Biotechnology and Bioprocess Engineering **2004**, 9: 223-228

The Production of Xylitol by Enzymatic Hydrolysis of Agricultural Wastes

Lien Ha Tran[1,4], Masanori Yogo[1], Hiroshi Ojima[1], Osamu Idota[1], Keiichi Kawai[3], Tohru Suzuki[2], and Kazuhiro Takamizawa[3]*

[1] United Graduate School of Agricultural Science, Gifu University, 1-1Yanagido 501-1193, Japan
[2] Life Science Research Center, Gifu University, 1-1Yanagido 501-1193, Japan
[3] Faculty of Applied Biological Science, Gifu University, 1-1Yanagido 501-1193, Japan
[4] Institute of Biological-Food Technology, Hanoi University of Technology, 1-Dai Co Viet Road, Hanoi, Vietnam

Abstract Agricultural waste products, beech wood and walnut shells, were hydrolyzed at 40°C using mixed crude enzymes produced by *Penicillium* sp. AHT-1 and *Rhizomucor pusillus* HHT-1. D-xylose, 4.1 g and 15.1 g was produced from the hydrolysis of 100 g of beech wood and walnut shells, respectively. For xylitol production, *Candida tropicalis* IFO0618 and the waste product hydrolyzed solutions were used. The effects on xylitol production, of adding glucose as a NADPH source, D-xylose and yeast extract, were examined. Finally, a 50% yield of xylitol was obtained by using the beech wood hydrolyzed solution with the addition of 1% yeast extract and 1% glucose at an initial concentration.

Keywords: xylitol, xylanolytic enzymes, xylose reductase, beech wood, walnut shells

INTRODUCTION

Xylitol is a five-carbon sugar alcohol. It has been used for some interesting applications, such as, to prevent dental caries by inhibiting the metabolism of dental plaque formation and the growth of bacteria that cause caries [1] as well as to provide an insulin-independent carbon source for diabetics [2]. It is also used as a sweetener in various food products, such as, chewing gum, sweets, soft drinks, and ice cream [3]. This has led to a rapidly increasing demand for xylitol. On the other hand, according to the FAO [4], more than 50 billion tons of agricultural products are produced every year and 10% of them go to waste with no economic utilization. Xylan is one of the abundant hemicellulose subcomponents of various agricultural waste products, which can be used for xylitol production. Currently, most of the xylitol production is manufactured from xylan by chemical method. However, the step to purify xylitol from other polyols and by-product sugars, makes its chemical production relatively complex [5]. In addition, it is hazardous to the environment because of the need for strong chemical treatment. As an alternative method, microbial production of xylitol is becoming increasingly more interesting and attractive. Therefore, the application of environmentally friendly biotechnological methods to produce xylitol from agricultural wastes will reduce the price of xylitol and at the same time solve the environmental waste problems.

***Corresponding author**
Tel: +81-58-293-2906 Fax: +81-58-293-2906
e-mail:tak2003@cc.gifu-u.ac.jp

As far as we know, almost all xylitol distributed in the world is made in Finland by a chemical method with approximatelly 50% yield from virgin beech wood plant. 30,000 tons of xylitol are yearly produced and Japan imports about 10,000 tons from Finland. Distributed price of xylitol is around 900 yen/kg. Recently, xylitol of China origin is coming a little bit. The resource of Chinese xylitol is shell of peanuts, agricultural wastes, and a chemical method is used.

From this information, it is considered that agricultural wastes is a candidate of xylitol production as well as other chemicals. In fact, our region, Gifu Prefecture produces higher amount of saw dust. If it will be used as a resource of xylitol, the cost of transportation will be reduced, providing lower price of xylitol. Japan has a big national project called "Biomass-Nippon" and one of the purposes of the project is to establish the oil-less society in future. Therefore, biomass, especially not utilized biomass at the time, turns a major concern.

In order to use xylan for xylitol production, many enzymes are required. Xylanolytic enzymes convert xylan to xylose and xylose reductase converts xylose to xylitol. Fungal species *Penicillium* sp. AHT-1 and *Rhizomucor pusillus* HHT-1, which are very strong producers of xylanolytic enzymes, have been isolated [6,7]. The highest xylitol production yield was found by using *Candida tropicalis* [8-10]. In this study *Penicillium* sp. AHT-1 and *Rhizomucor pusillus* HHT-1 were used for decomposition of xylan to D-xylose, while *Candida tropicalis* was used for conversion of D-xylose to xylitol. This paper describes the utilization of the mixture culture concept for xylitol production by enzymatic hydrolysis of beech wood and

walnut shells.

