J. Microbiol. Biotechnol. (2001), **11**(4), 564–569

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

High-Yield Production of Xylitol from Xylose by a Xylitol Dehydrogenase Defective Mutant of *Pichia stipitis*

KIM, MIN-SOO, YUN-SEUNG CHUNG[1], JIN-HO SEO[1], DO-HYUN JO, YUN-HEE PARK, AND YEON-WOO RYU*

Department of Molecular Science and Technology, Ajou University, Suwon 442-749, Korea
[1]Department of Food Science and Technology, Seoul National University, Suwon 441-744, Korea

Received: October 4, 2000
Accepted: May 15, 2001

Abstract This study was carried out in order to investigate the characteristics of xylitol fermentation by a xylitol dehydrogenase defective mutant PXM-4 of *P. stipitis* CBS 5776 and to determime optimum conditions for the high yield of xylitol production from xylose. Gluconic acid was selected as a cosubstrate for the xylitol fermentation, since gluconic acid neither blocked xylose transport nor repressed xylose reductase expression. An increase of gluconic acid concentration reduced the rates of xylitol production and cell growth by decreasing medium pH, and the optimal concentration of gluconic acid was determined to be 20 g/l with approximately 100% xylitol conversion yield. A fed-batch cell culture resulted in a 44.8 g/l xylitol concentration with 100% yield, based on the amount of xylose consumed.

Key words: *P. stipitis*, xylitol, xylitol dehydrogenase defective mutant, xylose reductase, gluconic acid

Xylitol, a five-carbon sugar alcohol, is a naturally occurring sweetener present in small quantities in a wide variety of fruits, vegetables, and mushroom [18]. It is also a normal metabolite in the mammalian carbohydrate metabolism, including human's [6]. The use of xylitol in the food industry is increasing due to its high sweetening power [7]. In addition, it has an anticariogenic property which does not cause acid formation and it can be used as a sugar substitute for diabetics since it does not require insulin for its metabolism [17]. Xylitol can also be effectively used as an acceptor for the enzymatic transglycosylation reaction for the production of glycosylated sugar alcohol [14].

Xylitol is currently produced by chemical hydrogenation of xylose in hemicellulose hydrolyzates using Ni/Al_2O_3 as a catalyst [16]. The product cost is high as a result of difficulties in the separation and purification of xylitol, removal of by-products from hemicellulose hydrolyzates, and the low yield of 40–50% [7]. Biotechnological processes for xylitol production using xylose-fermenting microorganisms have several advantages, such as selective conversion of xylose to xylitol with a high yield. Microorganisms employed for biotechnological production of xylitol include bacteria [8], fungi [4], and yeasts, especially *Pachysolen tannophilus* [24], *Pichia stipitis* [5, 11], *Candida pelliculosa* [19], *C. boidinii* [25, 26], *C. guilliermondii* [20], *C. tropicalis* [10, 22], and *C. parapsilosis* [12, 20].

The xylose metabolism in yeasts starts with the reduction of xylose to xylitol, catalyzed by xylose reductase (XR: EC 1.1.1.22) with cofactor NAD(P)H [24]. Xylitol is subsequently oxidized to xylulose by xylitol dehydrogenase (XDH: EC 1.1.1.9) with cofactor $NAD(P)^+$ [24]. Xylulose is then used for cell growth and NADPH regeneration through the pentose phosphate pathway after conversion to xylulose-5-phosphate by xylulose kinase with ATP as a cofactor [9]. The first two enzymes, XR and XDH, are known as the rate-limiting enzymes in xylitol production by yeasts. Several studies showed a correlation between the key enzyme activities and xylitol production in yeasts [20, 26]. XR and XDH in most yeasts are inducible in a medium containing xylose as a carbon source [1], however, the presence of hexoses such as glucose in the fermentation medium represses the expression of XR and XDH [10, 13], which results in a decrease in xylitol yield and productivity.

Xylose is an expensive substrate for the production of xylitol. Hence, it is important to increase the yield and productivity of xylitol by improving the strain and by optimizing the fermentation processes [3, 13]. This study was undertaken to investigate the characteristics of xylitol fermentation by a xylitol dehydrogenase defective mutant PXM-4 of *P. stipitis* CBS 5776, in an effort to determine the optimum bioconversion scheme for the high-yield production of xylitol from xylose.

