for the following experiments.

Effect of yeast extract and peptone concentrations *C. mogii* was grown under aerobic conditions in shake flask cultures (250 rpm) on synthetic or YMP medium containing a mixture of 20 g/*l* glucose and 5 g/*l* xylose. After depletion of both monosaccharides (28 h) D-xylose was added to the cultures resulting in a final concentration of 30 g/*l*. Samples were taken at distinct time intervals to determine concentrations of biomass, D-xylose and xylitol. Table 2 summarizes the yields and maximal specific rates for growth and xylitol formation.

TABLE 1. Screening of yeasts for xylitol producer

Microorganism	Biomass yield ($Y_{X/S}$) (g/g)	Xylitol yield ($Y_{P/S}$) (g/g)
C. mogii ATCC 18364	0.08	0.62
C. tropicalis ATCC 7349	0.23	0.40
C. parapsilosis ATCC 34078	0.10	0.40
Candida kefyr Hadmad 21a	0.20	0.29
Kluyveromyces marxianus Hamad 21b	0.13	0.26
Candida utilis ATCC 22023	0.19	0.18
Hansenula polymorpha Hamad 35a	0.27	0.15
C. tropicalis ATCC 20240	0.47	0.11
P. stipitis ATCC 7124	0.20	0.0
Saccharomyces diastaticus IFGB 1110	0.19	0.0
Saccharomyces cerevisiae ATCC 28338	0.07	0.0

The addition of yeast extract and peptone to the defined medium enhanced the biomass yield and maximum specific growth rate remarkably and had no significant effect on the product yield and the specific rate of xylitol formation. These results are in agreement with experimental observations found during aerobic growth of *P. stipitis* on D-xylose, where yeast extract acts as a non-essential activator according to Bui-Thanh *et al.* (17). During growth on complex medium various organic constituents of peptone and yeast extract are incorporated into cellular materials which results in a higher growth yield. Since the addition of yeast extract and peptone had no remarkable effect on yield and specific rate of xylitol formation, the following experiments were carried out under defined conditions in a synthetic medium without supplimentation.

Effect of initial D-xylose concentrations *C. mogii* was grown under oxygen-limited conditions (shaking rate 100 rpm) in synthetic medium containing different initial concentrations of D-xylose (5–53 g/*l*). Table 3 summarizes the maximum specific rates of growth, D-xylose uptake and xylitol formation as well as overall yields as a function of initial D-xylose concentrations.

The specific D-xylose uptake (Fig. 1) and xylitol formation rates show a hyperbolic dependency on the initial substrate concentration while the specific growth rate remains constant in the range of experimental errors observed.

The experimental data as illustrated in Fig. 1 were fitted to Eq. 1 by non-linear regression analysis by estimating its two parameters.

$$q_s = q_s^{max} \frac{C_s}{K_s + C_s} \quad (1)$$

with: $q_s^{max} = 0.029$ g/g/h
$K_s = 9.2$ g/*l*

TABLE 2. Effect of yeast extract and peptone on xylitol production

Yeast extract (g/*l*)	Peptone (g/*l*)	μ (1/h)	$Y_{X/S}$ (g/g)	$Y_{P/S}$ (g/g)	q_s (g/g/h)	q_p (g/g/h)
3	5	0.065	0.23	0.49	0.24	0.15
0	0	0.040	0.15	0.48	0.25	0.12

TABLE 3. Effect of initial D-xylose concentrations on xylitol production

Xylose (g/l)	$Y_{X/S}$ (g/g)	$Y_{P/S}$ (g/g)	μ (1/h)	q_s (g/g/h)	q_p (g/g/h)
5.3	0.44	0	0.006	0.009	0
10.1	0.29	0.17	0.005	0.014	0.004
19.3	0.18	0.44	0.004	0.022	0.011
28.9	0.16	0.50	0.004	0.023	0.012
53.3	0.12	0.70	0.003	0.024	0.017

Under these conditions, the yield for xylitol ($Y_{P/S}$) has been increased from zero to 0.70 by changing the initial D-xylose concentration from 5.3 to 53 g/l. A similar effect was found during xylitol production from D-xylose using *C. tropicalis* (9), where optimal product formation was obtained at 172.0 g/l D-xylose.