MATERIALS AND METHODS

Materials

Agricultural waste, walnut shells and beech wood were used in this study. Walnut shells and beech wood were pretreated by milling into small particles, no larger than 90 μm, to be used as a xylan source. All chemicals used in this study were of analytical grade. Oat spelt xylan and beech wood xylan were purchased from Sigma Chemical Co., St. Louis. Mo., USA and other chemicals used were from Wako Pure Chemicals Industries Ltd. (Osaka, Japan).

Fungal Strains and Culture Conditions for Xylose Production

Penicillium sp. AHT-1, *Rhizomucor pusillus* HHT-1 and *Bacillus* sp. were obtained from cultural collection in this laboratory. *Penicillium* sp. AHT-1 and *Rhizomucor pusillus* HHT-1 were isolated from the Ibigawa river, Gifu Prefecture, Japan and a jute compost in Bangladesh, respectively.

Penicillium sp. AHT-1 was grown in a 27 mL L type tube (Φ18 × 120 × 170 mm) with 10 mL of liquid culture basal medium A, containing oat spelt xylan 1.0%, K_2HPO_4 0.1%, $MgSO_4$ 0.05%, $NaNO_3$ 0.2%, KCl 0.05%, $FeSO_4$ 0.001%, polypeptone 0.1% at pH 7.0. The culture was incubated at 150 rpm and 30°C for 24 h. Subsequently, 10 mL of the culture (contained 42 mg cell in dry weight) was then inoculated into a 500 mL-flask containing 100 mL medium A and incubated at 150 rpm and 30°C for 5 days. After incubation, the culture was centrifuged at 10,000 rpm for 30 min and the supernatant was filtered using 0.22 μm membrane filters.

Rhizomucor pusillus HHT-1 and *Bacillus* sp. were grown in a 27 mL L type tube at 150 rpm and 30°C for 24 h. The medium of *Rhizomucor pusillus* HHT-1 was medium B, containing oat spelt xylan 1.0%, D-xylose 1.0%, KH_2PO_4, 1.5%, $(NH_4)_2SO_4$ 0.12%, polypeptone 0.6% at pH 5.5, whereas the medium of *Bacillus* sp. was a nutrient broth medium. It was found that the production of α-L-arabinofuranosidase and xylosidase by *Rhizomucor pusillus* was stimulated by the existence of *Bacillus* sp. The reason will be reported elsewhere. Therefore, a 10 mL culture (contained 59 mg cell in dry weight of *Rhizomucor pusillus* HHT-1 and 3 mg cell in dry weight of *Bacillus* sp.) of *Rhizomucor pusillus* HHT-1 and the *Bacillus* sp. were inoculated into a 500 mL flask containing 100 mL medium B and incubated at 150 rpm and 30°C for 5 days. Following this, the culture was centrifuged at 10,000 × g for 30 min and the supernatant was filtered using 0.22 μm membrane filters. Then the clear supernatant was freeze-dried for 24 h, resulting in a powder.

The resulting powder was combined with 100 mL of the clear *Penicillium* sp. AHT-1 supernatant and the solution was used as a crude enzyme solution for xylose production. An amount of 1 g of walnut shell milling or beech wood was placed into 50 mL of the crude enzyme solution and the reaction mixture was carried out at 40°C. The D-xylose concentration of the mixture was measured every 12 h. The mixture reaction was prolonged for 72 h, filtered using 0.22 μm membrane filters and used as a hydrolyzing solution for xylitol production.

Candida tropicalis IFO 0618 and Culture Conditions for Xylitol Production

An inoculum of *Candida tropicalis* IFO 0618 was grown in a 27 mL L type tube at 10 rpm and 30°C for 24 h. The inoculum medium for this strain was composed of D-xylose 6.0%, yeast extract 1.0%, KH_2PO_4, 1.5%, $(NH_4)_2HPO_4$ 0.3%, $MgSO_4 \cdot 7H_2O$ 0.1%, and the pH was adjusted to 5.5 by 1 M HCl. The culture was incubated at 150 rpm and 30°C for 24 h, then centrifuged at 13,000 rpm for 10 min and the cell mass used as an inoculum. For xylitol production, the medium was added with 0.001% of an antifoaming agent (Biott-Iable, Japan) and the pH was adjusted to 4.0 by 1 M HCl. The cell mass of 1 mL (6 mg in dry weight) of the subculture was inoculated into 10 mL of the hydrolyzed solution (inoculum ratio 10%) and the culture was incubated at 150 rpm and 30°C. Experiments using different concentrations of glucose, yeast extract, D-xylose and inoculum were carried out.

