0273-2289/01/91–93/0423/$13.25

Model Compound Studies

Influence of Aeration and Hemicellulosic Sugars on Xylitol Production by Candida tropicalis

Thomas Walthers,[1] Patcharee Hensirisak,[2] and Foster A. Agblevor*,[2]

[1]Department of Mechanical Engineering, Technical University of Dresden, Dresden, Germany; and [2]Department of Biological Systems Engineering, Virginia Polytechnic Institute and State University, 212 Seitz Hall, Blacksburg, VA 24061-0303, E-mail: fagblevo@vt.edu

Abstract

The influence of other hemicellulosic sugars (arabinose, galactose, mannose, and glucose), oxygen limitation, and initial xylose concentration on the fermentation of xylose to xylitol was investigated using experimental design methodology. Oxygen limitation and initial xylose concentration had strong influences on xylitol production by *Candida tropicalis* ATCC 96745. Under semiaerobic conditions, xylitol yield was highest (0.62 g/g), whereas under aerobic conditions volumetric productivity was highest (0.90 g/[L·h]). In the presence of glucose, xylose utilization was strongly repressed and sequential sugar utilization was observed. Ethanol produced from the glucose caused a 50% reduction in xylitol yield when the ethanol concentration exceeded 30 g/L. When complex synthetic hemicellulosic sugars were fermented, glucose was initially consumed followed by a simultaneous uptake of the other sugars. The highest xylitol yield (0.84 g/g) and volumetric productivity (0.49 g/[L·h]) were obtained for substrates containing high arabinose and low glucose and mannose contents.

Index Entries: Xylitol; fermentation; aeration; hemicellulose.

Introduction

Xylitol is a naturally occurring sugar alcohol. Its high sweetening power, anticariogenic properties, and possibilities for use in diabetic food products *(1)* makes xylitol an attractive sucrose substitute in a wide variety of foods and beverages. Xylitol can be produced by biologic reduction of xylose, a five-carbon sugar, using microorganisms such as *Candida guilliermondii (2)*, *Pichia stiptis*, *Pachysolen tannophilus (3)*, and *Candida tropicalis (4)*.

Xylose is a major component of hemicellulose, an abundant raw material. Hemicellulose hydrolyzes into a complex mixture of sugars that

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

Table 1
Composition of Hemicellulose Hydrolysates Expressed as Percentage of Total Sugar

Hydrolysate	Xylose	Glucose	Arabinose	Galactose	Mannose	Ref.
Sugarcane	58	16	26	—	—	*7*
bagasse	75	14	11	—	—	*2*
Rice straw	67	21	12	—	—	*2*
Hardwood	70	14	5	5	5	*3*
	62	16	4	8	9	*8*
	27	11	5	14	43	*9*
Corn fiber	16	71	11	2	—	*10*
	31	41	25	4	—	*5*
Isolated corn fiber xylan	26–60	16–37	24–46	—	—	*5*

include arabinose, glucose, galactose, and mannose *(2,3,5)*. These sugars may influence xylitol yield and productivity during xylose fermentation.

Corncobs, hardwoods, sugarcane bagasse, the seed coats of rice, soybeans, and corn are sources of low-cost hemicellulose *(6)*. The composition of hemicellulose hydrolysates varies widely depending on the raw material used, hydrolysis procedures, and pretreatment methods employed *(5,7)*. Table 1 gives the compositions of several hemicellulosic substrates reported in the literature. For xylitol production using hemicellulose hydrolysate, the process is affected by the concentrations of the sugars in the fermentation medium, the ratios at which these sugars occur, as well as the toxic compounds released during the hydrolysis. For instance, high concentrations of monomeric sugars could cause osmotic stress, inhibit induction of xylose reductase enzymes, or lead to ethanol production that exceeds the tolerance level of the yeast. Additionally, the ratio of monomeric sugars may influence transport or enzyme kinetics, in cases in which both sugars compete for the same transport system or are metabolized simultaneously.

The goal of the present study was to use hemicellulose hydrolysate as the feedstock for the microbial production of xylitol. Because some hemicellulose hydrolysates contain high levels of glucose and arabinose in addition to other sugars, we conducted model sugar studies designed to simulate the influence of sugar composition and other fermentation parameters (in the absence of toxic hydrolysate components) on xylitol yield and productivity using *C. tropicalis* ATCC 96745 as a reference organism.

Materials and Methods

Microorganism

C. tropicalis ATCC 96745 was acquired from the American Type Culture Collection (Rockville, MD) and maintained on yeast extract,

peptone, dextrose agar slants at 4°C. The microorganisms were subcultured every 2 wk.