It can be seen from the experimental results obtained at low D-xylose concentrations, that the net rates for D-xylose transport, D-xylose reductase and xylitol dehydrogenase are equal (q_s=0.009 g/g/h), because no xylitol was excreted from the cells. An increase in D-xylose concentration results in increasing rates of D-xylose uptake and D-xylose reductase, but not for the rate for the xylitol dehydrogenase. It may be speculated that during oxygen limited growth the maximal rate for xylitol dehydrogenase is approximately 0.009 g/g/h and the xylitol excretion rate is the difference between the rate of D-xylose uptake and xylitol dehydrogenase.

To study the effect of D-xylose concentration on the excretion rate of xylitol under defined conditions, repeated batch experiments were carried out in a 30-l fermentor using cells pre-grown in shaking flasks on a mixture of glucose and D-xylose as described in Materials and Methods. During aerobic growth of *C. mogii* the rates for D-xylose uptake and xylitol excretion were determined at an initial D-xylose concentration of 10 g/l and after addition of D-xylose to an initial concentration of approximately 50 g/l. Samples were taken in short time intervals (every 5 min) to determine the concentrations of biomass, D-xylose and xylitol. Table 4 shows the experimental results.

During aerobic growth (DO > 200 μmol/l) the specific growth rate (μ=0.045 1/h) and the specific oxygen uptake rate (q_{O2}=1.65 mmol/g/h) were found to be the same at a D-xylose concentration of 10 or 50 g/l. The results shown in Table 4 are in good agreement with the experimental observations during oxygen limited growth (Table 3), since increasing D-xylose concentrations result in an increase of the rates of D-xylose uptake and xylitol excretion. After pulsing with D-xylose the specific rates were calculated from the slope of the changes in D-xylose and xylitol concentrations during the first 20 min. At a D-xylose concentration of 10 g/l the reaction rate for the xylitol dehydrogenase ($q_{Xit\text{-}DH}=q_s-q_p$) was calculated to be 0.11 g/g/h. Since the time scale for the synthesis of new enzymes is in the range of hours (18), it can be assumed that during the time period of determination of specific rates (20 min) the levels of the monosaccharide transporter(s), D-xylose reductase and xylitol dehydrogenase, are frozen in the initial state. A sudden increase in the D-xylose concentration from 10 g/l to 50 g/l leads to increasing rates of D-xylose uptake (q_s= 0.19 g/g/h) and xylitol excretion (q_p=0.09 g/g/h). It can be summarized from these results that the xylitol dehydrogenase seems to be the rate limiting step in D-xylose metabolism of *C. mogii* during aerobic and oxygen limited conditions.

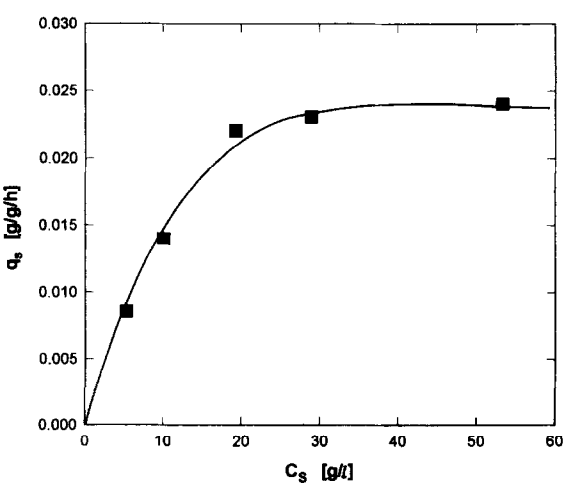

FIG. 1. Effect of initial D-xylose concentration on xylose uptake rate during oxygen-limited growth.

TABLE 4. Effect of D-xylose concentrations on yields and specific rates of D-xylose uptake and xylitol excretion

Xylose (g/l)	Yields (g/g)		Specific rates (g/g/h)	
	$Y_{X/S}$	$Y_{P/S}$	q_s	q_p
10	0.45	0.08	0.12	0.01
50	0.20	0.44	0.19	0.09

Effect of dissolved oxygen Since the specific excretion rate and the yield for xylitol formation are influenced by the D-xylose concentration (Table 4), the batch experiments were performed to estimate specific rates and yields for growth and xylitol formation. Cells were grown under aerobic conditions at an initial D-xylose concentration of 50 g/l until the substrate concentration decreased to 40 g/l (C_X=3.5 g/l), the oxygen uptake rate was then kept constant as indicated in Table 5. After a transient period of approximately 4 h, specific growth rate (μ) and substrate uptake rate (q_s) were estimated at a D-xylose concentration of 35 g/l. To investigate the influence of oxygen uptake at constant D-xylose concentration under pseudo-steady state conditions, the feeding rate (F) was changed exponentially as shown by Eq. 2 using kinetic parameters estimated from batch experiments as described previously.

$$F(t)=\frac{q_s C_{X0} V_{F0}}{C_s^0} e^{\mu t} \qquad (2)$$

Index 0 stands for the biomass concentration and the fermentor volume when the feed started. To keep the specific oxygen uptake rate constant, oxygen transfer rates were adjusted by varying the agitation rate according to the concentration of biomass.