Enzyme Assay

Xylanase Activity

Xylanase activity was determined in a reaction mixture containing 1.8 mL of 1% birchwood xylan in 50 mM Na-citrate buffer (pH 6.8) and 0.2 mL of enzyme solution [11]. The reaction was incubated at 50°C for 5 min. Then it was stopped by adding 3 mL of 3,5-Dinitrosalicylic acid solution (containing 10.0 g of 3,5 Dinitrosalicylic acid, 2.0 g crystal phenol, 0.5 g Na_2SO_3, 200 g potassium sodium (+) tartrate tetrahydrate and 10.0 g KOH per liter) and boiling for 15 min. The absorbance was measured at 540 nm using a UV-Vis spectrophotometer (Shimadzu UV-1200) at room temperature. The D-xylose concentration was determined from a standard curve of D-xylose. One unit of enzyme activity was defined as the formation of 1 μmol of D-xylose per min at 50°C.

α-L- Arabinofuranosidase Activity

α-L-arabinofuranosidase was measured according to the method of Rahman *et al.* [6]. α-L-arabinofuranosidase activity was assayed quantitatively in a reaction mixture (0.3 mL) containing 180 μL of 2.5 mM *p*-nitrophenyl α-L-arabinofuranoside (pNPA) in 50 mM citrate buffer (pH 5.0) and 20 μL of enzyme solution. The reaction was carried out at 50°C for 20 min and stopped by adding 100 μL of 1 M Na_2CO_3. The released *p*-nitrophenol was measured at 414 nm. One unit of enzyme activity was defined as the amount of enzyme re-

Table 1. Activities of enzymes in the crude enzymes solution of *Penicillium* sp. AHT-1 and *Rhizomucor pusillus* HHT-1

Xylanase (U/mL)	β-D-xylosidase (U/mL)	α-L-arabinofuranosidase (U/mL)
31.8 (±5.09)	0.55 (±0.152)	1.34 (±0.157)

quired to liberate 1.0 μmol of *p*-nitrophenol from the substrate, per minute.

β-Xylosidase

β-xylosidase activity was determined by a similar method as described for α-L-arabinofuranosidase, except that the substrate was *p*-nitrophenyl β-D-xylopyranoside.

Analysis

Xylitol, D-xylose and glucose, were analyzed by high-performance liquid chromatography (Shimadzu LC-10 AT) using a Shodex SUGAR SZ5532 column (Showa Denco, K.K) with a refractive index detector (Shimadzu RID-10A). The column was eluted with 80% acetonitrile, at a flow rate of 0.7 mL/min.

All experiments were performed 3 times and mean values are shown in each table and figure.

RESULTS AND DISCUSSION

Xylose Production

In order to digest xylan into xylose, a number of xylanolytic enzymes, which have the ability to cleave main chain and site chains, were used. This laboratory had isolated two microorganisms, which are abundant producers of xylanolytic enzymes. *Penicillium* sp. AHT-1 produces xylanase, α-L-arabinofuranosidase, β-D-xylosidase and *Rhizomucor pusillus* HHT-1 produces extracellular α-L-arabinofuranosidase [6,7]. In this study, two strains were used for the production of xylanolytic enzymes. The crude enzyme solution, prepared by *Penicillium* sp. AHT-1 and *Rhizomucor pusillus* HHT-1, had a xylanase, α-L-arabinofuranosidase and β-D-xylosidase activities of 31.8 U/mL, 1.34 U/mL, and 0.55 U/mL, respectively (Table 1).

Cho *et al.* [12] had chemically analyzed the sugar composition of agricultural waste. They found that walnut shells and beech wood contain 18.7 g D-xylose/100 g and 16.9 g D-xylose/100 g, respectively. In the reaction containing the beech wood hydrolyzed solution, D-xylose production was 3.3 g/100 g material and 4.6 g/100 g material after 12 h and 72 h, respectively with a yield of 24% (Fig. 1).