Culture Media

The preculture medium contained 60 g/L of xylose, 10 g/L of yeast extract, 15 g/L of KH_2PO_4, 3 g/L of $(NH_4)_2HPO_4$, 1 g/L of $MgSO_4 \cdot 7H_2O$, and three drops of Sigma 289 antifoaming agent (Sigma, St. Louis, MO). The pH was adjusted to 5.0 using 1 *M* HCl *(4)*.

The production medium contained 20 g/L of yeast extract, 15 g/L of KH_2PO_4, 3 g/L of $(NH_4)_2HPO_4$, 1 g/L of $MgSO_4 \cdot 7H_2O$, and three drops of Sigma 289 antifoaming agent. The concentrations of xylose, glucose, arabinose, galactose, and mannose were adjusted according to the experimental design. Sugar and salt solutions were autoclaved separately for 20 min at 121°C. Weight loss, which occurred after sterilization, was made up with the addition of sterile water. The pH was adjusted to 4.0 using 1 *M* HCl. All chemicals were reagent grade, obtained from Sigma.

Fermentation Conditions

Precultures were grown at 30°C in 500-mL Erlenmeyer flasks containing 250 mL of medium that were agitated at 130 rpm on a rotary platform shaker (Innova 2050). The cells were harvested after 14–16 h during the midexponential growth phase. The cells were centrifuged (11,000*g* for 10 min), decanted, washed with sterile water, and recentrifuged. The inoculum was transferred to the production medium at an initial cell concentration of 0.5 g/L. Fermentation was carried out in 250-mL cotton-plugged Erlenmeyer flasks at 30°C and agitated at 130 rpm on a rotary platform shaker. Aeration levels were adjusted by varying the volume of medium *(11)* at a constant agitation speed of 130 rpm.

Analytical Methods

Dry cell mass was estimated from an optical density/dry cell weight calibration curve. Spectrophotometric measurements were carried out at 640 nm using a Spectronic 1001 instrument (Milton Roy, Rochester, NY). After determining the absorbance at 640 nm, the samples were dried to a constant weight at 105°C in a Thelco laboratory oven (Precision Scientific, Chicago, IL). The gravimetric and spectrophotometric data were used to develop a calibration curve. The fermentation samples for xylose and mixed sugars were diluted with deionized water 10- and 20-fold, respectively, before the spectrophotomeric analysis.

Sugar and sugar alcohols were analyzed using a high-performance liquid chromatograph (Shimadzu, Columbia, MD) equipped with a refractive index detector. A carbohydrate column (Supelcogel™ Ca, 30 cm × 7.8 mm; Supelco, Bellefonte, PA) was used for the analysis. Column temperature was 80°C and filtered deionized water was used as the mobile phase. The mobile phase flow rate was raised linearly over the course of

20 min, beginning at 0.5 mL/min with a final value of 2 mL/min. Peaks were detected by refractive index and were identified and quantified by comparison to retention times of authentic standards (xylose, glucose, mannose, galactose, arabinose, ethanol, and xylitol).

For high-performance liquid chromatography analysis, 400-µL aliquots were diluted with 1200 µL of deionized water in 2-mL plastic test tubes, centrifuged at 26,000*g* for 10 min, and decanted. The samples were filtered through a 0.2-µm syringe filter before injection of 20 µL into the column.

In cases in which the fermentation medium contained multiple sugar mixtures, the resolution of the mannose and galactose peaks on the Supelcogel Ca column was poor. Consequently, a complementary sugar analysis was carried out on a gas chromatograph (Shimadzu gas chromatograph, GC-14A) according to ASTM standard method E 1821-96. The following chromatographic conditions were used for the analysis: column, Supelco SP-2380 (30 m, 0.25 mm id, 0.2-µm film thickness); carrier gas, helium; column flow rate, 0.6 mL/min; total gas flow rate, 64 mL/min; split ratio, 101:1; detector, flame ionization at 220°C; injection temperature, 240°C; and sample size, 1 µL. Shimadzu CLASS-VP™ software was used for temperature programming and data retrieval.

Experimental Design

To quantify the influence of initial xylose concentration and aeration on the production of xylitol, a 2^2 factorial experimental design was applied with four star points ($a = 1.41$) and five replications at the center point *(12)*. Initial xylose concentration was varied from 23.5 to 156 g/L. The medium volume was varied from 50.5 to 150 mL to simulate microaerobic, semiaerobic, and aerobic conditions (*see* Table 2).

In the fermentation of glucose/xylose mixtures, glucose concentrations were varied between 0 and 80 g/L, and initial xylose concentration was kept constant at 60 g/L. To simulate different aeration conditions, three levels of medium volume were used (65, 100, and 135 mL) in 250-mL Erlenmeyer flasks. The three medium levels were classified according to Nolleau et al. *(11)* as aerobic (65 mL), semiaerobic (100 mL), and microaerobic (135 mL).

