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Bioconversion of Birch Wood Hemicellulose Hydrolyzate to Xylitol

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Abstract A sugar solution containing 42.9 g l^{-1} of xylose was prepared from the wood of Japanese white birch (*Betula platyphylla* var. *japonica*) by hydrolysis with 3 % sulfuric acid with a liquor-to-solid ratio of 4 (g g^{-1}) at 120 °C for 1 h. During the acid hydrolysis, undesirable by-products were generated, such as acetic acid, furfural, and low-molecular-weight phenols, which inhibit bioconversion of xylose to xylitol. These inhibitors were successfully removed from the hydrolyzate by sorption onto a steam-activated charcoal followed by treatment with an anion exchange resin. Bioconversion of the detoxified hydrolyzate to xylitol by the yeast *Candida magnoliae* was investigated under the microaerobic conditions. The oxygen transfer rate (OTR) varied from 9.6 to 22.3 mmol O_2 l^{-1} h^{-1}. The best fermentative performance of *C. magnoliae* in the birch wood hydrolyzate (xylitol yield 0.74 g xylitol g $xylose^{-1}$; volumetric productivity 1.0 g l^{-1} h^{-1}) was obtained at the OTR of 12.6 mmol O_2 l^{-1} h^{-1}.

Keywords *Betula platyphylla* var. *japonica* · Hemicellulose hydrolyzate · Fermentation · Xylitol · *Candida magnoliae*

Introduction

Xylitol, a naturally occurring five-carbon sugar alcohol, has received great attention in the food and oral care industries because of its power to create sweetness, negative heat of dissolution, and anticariogenic properties. It is also used clinically as a sucrose substitute for diabetes [1].

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Xylitol has been widely found in fruits and vegetables. However, the small quantities present in nature make its extraction impractical. It is conventionally produced by a catalytic reduction of xylose present in the spent sulfite liquor of hardwood chips or the hemicellulose hydrolyzates of agricultural wastes such as corn cobs. Because the hemicelluloses of these raw materials contain other monosaccharides units such as arabinose, galactose, and glucose, extensive separation and purification steps are necessary to remove these solubilized contaminants before the chemical reduction. The inefficiency of current xylose preparation techniques seriously affects the recovery of xylose. The yields of xylitol correspond to only 50 to 60 % of xylan present in the raw materials [2], and xylitol is relatively expensive at about \$7 kg^{-1} [3]. It has been suggested that a bioconversion process could offer a more economical alternative [4]. Among the microorganisms that can assimilate xylose, yeasts belonging to the genus *Candida* are the best xylitol producers [5].

Although corn cobs are a promising source of xylose, they are used exclusively for cattle feeding in Japan. On the other hand, it has been reported that xylose was obtained in good yields from several species of hardwoods by hydrolysis with dilute sulfuric acid [6–12]. Of the hardwood species, hemicelluloses of birch (*Betula* genus) woods are characterized by high content of xylose [13]. Japanese white birch (*Betula platyphylla* var. *japonica*), which is widely distributed in chilly and mountainous regions in Japan, is considered to be a potential source of xylose.

During hydrolysis of lignocelluloses with mineral acids or with water alone at elevated temperatures, some undesirable by-products for bioconversion of the resulting hydrolyzates are also generated from the raw materials, which are potential inhibitors of microbial metabolism [14]. Activated charcoal treatment is an efficient method for removing the inhibitors such asfuran derivatives and low molecular weight phenols [15, 16]. In our recent studies [17, 18], we examined the abilities of four different carbonaceous sorbents to remove fermentation inhibitors present in the birch wood hydrolyzate. Of the sorbents tested, sorption capacities of the steam-activated charcoal were superior to those of a ZnCl2-activated charcoal, a dehydration product from birch wood hydrolysis residue, and a commercially available larch wood charcoal. In this study, before fermentation was performed using the yeast, *Candida magnoliae*, the hemicellulose hydrolyzate of Japanese white birch wood was detoxified with a commercially available steam-activated charcoal. However, the activated charcoal treated hardwood hydrolyzates usually still contain considerable amounts of acetic acid [15, 18] which also acts as an inhibitor of microbial metabolism [14, 19]. Van Zyl et al. [20] reported that, when sugar cane bagasse hemicellulose hydrolyzate was treated by applying to a glass column containing a weak basic anion exchange resin (Amberlite IRA-45) followed by neutralization with calcium hydroxide, ethanol productivity and yield by *Pichia stipitis* could be greatly improved by removal of acetic acid (84 % originally present in the hydrolysate). In this study, the activated charcoal treated hydrolyzate was further treated with a weak basic anion exchange resin. The resulting mixture was stirred at 30 °C for 1 h. The effects of the detoxification procedures on the microbial conversion of the hydrolyzate to xylitol were examined. For batch fermentation runs, two phase aeration process was employed for xylitol production, because Ding and Xia [21] reported that the two-phase aeration is more effective than one-phase aeration. The first step was carried out under aerobic conditions to improve glucose consumption through cell proliferation [22, 23]. The second step under limited oxygen conditions is intended to increase the xylitol accumulation.