For walnut shells, the reaction contained the crude enzymes, xylanase (60 U/mL), α-L-arabinofuranosidase (30 U/mL) and β-D-xylosidase (10 U/mL) with added cellulase 20 (U/mL) and 1 g of milled walnut shell. Cellulase was added to stimulate degradation of xylan into D-xylose. After 12 h and 72 h incubation, D-xylose production was 10 g/100 g and 15.1 g/100 g material, respectively (Fig. 1).

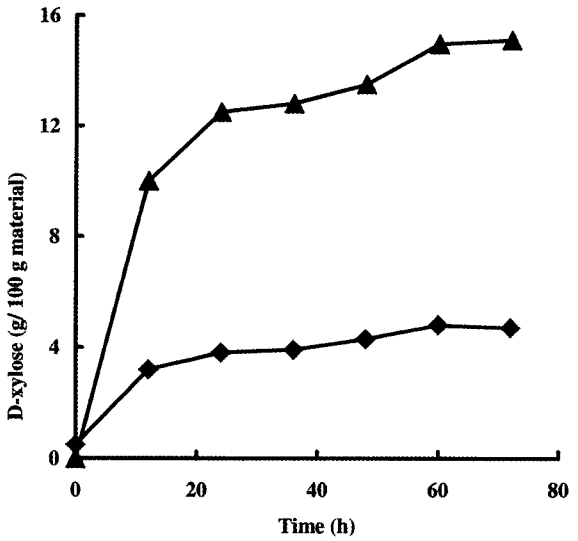

Fig. 1. Enzymatic hydrolysis of milled beech wood and walnut shells by the mixed crude enzymes of *Penicillium* sp. AHT-1 and *Rhizomucor pusillus* HHT-1.◆: Beech wood in the mixed crude enzymes contained xylanase 31.8 U/mL, α-L-arabinofuranosidase 1.34 U/mL and β-D-xylosidase 0.55 U/mL.▲: Walnut shells in the mixed crude enzymes contained xylanase 60 U/mL, α-L-arabinofuranosidase 30 U/mL, β-D-xylosidase 10 U/mL, and cellulose 20 U/mL (1 g of walnut shell milling or beech wood was placed into 50 mL of the crude enzyme solution and the reaction was carried out at 40°C).

Xylitol Production

Horitsu *et al.* [8] found that *Candida tropicalis* IFO 0618 has D-xylose reductase (XR) and xylitol dehydrogenase (XDH) but no xylose isomerase. This strain had a high activity of NADPH-linked XR (10.64 U/mL) and NAD-linked XDH (20.16 U/mL), and a low activity of NADH-linked XR (1.72 U/mL) and NADP-linked XDH (0.12 U/mL). The data showed that *Candida tropicalis* accumulated much more xylitol under oxygen limiting conditions. Therefore, all the experiments for xylitol production using this strain, were carried out in slower mixing conditions at 10 rpm and 30°C. The supply of NADPH and NADH is important. NADPH is produced by the EMP conversion pathway from glucose to glucose phosphate. In view of this, the effects of glucose addition on xylitol production were examined.

Using the Hydrolyzed Solution with or without Addition of Glucose

In this experiment, the cell mass of 1 mL of a subculture of *Candida tropicalis* IF0618 (inoculum ratio 10%) was inoculated into 10 mL of the hydrolyzed solution.

To determine the effects of glucose addition on xylitol production, the beech wood hydrolyzed solutions with and without a supplement of glucose, at an initial con-

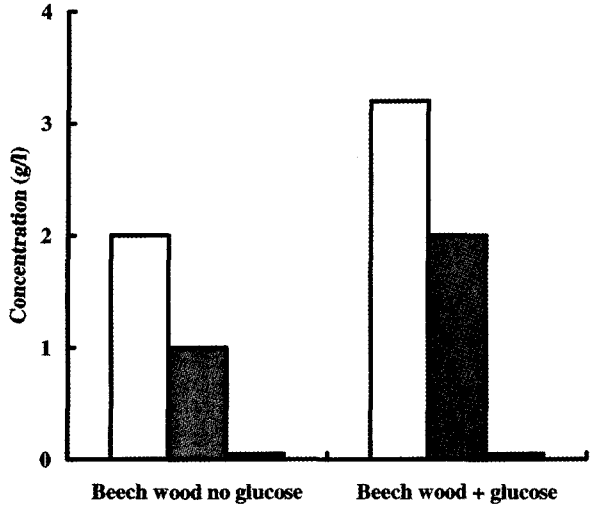

Fig 2. The growth of *Candida tropicalis* and the production of xylitol by using the beech wood hydrolyzed solution, with or without addition of glucose and D-xylose: □: dry cell weight after 12 h of incubation, ▩: D-xylose concentration at initial time, ■: xylitol production after 12 h of incubation