The kinetic parameters shown in Table 5 were estimated from the comparison between model prediction (integrated balance equations) and experimental observations using a nonlinear advanced optimization procedure (19).

TABLE 5. Effect of oxygen concentrations on yields and specific rates of D-xylose uptake and xylitol formation

C_{O_2} (μmol/l)	$Y_{X/S}$ (g/g)	$Y_{P/S}$ (g/g)	q_s (g/g/h)	q_p (g/g/h)	μ (1/h)	q_{o_2} (mmol/g/h)
100	0.22	0.48	0.25	0.12	0.040	1.65
8	0.19	0.54	0.13	0.07	0.023	1.05
2	0.05	0.63	0.08	0.05	0.003	0.50

$$\frac{dC_X}{dt}=\mu C_X-\frac{F(t)}{V_F(t)}C_X \quad (3)$$

$$\frac{dC_s}{dt}=\frac{F(t)}{V_F(t)}(C_s^0-C_s)-q_sC_X \quad (4)$$

$$\frac{dC_p}{dt}=q_pC_X-\frac{F(t)}{V_F(t)}C_p \quad (5)$$

$$\frac{dV_F}{dt}=F(t) \quad (6)$$

Using these experimental conditions D-xylose concentration was fairly constant over the period of balanced growth (app. twenty hours). Oxygen limitation results in a decrease of the growth yield and the specific rates of growth, D-xylose uptake and xylitol formation while the product yield increased. Furlan *et al.* (6) observed the same effect of oxygen limitation on the growth and product yield during growth of *C. parapsilosis* in continuous culture. In their study they found that decreasing specific oxygen uptake rate results in increasing specific xylitol production rate, which is different in *C. mogii*.

Anaerobic conditions Cells were grown on D-xylose in a batch culture (3-*l* fermentor) under aerobic conditions to obtain a higher biomass concentration. After an exponential growth phase of 36 h (C_X=10 g/*l*) nitrogen was continuously sparged through the fermentor at a flow rate of 1.5 *l*/min to obtain anoxic conditions. During anoxic conditions (48 h) the D-xylose and biomass concentrations remained constant in the range of experimental errors observed, therefore the rates of formation of xylitol and ethanol were negligibly low.

Effect of xylitol concentrations The influence of xylitol concentrations (10–100 g/*l*) on the specific production rate were studied at an initial D-xylose concentration of 50.0 g/*l* under micro-aerobic conditions in shake flask cultures (100 rpm). Table 6 shows the experimental results.

TABLE 6. Effect of initial xylitol concentrations on xylitol formation

Xylitol (g/l)	$Y_{X/S}$ (g/g)	$Y_{P/S}$ (g/g)	μ (1/h)	q_s (g/g/h)	q_p (g/g/h)
10	0.08	0.67	0.002	0.025	0.017
20	0.09	0.70	0.002	0.023	0.016
40	0.11	0.73	0.002	0.023	0.017
67	0.10	0.62	0.002	0.024	0.015
100	0.10	0.70	0.002	0.027	0.019

As can be seen the specific rates and yields for growth and xylitol production are not significantly influenced by the xylitol concentration up to a concentration of 100 g/*l*, this may be an advantage to avoid product inhibition during xylitol production by this strain of *C. mogii*. Small deviations exist due to the larger range of experimental errors observed during HPLC determination at higher xylitol concentrations.

Kinetic of D-xylose reductase and xylitol dehydrogenase During growth on glucose, activities of D-xylose reductase and xylitol dehydrogenase were negligibly low in contrast to growth on D-xylose where both enzymes were induced at a higher level (specific activities approximately 0.12 U/mg). The D-xylose reductase and xylitol dehydrogenase exhibited Michaelis-Menten kinetics with respect to its substrate and co-substrate. To determine the kinetic parameters for both enzymes, the co-substrate concentrations were varied at constant levels of the substrate as indicated in Table 7.