Experimental and Method

Hydrolysis

The ground wood of Japanese white birch (P32 R82 mesh) is composed of 17.4 % pentosan (including 16.8 % xylan), 38.8 % hexosan (including 35.5 % glucan), 28.6 % lignin (including 2.9 % acid soluble lignin), and 0.3 % ash. The ground wood was hydrolyzed directly with 3 % sulfuric acid with a liquor-to-solid ratio of 4 (g g^{-1}) at 120 °C for 1 h.

Detoxification

The hydrolyzate was treated with a commercially available steam-activated charcoal (ShirasagiM) in a SB-20 reciprocal shaker (shaking speed 160 strokes min^{-1}, AS ONE Corp., Osaka, Japan) at 30 °C for 1 h. The resulting sugar solution was filtered and neutralized with calcium carbonate, followed by centrifugation. The recovery rate of xylose after activated charcoal treatment followed by neutralization with calcium carbonate is about 80 % of the original hydrolyzate. After centrifugation, the supernatant was further treated with a weak basic anion exchange resin (80 g l^{-1} of Amberlite IRA 67, Organo Corp., Tokyo, Japan) at 30 °C for 1 h.

Microorganism and Inoculum

The strain of the yeast, *C. magnoliae* TISTR5663 (deposited in the National Institute of Bioscience and Human-Technology, Tsukuba, as FERM P-16522) was grown on an agar slant containing malt extract (3 g l^{-1}), yeast extract (3 g l^{-1}), peptone (5 g l^{-1}), D-glucose (10 g l^{-1}), and agar (20 g l^{-1}) at 4 °C for 3 days. A loop full of a slant culture was transferred to 5 ml of the preculture medium containing D-xylose (20 g l^{-1}), casamino acids (1 g l^{-1}), Difco yeast nitrogen base without amino acids and ammonium sulfate (1.7 g l^{-1}), and urea (2.27 g l^{-1}), and cultivation was performed at 30 °C for 24 h.

Adaptation

Adaptation of the yeast was performed according to the method of Amartey and Jeffries [24]. A loop full of a slant culture was transferred to 5 ml of the preculture medium containing increasing concentrations of the hydrolyzate (25, 50, 75, and 100 %) supplemented with D-xylose (30, 20, 10, and 0 g l^{-1}), casamino acids (1.0 g l^{-1}), yeast nitrogen base without amino acids and ammonium sulfate (1.7 g l^{-1}), and urea (2.2 g l^{-1}). Cultivation was performed at 30 °C for 24 h, and the system pH of the preculture was maintained at 5.0 during the cultivation.

Experimental Setup

Batch fermentation runs were performed in a BMZ-P type culture installation (ABLE Corp., Tokyo, Japan) containing baffles and two sets of disk turbines with six and four flat blades with a working volume of 1.55 l of medium. Fifty milliliters of the inoculum (average cell density 6.9 g l^{-1}) was transferred to each 1.5 l of detoxified birch wood hydrolyzate. This installation was equipped with controllers of pH, temperature, dissolved oxygen, and aeration

rate. At the fixed temperature (30 °C), the aerobic phase was applied in the first 17–20 h to promote the consumption of glucose. Ventilation volume was 1.0 l min^{-1}, and the medium was agitated vigorously. In the second aeration phase, the agitation was set at 325–450 rpm and the aeration was fixed at 0.67 volume of air per volume of medium per minute (vvm). The system pH of the medium was maintained at 5.0.