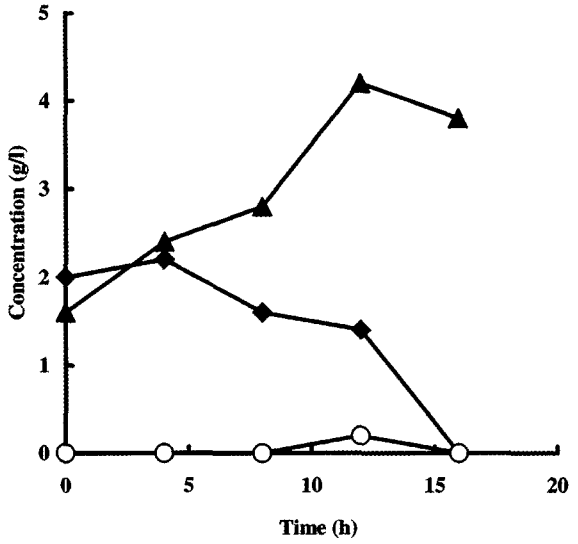

Fig. 3. Xylitol production in the beech wood hydrolyzed solution with addition of yeast extract 1%. ▲: dry cell weight, ◆: D-xylose concentration (g/L), ○: xylitol concentration (g/L).

centration of 10 g/L, were used. Fig. 2 shows that after 12 h of incubation, the dry cell weight of *Candida tropicalis* IF0 0618 was 3.2 g/L and 2 g/L in the beech wood hydrolyzed solution, with and without glucose addition, respectively. Obviously, the addition of glucose as a carbon source led to the increased growth rate. However, no xylitol was obtained from this culture.

Addition of Yeast Extract to the Beech Wood Hydrolyzed Solution

In this experiment, the hydrolyzed beech wood solution with 1% of yeast extract, 10% inoculum and without glucose supplementation was used. Cell growth increased from 1.6 to 4.2 g/L after 12 h of incubation (Fig 3). Cell concentration at 12 h of incubation was higher than that obtained by using only the hydrolyzed beech wood solution. After 12 h of incubation, xylitol produced was 0.2 g/L with a yield of 10%. These results showed that yeast extract is one of the essential factors for xylitol production.

Addition of Glucose and Yeast Extract to the Beech Wood Hydrolyzed Solution using 100% Inoculum

In this experiment, the beech wood hydrolyzed solution was combined with glucose to an initial concentration of 1%, yeast extract 1% and inoculum (ratio 100%). Cell growth was slow, from 6.7 to 8.9 g/L after 12 h of incubation (Fig 4). Glucose was completely consumed after 4 h incubation. For xylitol production, the concentration of xylitol at 0 h was 1.5 g/L. This is a carry-over from the subculturing, due to the 100% inoculum ratio. The total yield was calculated by subtraction of the initial value from the final value. 2.2 g/L xylitol was produced after 12 h incubation and yielded 50%.

Recently, extensive research on xylitol production was

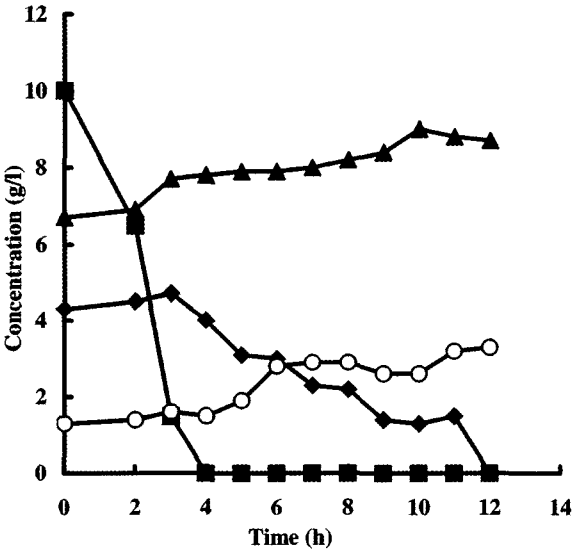

Fig. 4. Xylitol production in the beech wood hydrolyzed solution with addition of yeast extract 1% and glucose 1%, using 100% inoculum. ▲: dry cell weight (g/L), ◆: D-xylose concentration (g/L), ■: glucose concentration (g/L), ○: xylitol concentration (g/L).