DISCUSSION

From our screening program of 11 yeast strains tested only three species of *Candida* have emerged as efficient xylitol producers from D-xylose. Whereas most of these yeasts were characterized by a low yield and specific rate for product formation, *C. mogii* appeared to be promising for xylitol production from D-xylose. Of particular interest is *C. mogii* ATCC 18364, because this strain exhibited a high xylitol yield ($Y_{P/S}$=0.70), negligible product inhibition and is able to grow on synthetic medium for extended cultivation periods (up to 150 h), which

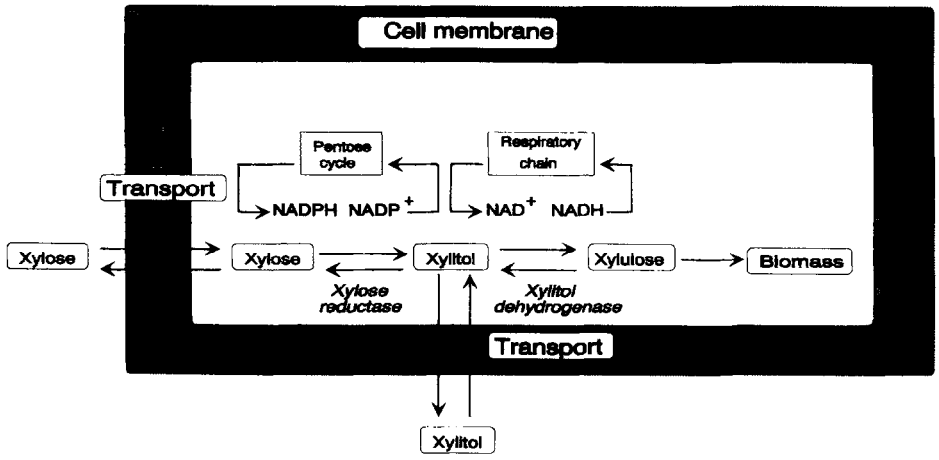

FIG. 2. Key reactions of D-xylose metabolism during xylitol production using *C. mogii*.

TABLE 7. Kinetic parameters for D-xylose reductase and xylitol dehydrogenase from *C. mogii*

	D-Xylose reductase		Xylitol dehydrogenase
	NADPH	NADH	NAD^+
V_{max} (U/mg)	0.16	0.06	0.22
K_m (mM)	0.036	0.085	0.70
Conc. range (mM)			
of co-substrate varied	0.025–1.5		0.1–3.0
at const. substrate	1000		1000

improves the overall process economics considerably.

As reported in Table 3 increasing D-xylose concentration leads to increasing rates of D-xylose uptake and xylitol formation while the specific growth rate remains fairly constant. Figure 1 shows that the D-xylose uptake followed Michaelis-Menten kinetics which is indicative for a carrier-mediated facilitated diffusion. This hypothesis is supported by a recent kinetic analysis of ^{14}C-xylose transport in intact cells of *C. mogii* performed by Sirisansaneeyakul (Ph. D. thesis, Universität Stuttgart, 1993).

According to the Michaelis constants for NADPH (K_M=0.036 mM) and NADH (K_M=0.085 mM) intracellular D-xylose seems to be preferably reduced to xylitol via NADPH-dependent D-xylose reductase and then oxidized to xylulose via NAD^+-dependent xylitol dehydrogenase (Fig. 2). As can be speculated from observations of other D-xylose consuming yeasts, NADPH is mainly regenerated by the enzymes glucose-6-phosphate and 6-phosphogluconate dehydrogenase (20). If D-xylose reduction is mainly dependent on NADPH as an electron donor, this first step results in an imbalance of the cell's NAD^+/NADH redox system under anaerobic conditions where no ethanol is produced. This seems to be the explanation why no growth of *C. mogii* can be observed under anaerobic conditions.