For the complex sugar mixtures, a second-order experimental design was developed. This experimental design covered the possible variations in actual sugar concentrations as well as the variation in the ratios of these sugars found in most hemicellulose hydrolysates (Table 1). Table 3 gives the design parameters for glucose, arabinose, galactose, mannose, and medium volume.

With second-order polynomials, only one local extremum can be modeled. Hence, the amount of glucose was varied below a concentration of 60 g/L. Preliminary experiments using high arabinose concentrations showed that although it was not significantly fermented by the yeast, it had a stimulating effect on xylitol production (data not shown). Thus, the influence of arabinose was tested at concentrations as high as 80 g/L. Galactose

Table 2
Experimental Design and Results
for Xylitol Yield and Productivity from Xylose Fermentation

Coded value	Xylose *A* (g/L)[a]	Medium *B* (mL)[b]	Xylitol yield (g/g) observed	Xylitol productivity (g/[L·h]) observed
1	120	135	0.65	0.41
–1	40	135	0.37	0.12
–1	40	65	0.50	0.39
1	120	65	0.64	0.74
+a	156	100	0.69	0.70
–a	23.5	100	0.48	0.22
0	80	150	0.57	0.22
8	80	50.5	0.59	0.67
9	80	100	0.61	0.43
10	80	100	0.61	0.44
11	80	100	0.63	0.46
12	80	100	0.63	0.46
13	80	100	0.63	0.46

[a]*A* = initial xylose concentration.
[b]*B* = medium volume in shake flask.

Table 3
Experimental Design for Complex Sugar Mixtures
Showing Star (±*a*), Axis (±1), and Center (0) Point Values

Coded variable	Parameter	Unit	+*a*	1	0	–1	–*a*
A	Glucose	g/L	60	42.6	30	17.4	0
B	Arabinose	g/L	80	56.8	40	23.2	0
C	Galactose	g/L	10	7.1	5	2.9	0
D	Mannose	g/L	5	3.55	2.5	1.45	0
E	Medium	mL	130.7	110	95	80	59.3

and mannose are minor components of most hemicellulose hydrolysates (Table 1) except in some wood hydrolysates, in which they are significant. To limit osmotic stress, these two sugars were varied in proportion to the composition of most hemicellulose hydrolysates except those found in some wood hydrolysates. Table 3 lists limits of the design parameters and their corresponding coded values. Xylose concentration was kept constant at 60 g/L.

Results and Discussion

Effect of Initial Xylose Concentration and Volume of Medium

C. tropicalis utilized the accumulated xylitol and ethanol when xylose concentrations were very low (<1.0 g/L). Consequently, xylitol yields and

Table 4
Regression Equations
for Xylitol Yield and Productivity from Fermentation of Xylose

Parameter	Regression equation[a]	R^2
Xylitol yield (g/g)	$0.6220 + 0.0896A - 0.0185B + 0.0350A \times B - 0.0291A^2 - 0.0316B^2$	0.92
Xylitol productivity (g/[L·h])	$0.4500 + 0.1669A - 0.1546B$	0.99

[a] A = coded value for initial xylose concentration; B = coded value for medium volume.

productivities were determined at the maximum product concentration and not at the end of the run. The experimental results in Table 2 were used to estimate the main effects of the variables and their interactions. Polynomial models were used to establish the relationships between the dependent variables (xylitol yield and xylitol productivity) and the independent variables (initial xylose concentration and aeration). The data were fitted to the following model equations:

$$\text{Xylitol yield} = \beta_o + \beta_1(A) + \beta_2(B) + \beta_3(A \times B) + \beta_4(A^2) + \beta_5(B^2)$$

$$\text{Xylitol productivity} = \alpha_o + \alpha_1(A) + \alpha_2(B) + \alpha_3(A \times B) + \alpha_4(A^2) + \alpha_5(B^2)$$

The xylitol yield was fitted to the initial xylose concentration and volume of medium (aeration) by a second-order polynomial model. Analysis of variance (ANOVA) found that the linear, quadratic, and interaction terms were significant ($p < 0.05$). Table 4 gives the empirical model equations. Xylitol yield was correlated with high initial xylose concentration and low aeration ($R^2 = 0.92$). This trend indicates that xylitol is an overflow metabolite *(13)*. The highest xylitol yield calculated from the empirical model equation was 0.7 g/g, which was achieved at an initial xylose concentration of 156.5 g/L under semiaerobic conditions (117 mL of medium).