*Corresponding author
Phone: 82-31-219-2449; Fax: 82-31-216-8777;
E-mail: ywryu@madang.ajou.ac.kr

MATERIALS AND METHODS

Chemicals

All carbohydrates, xylitol, and NADPH were supplied from Sigma Chemical Company (St. Louis, MO, U.S.A.), and acetonitrile was purchased from Fisher Scientific Co. (Rochester, NY, U.S.A.). All other chemicals were of the reagent grade.

Microorganism and Inoculum Preparation

Pichia stipitis CBS 5776 and its XDH defective mutant PXM-4, as reported previously [11], were used in this study. *P. stipitis* was maintained at 4°C on a YPX slant composed of 10 g/l yeast extract, 10 g/l peptone, and 20 g/l xylose. Twenty g/l glucose (YPD) instead of xylose was used for the mutant PXM-4.

For inoculum preparation, a loopful of cells grown on the YPD slant was transferred to a 250-ml Erlenmeyer flask containing 50 ml of the YPD medium. The cultures were grown aerobically in a shaking incubator at 150 rpm and 30°C for 12 h.

Xylitol Fermentation

Xylitol fermentation was conducted in a 500-ml Erlenmyer flask containing 200 ml of the fermentation medium composed of 5 g/l of yeast extract, 5 g/l of peptone, 5 g/l of KH_2PO_4, 2 g/l of $(NH_4)_2SO_4$, 0.4 g/l of $MgSO_4$ and appropriate amounts of xylose and a cosubstrate. Xylitol fermentation for determining XR activity profiles were performed in a 2.5-l fermenter (Korea Fermenter Co, Korea) at 250 rpm agitation, 1 vvm aeration, and 30°C. Fed-batch cultures for obtaining high cell concentration were performed in the same fermenter. The cultures were performed batchwise with fermentation medium containing 10 g/l glucose as a carbon source and switched to a fed-batch mode by feeding the growth medium containing 120 g/l of glucose and 30 g/l of yeast extract.

Determination of Xylose Reductase Activity

Xylose reductase activity was measured spectrophotometrically at 340 nm by the oxidation of NADPH with the cell free extract. The cells grown in the fermentation medium containing xylose and a cosubstrate were harvested by centrifugation at 4,000 rpm for 10 min. The cells were washed twice with 5 ml potassium phosphate buffer (0.2 mM pH 7.0), resuspended in 5 ml of the same buffer by mixing with 0.5 g glass bead (0.1 mm) and then disrupted in the Mini-BeadBeater (BioSpec Products, Inc.) for 3 min. The cell debris and glass beads were separated by centrifugation at 10,000 rpm and the supernatant was used for measuring the enzyme activity. The reaction mixture consisted of 1 ml of 1 M potassium phosphate buffer (pH 7.0), 0.2 ml of 1 M 2-mercaptoethanol, 1 ml of enzyme solution, and 0.2 ml of 0.5 M xylose. This reaction mixture was allowed to stand for 1 min to exhaust endogenous oxidation of NADPH. The activity of XR was determined by monitoring the oxidation of NADPH in a spectrophotometric cuvette at 340 nm at room temperature. One unit of enzyme activity was defined as the amount of enzyme capable of reducing 1 μmol of NADPH per min. Specific enzyme activity was expressed as units of enzyme per mg of protein. Protein was measured by the Lowry method [15] with bovine serum albumin as a standard.

Analysis

Cell mass was estimated by using the relationship between dry cell weight and optical density (OD) measured at 620 nm. One OD unit was equivalent to 0.323 g/l of the dry cell weight. Xylitol and all other sugars were determined by HPLC (Waters Co, U.S.A.) by using both a refractive index detector and carbohydrate analysis column (Waters Co, U.S.A.) with 80% (v/v) acetonitrile as mobile phase at a flow rate of 2 ml/min. Organic acids were determined by HPLC by using a UV/VIS detector at 215 nm and Aminex HPX-87H column (Bio-Rad, U.S.A.) with 5 mM H_2SO_4 as a mobile phase at a flow rate of 0.6 ml/min. All samples were centrifugated for 10 min at 4,000 rpm, filtered through a membrane filter (0.2 μm) and 20 μl of the sample was injected for HPLC analysis.