The volumetric oxygen transfer coefficient (*K*L*a*) was determined by the method of Taguchi and Humphrey [25]. The dissolved oxygen concentration (DOC) of the medium was decreased to zero by nitrogen sparging, and the *K*L*a* was calculated from the rate of DOC increased during subsequent aeration. The oxygen transfer rate (OTR) was calculated as OTR=*K*L*a* (*C**−*C*), where *C** and *C* are saturated DOC and DOC, respectively.

Analytical Methods

Neutral sugars, xylitol, and ethanol were determined by HPLC equipped with RI detection using an Aminex HPX-87P column (300×7.8 mm, Bio-Rad, Richmond, VA) eluted with water at a flow rate of 0.5 ml min^{-1} and at 70 °C. Furfural and acetic acid were determined by HPLC with RI detection using a Shodex SH column (300×8 mm, Showa Denko, Tokyo, Japan) eluted with 0.01 M sulfuric acid at a flow rate of 0.7 ml min^{-1} and at 50 °C. The overall content of phenols in the hydrolyzate was evaluated by the absorbance at 280 nm (A_{280}) at pH 12 [26]. The cell concentration was determined indirectly by correlation between the dry weight of the cell and the absorbance at 600 nm (A_{600}) [27].

Results and Discussion

Bioconversion of lignocellulose hydrolyzates to biofuels, polyols, or protein is usually depressed by a range of toxic compounds generated from cell wall components during hydrothermal treatment. Activated charcoal treatment is an efficient method for their reduction [15, 16]. Besides sugars, the hemicellulose hydrolyzate of Japanese white birch wood contained significant amounts of the inhibitors, such as acetic acid (12.6 g l^{-1}), furfural (1.2 g l^{-1}), and low-molecular-weight phenols (monitored as the A_{280} value). In this study, the birch wood hemicellulose hydrolyzate was treated with a steam-activated charcoal at 30 °C for 1 h. When 20 ml of the hydrolyzate was treated with 0.3 g of activated charcoal (15 g l^{-1} of carbon dose), the concentration of furfural decreased from 1.2 to 0.2 g l^{-1} (Table 1). The A_{280} value of the diluted hydrolyzate also decreased from 0.186 to 0.029. The results indicate that the activated charcoal eliminates selectively large parts of furfural and low-molecular-weight phenols from the hydrolyzate. The effectiveness of activated carbon treatment on removal of lignin degradation products and furan derivatives agrees with those in the literature [15, 26, 28–33]. In contrast, the activated charcoal treated hydrolyzate still contained a large amount of acetic acid (11.5 g l^{-1}). Van Zyl et al. [20] reported that under oxygen-limited conditions, the presence of 10 g l^{-1} acetic acid in the synthetic fermentation medium containing 50 g l^{-1} D-xylose caused a serious reduction in the ethanol production by *Pichia stipitis* (33 % reduction in the ethanol yield and 56 % reduction in the volumetric ethanol productivity). The authors also observed that when sugar cane bagasse hydrolyzate was treated with a weak basic anion exchange resin, 84 % of the acetic acid in the hydrolyzate was removed and the subsequent ethanol fermentation was greatly improved.

Table 1 Chemical composition of Japanese white birch (*Betula platyphylla* var. *japonica*) wood hydrolyzates

Components	Concentration (g l^{-1})		
	Original[a]	Detoxified with activated charcoal[b]	Detoxified with activated charcoal followed by treatment with anion exchange resin[c]
Arabinose	1.8	2.7	1.4
Xylose	42.9	41.3	37.6
Galactose	4.8	4.8	2.9
Glucose	3.2	2.7	2.4
Mannose	1.7	1.9	1.3
Acetic acid	12.6	11.5	3.7
Furfural	1.2	0.2	0.1
Phenolics[d]	0.186	0.029	0.028

[a] The ground wood was hydrolyzed with 3 % sulfuric acid with a liquor-to-solid ratio of 4 (g g^{-1}) at 120 °C for 1 h

[b] The original hydrolyzate was treated with a steam-activated charcoal (15 g l^{-1}) in a reciprocal shaker (160 strokes min^{-1}) at 30 °C for 1 h