The positive interaction between initial xylose concentration and aeration can be explained in terms of cell density *(4)*. At high initial xylose concentrations and high aeration, the cells grew rapidly at the beginning of fermentation. This led to high cell densities and low oxygen levels in the later stages of the fermentation and resulted in high production rates. At lower initial xylose concentrations, cell densities were low and the level of dissolved oxygen remained high; therefore, less xylitol was accumulated.

The negative coefficient for the quadratic terms in the xylitol yield model also suggests that extremely high initial xylose concentrations will be detrimental to xylitol yields. This prediction could be attributed to osmotic stress, which could be induced in the microorganism by the excess amount of sugar in the medium. Thus, for this microorganism, there is an upper limit for the initial xylose concentration (156 g/L) that will not induce osmotic stress for low aeration rates. However, the osmotic stress can be counteracted by increased aeration. Thus, careful manipulation of

Table 5
Xylitol Yield and Productivity Data for Fermentation of Mixtures Containing Xylose and Hemicellulosic Sugars[a]

Substrate	Fermentation time (h)	Ethanol concentration (g/L)	Y_{xyl} (g/g)	p_{xyl} (g/L·h)
Xylose	91.7	4.1	0.61	0.57
Xylose + 20 g/L of glucose	142	15.4	0.36	0.15
Xylose + 10 g/L of glucose	127	8.4	0.42	0.20
Xylose + 20 g/L of mannose	108.3	14.1	0.54	0.32
Xylose + 10 g/L of mannose	104.5	8.5	0.53	0.31
Xylose + 20 g/L of galactose	104.5	13.5	0.47	0.27
Xylose + 10 g/L of galactose	104.5	8.0	0.51	0.25
Xylose + 20 g/L of arabinose	129	4.2	0.54	0.37
Xylose + 20 g/L of arabinose	ND	ND	ND	ND

[a]All fermentations commenced with 60 g/L of xylose. ND, not determined.

both the aeration and initial xylose concentration probably can result in very high xylitol yields beyond those observed in these studies.

Xylitol productivity was fitted to initial xylose concentration and aeration by a linear regression model (Table 4). Xylitol productivity was correlated with high aeration rate and high initial xylose concentration (R^2 = 0.99). The highest productivity of 0.9 g/(L·h) was achieved at the highest aeration level (50.5 mL of medium volume).

In both the productivity and yield of xylitol, aeration appeared to have played a significant role. This is in agreement with the finding of Nolleau et al. *(11)*, who showed that by varying the volume of medium in a shaker flask, the aeration conditions could be varied sufficiently to enable investigations to be carried out. This approach was subsequently used to study the influence of aeration and hemicellulosic sugars on xylitol production.

Effect of Binary Sugar Mixtures

When *C. tropicalis* was cultivated in a medium containing single sugars as the carbon source, it fermented glucose, mannose, and galactose but was unable to utilize arabinose (data not shown). The yeast produced no xylitol from any of these sugars.

Similar to the xylose experiments, the xylitol yields and productivities for the xylose/glucose experiments were determined at the maximum product concentration instead of the end of the run. Table 5 gives the results of fermentation of binary sugar mixtures consisting of xylose (60 g/L) and other hemicellulosic sugars at two levels. Glucose strongly inhibited xylitol formation at all levels, and mannose and galactose caused moderate inhibition in xylitol production, whereas arabinose appeared to have a slightly positive effect on xylitol productivity.

The influence of initial glucose concentration and aeration regime on xylitol yield is shown in Fig. 1A. Xylitol productivity followed a trend