RESULTS AND DISCUSSION

Selection of Cosubstrate

P. stipitis produced ethanol from xylose as a major product in normal fermentation conditions [5, 27]. Therefore, it was necessary to use a XDH defective mutant strain of *P. stipitis* for xylitol production from xylose. An efficient conversion of xylose to xylitol by this mutant requires a good cosubstrate for cell growth and redox balance, especially a continuous regeneration of NAD(P)H, which is a cofactor of XR. Xylose and a cosubstrate should enter the cell simultaneously without inhibition of the required permeases and not repress the enzymes involved in cosubstrate metabolism and xylose conversion. Most hexoses, such as glucose, block xylose transport and repress XR induction [13], resulting in a low xylitol yield and productivity [1, 11]. For this reason, selection of an appropriate cosubstrate for xylitol production by the mutant PXM-4 was investigated.

Experiments were conducted in a 500-ml Erlenmyer flask containing 200 ml fermentation medium with 20 g/l of xylose and 20 g/l of various cosubstrates in a shaking incubator for 5 days, and the results are summarized in Table 1. Cell growth was favorable in a medium with glucose or galactose as a cosubstrate while glycerol, and

Table 1. Comparison of xylitol production and XR activity of mutant PXM-4 in the medium containing 20 g/l of xylose and 20 g/l of various cosubstrates.

Cosubstrate	Dry cell weight (g/l)	Residual xylose (g/l)	Residual cosubstrate (g/l)	Xylitol (g/l)	Xylitol yield (%)	Specific XR activity (unit/mg-protein)	
						10 h	24 h
Glucose	4.8	10.2	0.00	9.5	98	15	205
Galactose	4.7	10.0	0.00	10.3	100	31	211
Mannitol	0.4	20.0	20.0	0.0	0	ND[a]	ND
Sorbitol	0.4	20.0	20.0	0.0	0	ND	ND
Glycerol	2.5	6.0	17.4	13.2	97	107	167
Acetic acid	2.1	10.2	15.9	9.7	99	177	236
Gluconic acid	2.5	1.5	0.00	18.4	100	181	245
Lactic acid	1.5	17.1	16.7	3.0	100	46	59

[a]not detected.

gluconic and acetic acids were good cosubstrates for the production of xylitol. Since glycerol, acetic acid, and lactic acid were not completely consumed in 5 days of fermentation, these cosubstrates were not appropriate carbon sources for the mutant PXM-4. Also, *P. stipitis* could not use mannitol and sorbitol as a carbon source. Specific XR activities at 10 h of fermentation were low in the presence of glucose and galactose. As shown in Fig. 1, cell growth in a glucose medium rapidly increased in a 24 h fermentation and then slowly decreased. But specific XR activity increased up to 3 days of fermentation due to depletion of glucose beyond 10 h of fermentation and then rapidly decreased. Xylose was continuously converted to xylitol during the fermentation period, but not completely owing to the deficiency of an energy source. These results suggested that glucose repressed XR induction in the mutant PXM-4. Therefore, gluconic acid was selected as the best cosubstrate for xylitol production by the mutant PXM-4.

Xylose Transport and XR Activity

The first step in the metabolism of xylose is the transport of xylose across the cell membrane which could be a limiting factor in the overall rate of xylose utilization. Therefore, xylose transport and XR induction in the presence of gluconic acid as a cosubstrate were investigated.