conducted on the following 3 types. The first type uses D-xylose as a carbon source for different *Candida* strains or recombinant strains under optimum conditions for xylitol production as illustrated in the studies done by Horitsu *et al.* [8], Yahashi *et al.* [10], Oh and Kim [13], Kim *et al.* [14], Choi *et al.* [15], Azuma *et al.* [16], Chung *et al.* [17] and Kim *et al.* [18]. They have found the right conditions for a high yield, such as, the yield of 91% using *C. tropicalis* [13] or the yield of 95% using

recombinant *Saccharomyces cerevisiae* [19]. However, the different growth conditions employed in these studies make comparison between different strains and species difficult. This is due to the fact that in many articles, cell mass concentration is not given. The second type uses D-xylose produced by chemical methods, as shown by the research of Delgenes *et al.* [20]. In order to solve the difficulty of the xylitol purification step from sugar and by-product sugar, they used a mixed culture of *Candida guilliermondii* and *Lactobacillus reuterii*. *Lactobacillus reuterii* has the ability to consume L-arabinose without arabitol production and does not utilize xylose and xylitol. In these conditions, a 54% yield of xylitol was obtained and the xylitol was produced with a purity of 99.5%. The last method is the use of enzymes for production of D-xylose, as in the present study. The yield of D-xylose obtained was 0.24 g D-xylose/g of D-xylose in raw material and the total yield of xylitol was 0.5 g xylitol/g consumed xylose. We have already reported that yeast extract is a key substance for *Candida tropicalis* IFO 0618 to convert D-xylose to xylitol and optimizing the xylitol production rate using an experimental method, Box-Wilson method [8]. A maximum production rate of 2.67 g L^{-1} h^{-1} has obtained when initial D-xylose concentration and yeast extract concentration were 172.0 g/L and 21.0 g/L, respectively, and KLa was 451.50 /h [8]. Besides, we described that yield increased up to 82% with addition of D-glucose from 63% [10]. Because, D-xylose reductase of *Candida tropicalis* IFO 0618 is fully NADPH-linked and therefore, supplementation of D-glucose enhances the activity of D-xylose reductase, resulting in higher yield of xylitol [10]. These findings were obtained using pure D-xylose. This manuscript is related to application of above mentioned fundamental research. From our previous research, the possible reasons of lower xylitol production will be the lacking of some essential factors in the hydrolyzed solution, *e.g.* amino acids, vitamins or production of growth regulation compounds *etc.*, compared with those solution added yeast extract. We have also demonstrated that acetic acid, furfural, and vanillin have growth inhibition activity for this strain [21]. We suppose that ferulic acid or its derivatives are also harmful for the strain to convert D-xylose to xylitol. Clean-up of the hydrolyzed solution using ion exchange resin or using esterases to release side chain derivatives will lead more production of xylitol. Higher recovery yields of D-xylose without impurities for fermentation will lead to more efficient xylitol production from agricultural waste.

REFERENCES

[1] Rolla, G., A. A. Schele, and S. Assev (1987) Plaque formation and plaque inhibition. *Dutsch. Zahnarztl.* 42: 39-41.

[2] Van Eys, J., Y. M. Wang, S. Chan, S. Tanphaichitr, and S. M. King (1974) Sugar in nutrition: Xylitol as a therapeutic agent in glucose-6 phosphate dehydrogenase deficiency, 8ed, pp. 613-631 In: H. L. Sipple and K. W. McNatt (eds.). Academic Press, New York, USA.

[3] Pepper, T. and P. M. Olinger (1988) Xylitol in sugar-free confections. *Food Technol.* 42: 98-106.

[4] FAO (1999) Food and agriculture organization of the United Nations, *FAO production yearbook* 1999.

[5] Winkelhausen, E. and S. Kuzmanova (1998) Microbial conversion of D-xylose to xylitol. *J. Ferment. Bioeng.* 86: 1-14.