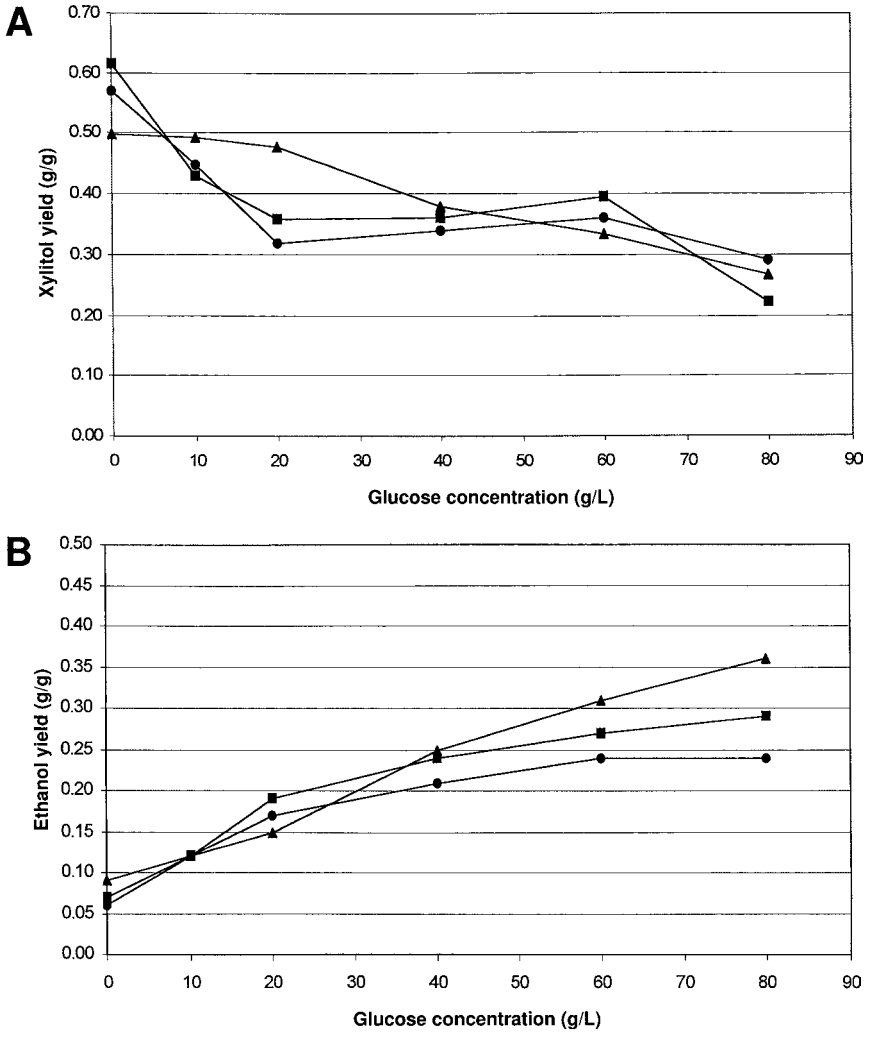

Fig. 1. **(A)** Observed xylitol yields in the fermentation of xylose/glucose mixtures. Aerobic, 65 mL of medium (●); semiaerobic, 100 mL of medium (■); and microaerobic, 135 mL of medium (▲). Initial xylose concentration was 60 g/L. **(B)** Observed ethanol yield from the fermentation of xylose/glucose mixtures. Aerobic, 65 mL of medium (●); semiaerobic, 100 mL of medium (■); and microaerobic, 135 mL of medium (▲). Initial xylose concentration was 60 g/L.

similar to that of the yield (data not shown). In the presence of glucose, xylitol yield was a function of the aeration regime. Xylitol yield was highest (0.62 g/g) under semiaerobic conditions in the absence of glucose. In the presence of glucose, xylitol yield was highest (0.50 g/g) under microaerobic conditions. Low initial glucose concentrations (10–20 g/L) did not have any appreciable effect on xylitol yield under microaerobic conditions, but above 20 g/L, xylitol yield decreased considerably. By contrast, for the aerobic and semiaerobic conditions, there were considerable decreases in

xylitol yield at low initial glucose concentrations. The initial decrease in xylitol yields leveled off for both aeration regimes for glucose concentrations between 20 and 60 g/L. However, at 60 g/L or higher of glucose, xylitol yield decreased further.

Xylitol production was always accompanied by ethanol production. Ethanol yield increased with the concentration of glucose for all aeration regimes (Fig. 1B). However, ethanol yield was sensitive to the aeration regime. Ethanol yield was highest (0.36 g/g of substrate) under microaerobic conditions. In all cases, ethanol production was very rapid and occurred within 10 h after the start of the fermentation and remained almost constant throughout the run. This clearly showed that most of the ethanol was produced from the fermentation of glucose and probably sugars in the yeast extract.

Independent of either aeration regime or glucose concentration, the glucose in the medium was always consumed first before xylose. Thus, the growth curve showed a diauxic pattern when glucose was in the medium (Fig. 2A). The secondary lag period between glucose depletion and initiation of xylose consumption was more pronounced at higher glucose concentrations. Cell densities were about threefold that for xylose fermentation.

Note that during the early stages of glucose/xylose mixture fermentation, the cell densities were significantly higher than those for xylose fermentation (Fig. 2A). However, contrary to the predictions of Yahashi et al. *(14)*, the higher cell densities did not result in higher specific xylose uptake, higher specific xylitol yield, or productivity; instead, there was an overall decrease in xylitol yield relative to xylose fermentation.

These observations suggest the presence of an additional regulatory mechanism that affects the metabolism of glucose/xylose mixtures. Several plausible explanations could be adduced for the aforementioned phenomena. The most convincing evidence is the effect of ethanol on xylitol production. The influence of ethanol concentration on xylitol yield is shown in Fig. 2B. For the aerobic and semiaerobic fermentation, the presence of low levels of ethanol (10 g/L) resulted in a 45% decrease in xylitol yield. The microaerobic condition was less sensitive to low ethanol concentration. However, all three aeration regimes had more than a 50% decrease in xylitol yield when the ethanol concentration in the medium was >30 g/L.