To examine the xylose transport and the profiles of specific XR activity in the mutant PXM-4, batch fermentations with 10 g/l of gluconic acid and 10 g/l of xylose were performed in a fermenter under 1 vvm aeration and 250 rpm agitation at 30°C. As shown in Fig. 2, both xylose and gluconic acid were consumed simultaneously, and intracellular xylose was detected in the presence of gluconic acid in the medium. In addition, specific XR activity was constantly sustained during the xylose and gluconic acid consumption period. It was clear from these results that gluconic acid neither inhibited xylose transport nor repressed XR expression. But xylose continuously accumulated inside the cell during

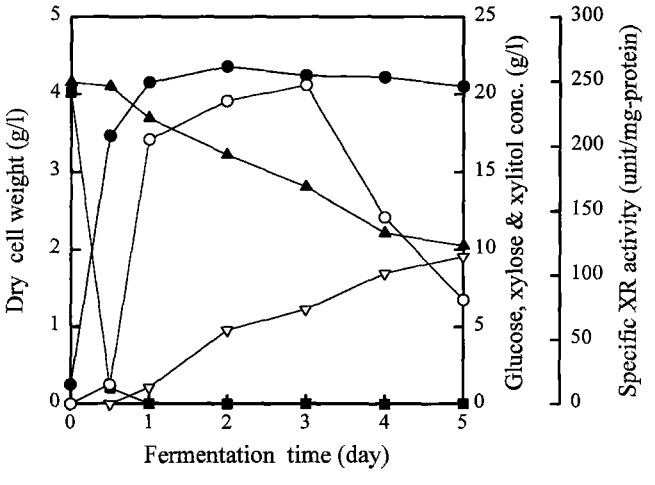

Fig. 1. Profiles of cell growth (●) and glucose (■), xylose (▲), and xylitol (▽) concentrations and specific XR activity (○) during the batch fermentation of mutant PXM-4.

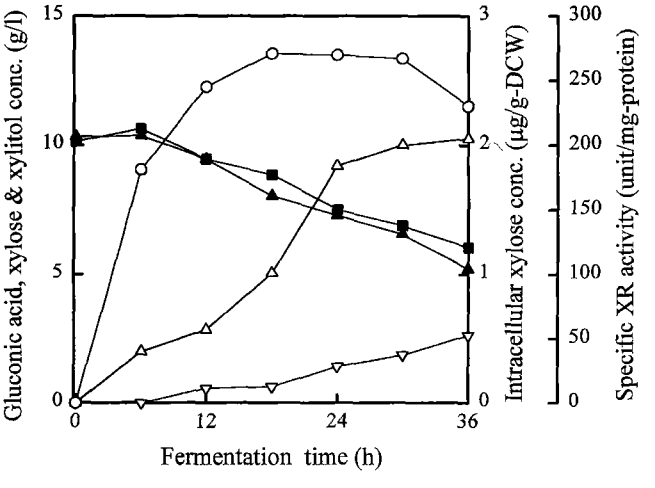

Fig. 2. Profiles of gluconic acid (■), xylose (▲), xylitol (▽), and intracellular xylose (△) concentrations and XR activity (○) during the batch fermentation of mutant PXM-4.

Table 2. Effect of initial pH on the cell growth and xylitol production by mutant PXM-4 in the medium containing 20 g/l of xylose and 20 g/l of gluconic acid at 5-days fermentation.

Initial pH	Dry cell weight (g/l)	Residual xylose (g/l)	Xylitol (g/l)	Xylitol yield (%)	Specific XR activity (unit/mg-protein)
3.00	1.5	4.0	16.1	100	154
3.56	2.2	1.8	18.1	99	205
4.00	2.2	1.4	18.5	100	211
5.00	2.9	1.5	18.4	99	206
6.00	2.9	5.7	14.0	98	227
7.00	2.5	6.0	13.5	96	253
8.00	2.0	9.9	10.5	100	129

the bioconversion period, although the specific XR activity was maintained at a level high enough for the conversion of xylose to xylitol to take place. This could be attributed to the fact that the xylose conversion required continuous regeneration of the cofactor NADPH from the gluconic acid metabolism.