During the course of oxygen-limited growth at constant oxygen transfer rates, the electron transfer system is not able to oxidize the cata- and anabolically produced NADH by respiration completely. As a consequence the concentration of NADH increases. It can be seen from equilibrium studies of D-xylose reductase [$K_{eq}=1.74\times10^{-10}$ M (15)] and xylitol dehydrogenase [$K_{eq}=6.9\times10^{-11}$ M (21)] that under physiological pH conditions [den Hollander *et al.* (22)] the accumulation of xylitol is favoured and can be enhanced by increasing NADH concentrations. This fact results in a higher yield for xylitol formation under oxygen limited conditions as reported in Table 5. If D-xylulose is exclusively used for growth and regeneration of NADPH via PP-shunt, decreasing rates for xylitol oxidation due to competitive inhibition of NADH against NAD^+ (21, 23) lead to smaller specific rates for growth and NADPH formation via PP-shunt under oxygen limitation. Therefore the NADP level increased, and the reaction rate of D-xylose reductase decreased due to competitive inhibition of NADP against NADPH (15). The experimental observations shown in Table 5 are in agreement with the inhibition pattern obtained from *in-vitro* studies of purified D-xylose reductase (15). It may be speculated from these experimental observations, that the conversion of xylitol to D-xylulose is the rate limiting step in D-xylose metabolism in *C. mogii*.

The results obtained from the studies of xylitol inhibition on growth and product formation are shown in Table 6. It is obvious from these results that xylitol concentrations up to 100 g/*l* have only negligible influence on the product formation. Product inhibition studies using purified D-xylose reductase showed that xylitol acts as a weak competitive inhibitor against D-xylose (15, 23, 24).

Horitsu *et al.* (9) studied the influence of culture conditions on xylitol formation by *C. tropicalis* and optimized the volumetric xylitol production rate by the Box-Wilson method. In this respect, initial D-xylose concentration, yeast extract concentration and k_La were chosen as the independent factors in 2^3-factorial experimental design. During these studies, optimal product formation ($r_{xylitol}$ =2.67 g/*l*/h, $C_{xylitol}$=110 g/*l*) was obtained at 172.0 g/*l* D-xylose, 21.0 g/*l* yeast extract and a k_La of 451.5 1/h. To compare the experimental results reported by Horitsu *et al.* (9) with those obtained with *C. mogii*, kinetic parameters summarized in Table 2 were taken into consideration. Using an initial substrate concentration of 172 g/*l* approximately 26 g/*l* biomass and 83 g/*l* xylitol can be produced which results in a maximal volumetric production rate of 3.1 g/*l*/h which is 1.2 times higher than the value reported by Horitsu *et al.* (9). For the production of 110 g/*l* xylitol in the case of *C. tropicalis* it was necessary to supplement the medium with 21.0 g/*l* yeast extract and to adjust the inlet gas to 90% of oxygen which increased the production cost remarkable.

It can be concluded from the results reported in this paper, that oxygen limitation leads to enhanced levels of NADH and due to the equilibrium between D-xylose and xylitol to higher product yields. Enhanced specific xylitol formation rates can be obtained by efficient regeneration steps for NADPH which results in low levels of NADP and therefore higher rates for D-xylose reductase. In order to gain a deeper insight into the D-xylose metabolism of *C. mogii* ATCC 18364 the D-xylose transport across the cell membrane as well as the role of NADPH-dependent D-xylose reductase, NAD^+-dependent xylitol dehydrogenase and the levels of co-metabolites ($NAD(P)^+$, NAD(P)H) are under current investigation and will be considered in a structured metabolic model which is applied to purposes of metabolic modelling and metabolic design to enhance the xylitol production from D-xylose.

NOMENCLATURE

C_P : xylitol concentration, g/*l*
C_S^0 : D-xylose inlet concentration, g/*l*
C_S : D-xylose concentration, g/*l*
C_X : biomass concentration, g/*l*
DO : dissolved oxygen concentration, μmol/*l*
$F(t)$: feeding rate, 1/h
k_La : volumetric gas-liquid mass-transfer coefficient, 1/h
K_{eq} : equilibrium constant, M
K_m : Michaelis constant for substrate, mM
K_S : half saturation constant for D-xylose uptake, g/*l*
μ : specific growth rate, g/g/h
q_{O2} : specific oxygen uptake rate, mmol/g/h
q_P : specific xylitol formation rate, g/g/h
q_S : specific D-xylose uptake rate, g/g/h
$q_{Xit\text{-}DH}$: reaction rate of xylitol dehydrogenase, g/g/h
t : process time, h
$V_F(t)$: fermentor volume, *l*
V_{max} : maximal reaction rate, U/mg
$Y_{P/S}$: product yield, g/g

$Y_{X/S}$: biomass yield, g/g

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