The effect of ethanol was further investigated by the addition of similar concentrations of ethanol to xylose fermentation medium 24 h after the start of the experiment. There was a reduction in xylitol yield, but additionally, the yeast used ethanol as a cosubstrate to produce cell mass when the ethanol concentrations were <30 g/L. However, when the ethanol concentration was raised to 50 g/L, cell growth ceased and no xylitol was produced (data not shown).

The presence of ethanol in the medium could also account for the decrease in xylitol yield in the presence of the other hemicellulosic sugars (Table 5). The addition of glucose (20 g/L) caused the greatest reduction in xylitol yield (41%). Because glucose is a catabolite repressor, all the glucose

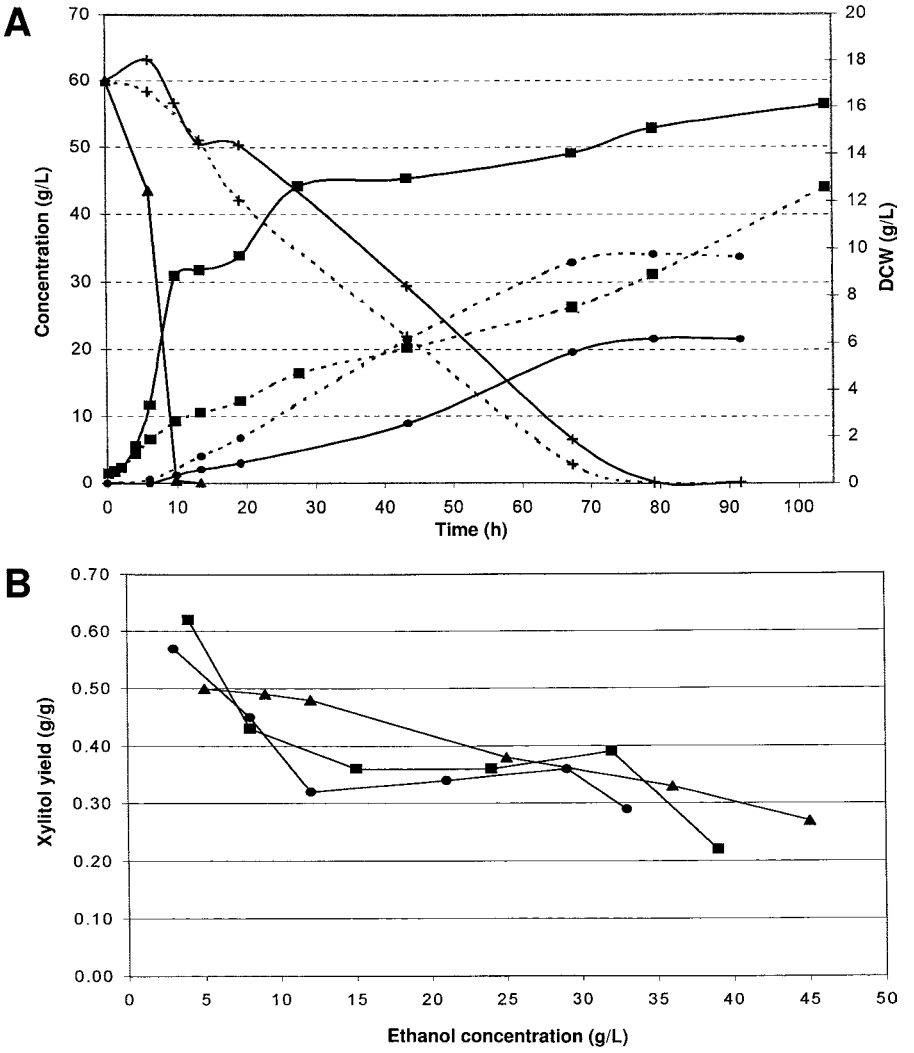

Fig. 2. **(A)** Influence of glucose on xylitol production. (– – –) Fermentation of xylose only; (——) fermentation of xylose/glucose mixture; (+) xylose consumption; (▲) glucose consumption; (■) cell concentration; and (●) xylitol formation. Initial xylose concentration was 60 g/L, glucose concentration was 60 g/L, and medium volume was 65 mL. **(B)** Influence of coproduct ethanol concentration on xylitol yield. Aerobic, 65 mL of medium (●); semiaerobic, 100 mL of medium (■); and microaerobic, 135 mL of medium (▲). Initial xylose concentration was 60 g/L. DCW, dry cell weight.