Effects of Initial pH on Xylitol Production

In general, initial pH is extremely crucial to cell growth and xylitol production. The addition of gluconic acid to a growth medium caused a decrease in pH. To investigate the effects of initial pH on cell growth and xylitol production, experiments were carried out in a 500-ml Erlenmyer flask containing 200 ml of fermentation medium at different initial pHs ranging from 3.0 to 8.0. The initial pH of fermentation medium was adjusted by 1 N HCl or 1 N NaOH. The initial pH of the fermentation medium (control) was 3.56. As shown in Table 2, the optimum initial pH values for cell growth were in the range of 5.0 and 6.0. When the initial pH was set between 4.0 and 5.0, the maximum amounts of xylitol obtained was 18.5 g/l with its yield of 100%, based on xylose consumed. But the xylitol production was hampered by high initial pH, although the specific XR activity was kept at a high level. This observation indicated that xylitol production was influenced by initial pH but cell growth was independent, as reported elsewhere [20]. In particular, the maximum specific XR activity was obtained at initial pH 7.0. However, cell growth and xylitol production were not favorable as compared to those at the initial pH of 4.0. It was suggested that *P. stipitis* was sensitive to cell growth and xylitol production in high Na^+ concentrations. Sirisansaneeyakul *et al.* [23] reported that the optimum initial pH for xylitol production was 5.5 for *C. mogii*, while it was in the range of 4.0–6.0 for *Candida* sp. [2], and 7.0 for *C. boidinii* [25]. But Silva and Afschar [22] reported that the maximum xylitol yield was attained at pH 2.5 for *C. tropicalis* DSM 7524. These studies suggest that the optimum value of initial pH is dependent on the yeast strains employed.

Effects of Initial Gluconic Acid Concentration

To investigate the effects of gluconic acid concentration on cell growth and xylitol production by the mutant PXM-4, experiments were conducted in a shaking incubator with fermentation media containing 20 g/l of xylose and various concentrations of gluconic acid ranging from 5 to 40 g/l. As shown in Table 3, cell concentration increased by increasing the gluconic acid concentration up to 20 g/l, and decreased at 40 g/l gluconic acid, suggesting the optimal gluconic acid concentration of 20 g/l at a fixed xylose concentration of 20 g/l. As a result, 17.3 g/l xylitol was produced.

These experimental results were verified in a 2.5-l jar fermenter with 1.0-l of the fermentation medium containing 20 g/l of xylose and 20 g/l of gluconic acid for 5 days. As shown in Fig. 3, xylose and gluconic acid were simultaneously consumed during the fermentation period, and the specific XR activity greatly increased up to 24 h in the fermentation process and maintained at a constant value after 24 h fermentation. The maximum concentration of xylitol was 18.4 g/l with a conversion yield of 100%, based on xylose consumed in 5 days of fermentation.

Batch Fermentation

To investigate the kinetics of cell growth and xylitol production and the pattern of XR expression in the mutant

Table 3. Effect of initial gluconic acid concentration in the medium containing 20 g/l xylose on the cell growth and xylitol production by mutant PXM-4.

Initial gluconic acid (g/l)	Dry cell weight (g/l)	Residual xylose (g/l)	Xylitol (g/l)	Xylitol yield (%)	Initial pH
5	1.3	15.2	4.7	98	4.27
10	2.1	6.1	13.3	96	3.84
20	2.5	2.7	17.3	100	3.47
40	1.5	13.5	6.3	98	3.17

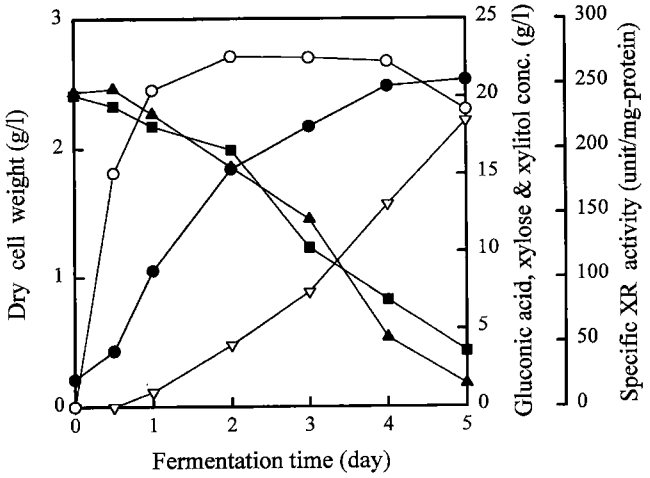

Fig. 3. Profiles of cell growth (●) and gluconic acid (■), xylose (▲), and xylitol (▽) concentrations and specific XR activity (○) during the batch fermentation of mutant PXM-4.