[6] Rahman, A. K. M. S., S. Kawamura, M. Hatsu, M. M. Hoq, and K. Takamizawa (2001) Physicochemical properties of a novel α-L-arabinofuranosidase from *Rhizomucor pusillus HHT-1*. *Can. J. Microbiol.* 47: 67-772.

[7] Rahman, A. K. M. S., N. Sugitani, M. Hatsu, and K. Takamizawa (2003) A role of xylanase, α-L-arabinofuranosidase, and xylosidase in xylan degradation. *Can. J. Microbiol.* 49: 58-64.

[8] Horitsu, H., Y. Yahashi, K. Takamizawa, K. Kawai, K. Suzuki, and N. Watanabe (1992) Production of xylitol from D-xylose by *Candida tropicalis*: optimization of production rate. *Biotech. Bioeng.* 40: 1085-1091.

[9] Yokoyama, S., T. Suzuki, K. Kawai, H. Horitsu, and K. Takamizawa (1995) Purification, characterization and structure analysis of NADPH dependent D-xylose reductase from *Candida tropicalis*. *J. Ferment. Bioengi.* 79: 217-223.

[10] Yahashi, Y., H. Horitsu, K. Kawai, T. Suzuki, and K. Takamizawa (1996) Production of xylitol from D-xylose by *Candida tropicalis* -the effect of D-glucose feeding. *J. Ferment. Bioeng.* 81: 148-152.

[11] Bailey, M. J., P. Biely, and K. Poutanen (1992) Interlaboratory testing of methods for assay of xylanase activity. *J. Biotechnol.* 23: 257-270.

[12] Cho, C. H., M. Hatsu, and K. Takamizawa (2002) The production of D-xylose by enzymatic hydrolysis of agricultural wastes. *Water Sci. Technol.* 45: 97-102.

[13] Oh D. K. and S. Y. Kim (1998) Increase of xylitol yield by feeding xylose and glucose in *Candida tropicalis*. *Appl. Microbiol Biotechnol.* 50: 419-425.

[14] Kim, Y. S., S. Y. Kim, J. H. Kim, and S. C. Kim (1999) Xylitol production using recombinant *Saccharomyces cerevisiae* containing multiple xylose reductase genes at chromosomal δ-sequences. *J. Biotechnol.* 67: 159-171.

[15] Choi, J.-H., K.-H. Moon, Y.-W. Ryu, and J.-H. Seo (2000) Production of xylitol in cell recycle fermentation of *Candida tropicalis*. *Biotechnol Lett.* 22: 1625-1628.

[16] Azuma, M. I., J. Keuchi, R. Kinitani, J. Kato, and H. Ooshima (2000) Increase in xylitol production of *Candida tropicalis*. *Biotechnol. Lett* 22:1625-1628.

[17] Chung, Y. S., M. D. Kim, W. L. Lee, Y. W. Ryu, J. H. Kim, and J. H. Seo (2002) Stable expression of xylose reductase gene enhance xylitol production in recombinant *Saccharomyces cerevisiae*. *Enzyme Microb. Technol.* 20: 809-816.

[18] Kim, M. D., Y. S. Jeun, S. G. Kim, Y. W. Ryu, and J. H. Seo (2002) Comparison of xylitol production in recombinant *Saccharomyces cerevisiae* strain harboring XYL1 gene of *Pichia stipitis* and GRE3 gene of *S. cerevisiae*. *Enzyme Microb. Technol.* 31: 862-866.

[19] Hallborn, J., M. Waldfridsson, U. Airakisinen, H. Ojamo, B. Haln-Hägerdal, M. Penttila, and S. Keränen (1991) Xylitol production by recombinant *Saccharomyces cere-*

visiae. *Bio/Technology* 9: 1090-1095.

[20] Delgenes, J. P., M. C. Escare, J. M. Laplace, R. Moletta, and J. M. Navarro (1998) Biological production of industrial chemicals, *i.e.* xylitol and ethanol, from lignocellulose by controlled mixed culture. *Ind. Crops Products.* 7:101-111.

[21] Cho, C. H., S. Aruga, M. Hatsu, T. Suzuki, K. Kawai, and K. Takamizawa (2000) Development of medium by the combined treatment of steam explosion and chemical decomposition of peanut shells for production of xylitol by *Candida tropicalis*. *J. Japan Soc. Waste Manage. Experts* 11: 11-20.

[Received March 26, 2004; accepted June 5, 2004]