in the medium was converted to ethanol before xylose utilization started. The production of xylitol was therefore started in the presence of ethanol (approx 10 g/L). However, in the case of the fermentation of xylose with other sugars (mannose and galactose), there was a simultaneous uptake of xylose and the sugar, and, therefore, the concentration of ethanol in the medium was relatively low and rose gradually with time. Consequently, its

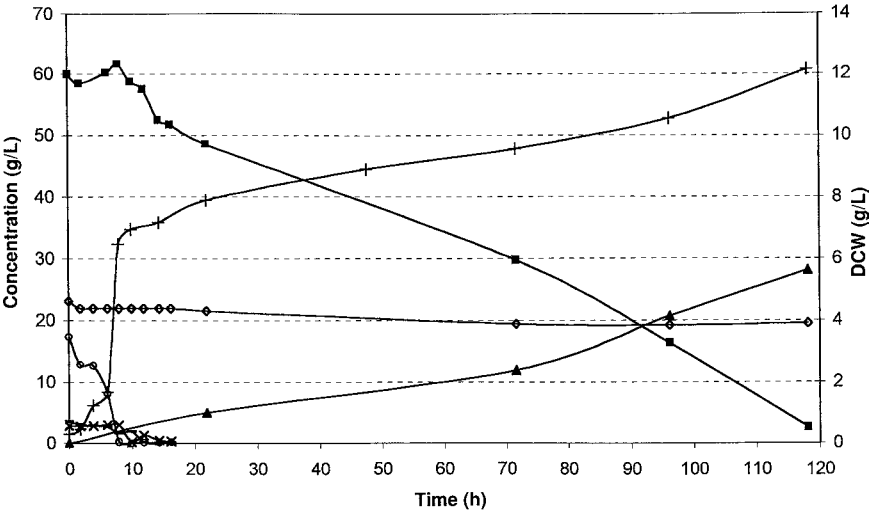

Fig. 3. Fermentation of complex sugar mixture with initial concentration of 60 g/L of xylose (■), 17.4 g/L of glucose (○), 23.2 g/L of arabinose (◇), 3.55 g/L of mannose (–), and 2.9 g/L of galactose (×). (+) Dry cell mass; (▲) xylitol. DCW, dry cell weight.

effect on xylitol yield was less drastic, and the overall xylitol yield decreased by 22% for 20 g/L of galactose and 11% for 20 g/L of mannose.

Complex Sugar Mixtures

In the fermentation of complex sugar mixtures, sugar utilization by *C. tropicalis* was sequential. Glucose was utilized first followed by the consumption of the other sugars. Mannose, galactose, and xylose were consumed simultaneously after glucose was depleted. Arabinose was not fermented significantly (Fig. 3). Interestingly, the pronounced diauxic growth pattern obtained for the binary sugars was almost absent from the complex sugar growth curve (Fig. 3). This growth pattern can be attributed to the simultaneous uptake of xylose, mannose, and galactose after the depletion of glucose in the medium and the ability of *C. tropicalis* to grow on mannose and galactose.

To predict and quantify the level of factors on xylitol yield and productivity, the experimental data were fitted to a second-order polynomial model. For xylitol yield, ANOVA found the influence of glucose, arabinose, mannose, and aeration to be significant ($p < 0.05$), whereas the contribution of galactose was statistically insignificant. Interactions between glucose and arabinose, mannose and arabinose, glucose and aeration, and mannose and aeration were also found to be statistically significant ($p < 0.05$). The regression model (Table 6) was significant ($p < 0.05$) with a good correlation ($R^2 = 0.90$). The model also indicates that except for arabinose/mannose, all the other interactions were negative. The antagonistic effect of glucose/arabinose, glucose/aeration, and mannose/aeration indicates that simultaneous increases in the levels of any of these factors

Table 6
Regression Equations for Fermentation of Complex Sugar Mixtures

Parameter	Regression equation[a]	R^2
Xylitol yield (g/g)	$0.5033 - 0.02362A + 0.0369B - 0.01094A \times B - 0.0178A \times E + 0.00781B \times D - 0.00969DE - 0.01252E^2$	0.90
Xylitol productivity (g/[L·h])	$0.27753 - 0.005A + 0.02226B + 0.01342C - 0.02324E - 0.00781A \times B - 0.01281A \times E - 0.00719B \times D - 0.01094D \times E + 0.00599A^2 - 0.01523E^2$	0.91

[a]A, B, C, D, and E are the coded values for glucose, arabinose, galactose, mannose, and medium volume, respectively, as given in Table 3.

will decrease xylitol yield. By contrast, a simultaneous increase in arabinose and mannose levels will improve xylitol yield.