PXM-4, batch fermentations with 25 g/l of gluconic acid and 50 g/l of xylose were performed under 1 vvm aeration and 250 rpm agitation at 30°C. As shown in Fig. 4, cell growth continuously proceeded up to 6 days of fermentation, reaching a maximum concentration level of 4.7 g/l. Xylose and gluconic acid were consumed simultaneously up to 5 days of fermentation. Specific XR activity greatly increased in 2 days of fermentation and maintained at 206 to 224 unit/mg-protein until 5 days of fermentation. These results indicated that xylose transport and XR production were not inhibited by gluconic acid. Xylitol continuously accumulated in the medium until 6 days of fermentation, with a final concentration of 36.7 g/l. The volumetric productivity and yield of xylitol were 0.25 g/l-h and 100%, respectively. Low productivity could be improved by using the fed-batch fermentation for the high cell density culture.

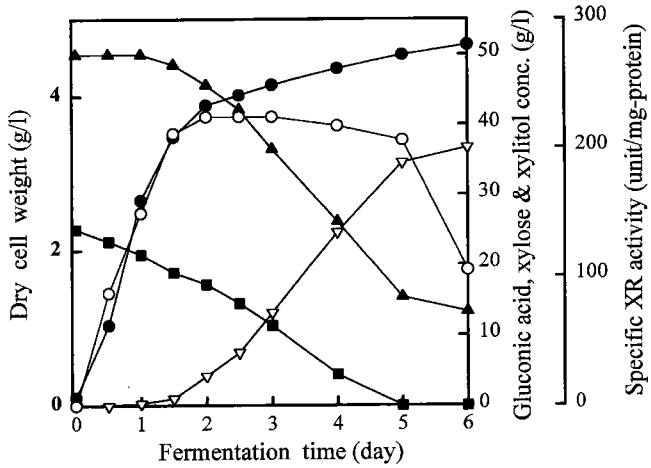

Fig. 4. Profiles of cell growth (●) and gluconic acid (■), xylose (▲), and xylitol (▽) concentrations and specific XR activity (○) during the batch fermentation of mutant PXM-4.

Fed-Batch Fermentation

Fed-batch culture has been the most popular method used to achieve a high productivity and final concentration level of desired product by maintaining a high cell concentration [28] and controlling a feeding medium during the production stage [21]. A two-stage fed-batch fermentation was employed to enhance xylitol productivity by a mutant PXM-4, the first stage for cell growth being followed by a xylitol production. The growth stage was performed in the fed-batch mode where the growth medium was intermittently fed after batch culture. Glucose concentration in the culture broth was maintained between 2 to 30 g/l, which were found to be optimum range for cell growth (data not shown). The cells concentration increased continuously by feeding with growth medium and reached 22.6 g/l after 12 h (Fig. 5A). The xylitol production stage was initiated by adding xylose and gluconic acid to maintain 50 g/l of xylose and 25 g/l of gluconic acid (Fig. 5B). The cell concentration in the fermenter was maintained at a constant level of 22 to 24 g/l during the xylitol production stage. Xylose and gluconic acid were simultaneously consumed

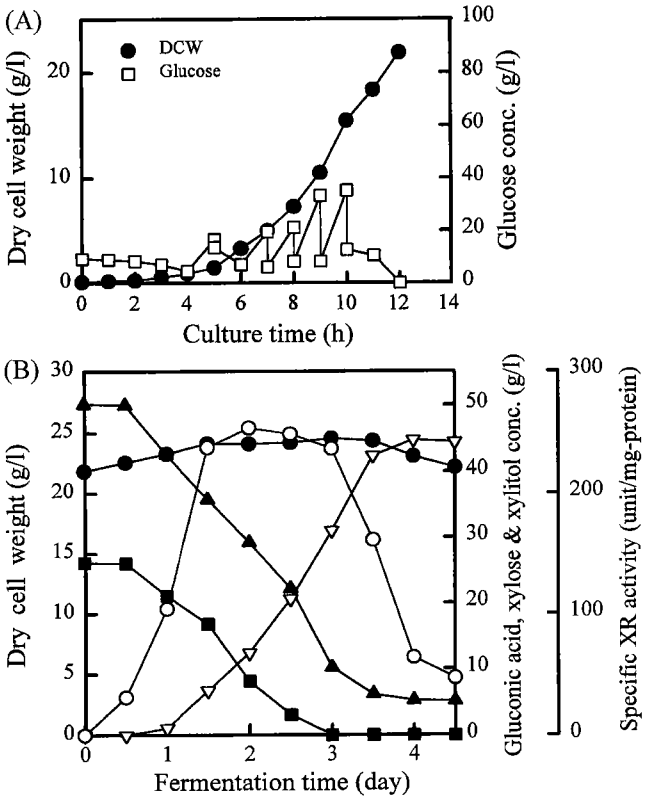

Fig. 5. Profiles of cell growth (●) and gluconic acid (■), xylose (▲), and xylitol (▽) concentrations and specific XR activity (○) during the two-stage fed-batch fermentation followed by batch culture of mutant PXM-4.
(A) represents the intermittent fed-batch culture for cell growth by feeding of growth medium and (B) is profiles of xylitol fermentation after feeding of xylose and gluconic acid.

after about 12 h of lag period. Gluconic acid was exhausted after 3 days, but xylose was not completely consumed after 4 days. The xylitol concentration was 44.8 g/l in 4 days of fermentation with almost 100% yield of xylitol. The volumetric productivity including the period of growth stage was 0.42 g/l-h, corresponding to a 1.7-fold enhancement compared with the previous batch fermentation.

Acknowledgments

This study was supported by grant No. 95-0402-05-01-3 from the Korea Science and Engineering Foundation and from the Ministry of Education by the BK21 program.