Similar to xylitol yield, xylitol productivity was fitted to a second-order polynomial. ANOVA showed that the relationship between xylitol productivity and various factors was more complex than that for xylitol yield. The model was statistically significant ($p < 0.05$) with a satisfactory correlation ($R^2 = 0.91$). Glucose, arabinose, galactose, and aeration had a statistically significant influence on xylitol productivity ($p < 0.050$), whereas mannose had only an interactive effect. Interactions between glucose and arabinose, mannose and arabinose, glucose and aeration, and mannose and aeration were all statistically significant ($p < 0.05$). Unlike xylitol yield, interaction between various factors had a negative effect on xylitol productivity (Table 6).

The data show that for effective xylitol production, the glucose concentrations in the medium should be very low. When the fermentation medium contained glucose, higher yields and productivities were obtained under aerobic conditions, and in the absence of glucose, microaerobic conditions improved yields. This observation can be attributed to increased oxygen demand by the high cell densities achieved in the presence of glucose.

Arabinose appears to be a gratuitous inducer of xylose reductase enzymes; therefore, high arabinose concentrations stimulated both yield and productivity. Galactose consumption did not have any effect on xylitol yield but had a stimulating effect on xylitol productivity when its concentration was 10 g/L. Although the concentration of mannose was low, it caused a decrease in xylitol yield, because the yeast converted it to ethanol, which inhibits xylitol formation.

Conclusion

The empirical models developed can be used to estimate the achievable xylitol yields and productivities under different aeration conditions if the composition of the hemicellulose hydrolysate is known. For the hydrolysis of a raw material, the model can provide useful information for

the design of the hydrolysis process, because varying the process conditions can influence the ratio and concentration of different sugars in the hydrolysate.

When glucose is present in the medium, high cell densities are obtained without the consumption of xylose, and xylitol formation is strongly inhibited by two mechanisms. Initially, xylose uptake is repressed and then the ethanol produced from the utilization of the glucose partially inhibits xylitol formation. Thus, for hemicellulosic feedstocks with high glucose content, it will be necessary to remove the initial ethanol formed from the fermentation of the glucose in order to attain high xylitol yield and productivity.

C. tropicalis utilized both accumulated xylitol and ethanol for cell growth when xylose concentration was very low (<1 g/L). It will, therefore, not be possible to ferment all the xylose in the medium without losing some of the xylitol accumulated.

References

1. Pepper, T. and Olinger, M. (1988), *Food Technol.* **10,** 98–106.
2. Roberto, I., Felipe, M. G. A., Mancilha, I. M., Vitolo, M., Sato, S., and da Silva, S. S. (1995), *Bioresour. Technol.* **51,** 255–257.
3. Perego, P., Converti, A., Palazzi, E., Del Borghi, M., and Ferraiolo, G. (1990), *J. Ind. Microbiol.* **6,** 157–164.
4. Horitsu, H., Yahashi, Y., Takamizawa, K., Kawai, K., Suzuki, T., and Watanabe, N. (1992), *Biotechnol. Bioeng.* **40,** 1085–1091.
5. Hespell, R. B., O'Bryan, P. J., Moniruzzaman, M., and Bothast, R. J. (1997), *Appl. Biochem. Biotechnol.* **62,** 87–96.
6. Whistler, R. L. (1993), in *Industrial Gums—Polysaccharides and Their Derivatives*, Whistler, R. D. and BeMiller, J. N., eds., Academic, San Diego, pp. 295–308.
7. Chen, L.-F. and Gong, C.-S. (1985), *J. Food Sci.* **50,** 226–228.
8. Jeffries, T. W. and Screenath, H. K. (1988), *Biotechnol. Bioeng.* **31,** 502–506.
9. Olsson, L. and Hahn-Hägerdahl, B. (1993), *Process Biochem.* **28,** 249–257.
10. Saha, B. C., Dien, B. S., and Bothast, R. J. (1998), *Appl. Biochem. Biotechnol.* **70–72,** 115–125.
11. Nolleau,V., Preziosi-Belloy, L., Delgenes, J. P., and Navarro, J. M. (1993), *Curr. Microbiol.* **27,** 191–197.
12. Box, G. E. P. and Draper, N. R. (1987), *Empirical Model Building and Response Surfaces*, John Wiley & Sons, New York.
13. Ojamo, H. (1994), *Yeast Xylose Metabolism and Xylitol Production*, Technical Research Centre of Finland, VTT Publications, Espoo, Finland.
14. Yahashi, Y., Horitsu, H., Kawai, K., Suzuki, T., and Takamizawa, K. (1996), *J. Ferment. Technol.* **81,** 148–152.