REFERENCES

1. Bicho, P. A., P. L. Runnals, J. D. Cunningham, and H. Lee. 1988. Induction of xylose reductase and xylitol dehydrogenase activities in *Pachysolen tannophilus* and *Pichia stipitis*. *Appl. Environ. Microbiol.* **54:** 50–54.
2. Cao, N. J., R. Tang, C. S. Gong, and L. F. Chen. 1994. The effect of cell density on the production of xylitol from D-xylose by yeast. *Appl. Biochem. Biotechnol.* **45/6:** 515–519.
3. Choi, J. H., K. W. Moon, Y. W. Ryu, and J. H. Seo. 2000. Production of xylitol in cell recycle fermentations of *Candida tropicalis*. *Biotechnol. Lett.* **22:** 1625–1628.
4. Dahiya, J. S. 1991. Xylitol production by *Petromyces albertensis* grown on containing D-xylose. *Can. J. Microbiol.* **37:** 14–18.
5. Furlan, S. A., P. Bouilloud, and H. F. de Castro. 1994. Influence of oxygen on ethanol and xylitol production by xylose fermenting yeasts. *Process Biochem.* **29:** 657–662.
6. Hollmann, S. and O. Touster. 1957. The xylulose-xylitol enzyme and other polyol dehydrogenases of guinea pig liver mitochondria. *J. Biochem.* **225:** 87–102.
7. Hyvönen, L., P. Koivistoinen, and F. Voirol. 1982. Food technological evaluation of xylitol. *Adv. Food. Res.* **28:** 373–403.
8. Izumori, K. and K. Tuzaki. 1988. Production of xylitol from D-xylose by *Mycobacterium smegmatis*. *J. Ferment. Technol.* **66:** 33–36.
9. Jin, Y. S., T. H. Lee, Y. D. Choi, Y. W. Ryu, and J. H. Seo. Conversion of xylose to ethanol by recombinant *Saccharomyces cerevisiae* containing genes for xylose reductase and xylitol dehydrogenase from *Pichia stipitis*. *J. Microbiol. Biotechnol.* **10:** 564–567.
10. Kim, J. H., Y. W. Ryu, and J. H. Seo. 1999. Analysis and optimization of a two-substrate fermentation for xylitol production using *Candida tropicalis*. *J. Ind. Microbiol. Biotechnol.* **22:** 181–186.
11. Kim, M. S., C. Kim, J. H. Seo, and Y. W. Ryu. 2000. Enhancement of xylitol yield by xylitol dehydrogenase defective mutant of *Pichia stipitis*. *Kor. J. Biotechnol. Bioeng.* **15:** 113–119.
12. Kim, S. Y., J. H. Kim, and D. K. Oh. 1997. Improvement of xylitol production by controlling oxygen supply in *Candida parapsilosis*. *J. Ferment. Bioeng.* **83:** 267–270.
13. Lee, W. J., Y. W. Ryu, and J. H. Seo. 2000. Characterization of two-substrate fermentation processes for xylitol production using recombinant *Saccharomyces cerevisiae* containing xylose reductase gene. *Process Biochem.* **35:** 1199–1203.
14. Lee, Y. H. and D. C. Park. 1999. Novel heterogeneous carbohydratase reaction systems for the direct conversion of insoluble carbohydrates: Reaction characteristics and their applications. *J. Microbiol. Biotechnol.* **9:** 1–8.
15. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin-Phenol reagent. *J. Biol. Chem.* **193:** 265–275.
16. Melaja, A. and L. Hämäläinen. 1977. Process making for xylitol. US patent 4,0008,285.
17. Mäkinen, K. K. 1979. Xylitol and oral health. *Adv. Food Res.* **25:** 137–158.
18. Mäkinen, K. K. and E. Söderling. 1980. A quantitative study of mannitol, sorbitol, xylitol and xylose in wild berries and commercial fruits. *J. Food Sci.* **45:** 367–371.
19. Nishio, N., K. Sugawa, N. Hayase, and N. Nagai. 1989. Conversion of D-xylose into xylitol by immobilized cells of *Candida pelliculosa* and *Methanobacterium* sp. HU. *J. Ferment. Bioeng.* **67:** 356–360.
20. Nolleau, V., L. Preziosi-Belloy, and J. M. Navarro. 1995. The reduction of xylose to xylitol by *Candida guilliermondii* and *Candida parapsilosis*; Incidence of oxygen and pH. *Biotechnol. Lett.* **17:** 417–422.
21. Ryu. H. W., K. S. Cho, B. S. Kim, Y. K. Chang, H. N. Chang, and H. J. Shim. 1999. Mass production of poly(3-hydroxybutyrate) by fed-batch cultures of *Ralstonia eutropha* with nitrogen and phosphate limitation. *J. Microbiol. Biotechnol.* **9:** 751–756.
22. Silva, S. S. and A. S. Afschar. 1994. Microbial production of xylitol from xylose using *Candida tropicalis*. *Bioproc. Eng.* **11:** 129–134.
23. Sirisansaneeyakul, S., M. Kuzmanova, and T. W. Jeffries. 1995. Screening of yeasts for production of xylitol from D-xylose. *J. Ferment. Bioeng.* **79:** 567–570.
24. Smiley, K. L. and P. L. Bolen. 1982. Demonstration of D-xylose reductase and D-xylitol dehydrogenase in *Pachysolen tannophilus*. *Biotechnol. Lett.* **4:** 607–610.
25. Vandeska, E., S. Amartey, S. Kuzmanova, and T. W. Jeffries. 1995. Effects of environmental conditions on production of xylitol by *Candida boidinii*. *World J. Microbiol. Biotechnol.* **11:** 213–218.
26. Vandeska, E., S. Kuzmanova, and T. W. Jeffries. 1989. Xylitol formation and key enzyme activities in *C. boidinii* under different oxygen transfer rate. *J. Ferment. Bioeng.* **67:** 513–516.
27. Yoon, G. S., T. S. Lee, C. Kim, J. H. Seo, and Y. W. Ryu. 1996. Characterization of alcohol fermentation and segregation of protoplast fusant of *Saccharomyces cerevisiae* and *Pichia stipitis*. *J. Microbiol. Biotechnol.* **6:** 286–291.
28. Yu, X. B., H. S. Yun, and Y. M. Koo. 1999. Cellulase production in fed-batch culture by *Trichoderma reesei* Rut C30. *J. Microbiol. Biotechnol.* **9:** 44–49.