[c] The original hydrolyzate was treated with a steam-activated charcoal (15 g l^{-1}) followed by treatment with an anion exchange resin (80 g l^{-1})

[d] The overall content of phenols was evaluated by the absorbance at 280 nm (A_{280}) at pH 12

In this study, the activated charcoal treated birch wood hydrolyzate was further treated with a weak basic anion exchange resin (Amberlite IRA 67). A large portion of the acetic acid (68 %) present in the activated charcoal treated hydrolyzate could be removed by the anion exchange resin (Table 1). The system pH of the hydrolyzate rose from 2.0 to 5.0 after the ion exchange resin treatment, due to the weakly basic nature of the resin. Recently, Shen et al. [34] reported that, when the spent liquor prepared from a hardwood chips mixture (70 wt.% maple, 20 wt.% poplar, and 10 wt.% birch) by the kraft-based dissolving pulp cooking process was treated with 10 g g^{-1} of the same type of ion exchange resin (Purolite A103S), about 70 % of acetic acid was removed, and the system pH of the spent liquor rose from 4.0 to 6.0 after the ion exchange resin treatment. De Mancilha and Karim [35] also reported that acetic acid was substantially removed from the corn stover hydrolyzate by using the same type of ion exchange resin, which agrees with the present result. Acetic acid concentration of the birch wood hydrolyzate treated with the anion exchange resin decreases by its harmless level to *C. magnoliae*. Rao et al. [36] reported the presence of other type of inhibitors in the corn fiber and sugarcane bagasse hydrolyzates treated with activated charcoal and anion exchange resin. The sensitivity to each inhibitor is, however, yeast species dependent. Further, the fermentative xylitol production can be greatly enhanced by sequential adaptation, as pointed out by the authors.

The proper conditions for oxygen supply to *C. magnoliae* were investigated under strictly controlled conditions. Table 2 shows the effects of the OTR within the range of 9.6–22.3 mmol O_2 l^{-1} h^{-1} on xylitol production. Glucose in the fermentation media (2.4 g l^{-1}) was completely consumed during the first 17–20 h under aerobic conditions. A slow rate of xylose consumption was observed before the glucose in the fermentation media was completely assimilated. Then, *C. magnoliae* metabolized xylose at a higher rate.

Table 2 Effects of oxygen transfer rate (OTR) on xylitol production from the detoxified hydrolyzates of Japanese white birch wood by the yeast, *Candida magnoliae*

	OTR (mmol O_2 l^{-1} h^{-1})				
	9.6	12.6	15.4	17.5	22.3
Initial xylose concentration (g l^{-1})	37.0	40.5	40.0	40.8	40.2
Final xylose concentration (g l^{-1})	2.6	1.2	2.2	3.0	2.6
Maximum xylitol concentration (g l^{-1})	19.7	30.0	28.2	26.2	25.7
Xylitol yield (g xylitol g $xylose^{-1}$)	0.53	0.74	0.70	0.64	0.64
Volumetric productivity (g l^{-1} h^{-1})	0.6	1.0	1.3	1.6	2.0
Initial acetic acid concentration (g l^{-1})	3.3	3.0	3.2	3.4	3.4
Final acetic acid concentration (g l^{-1})	0.9	0.0	0.2	0.1	0.0
Final ethanol concentration (g l^{-1})	3.1	2.3	1.3	1.3	1.1
Final dry cell concentration (g l^{-1})	4.5	4.9	5.9	6.0	6.1

The birch wood hydrolyzate was detoxified with a steam-activated charcoal (15 g l^{-1}) followed by treatment with an anion exchange resin (80 g l^{-1})

At the OTR of 9.6 mmol O_2 l^{-1} h^{-1}, xylitol concentration of 19.7 g l^{-1} was obtained after 50 h of fermentation, which corresponds to a xylitol volumetric productivity of 0.6 g l^{-1} h^{-1}, and a xylitol yield of 0.53 g xylitol g $xylose^{-1}$. After complete glucose consumption, ethanol also accumulated gradually in the fermentation medium and reached the maximum concentration (3.1 g l^{-1}) after 50 h of fermentation. The OTR of 9.6 mmol O_2 l^{-1} h^{-1} is slightly anaerobic for the xylitol production from the detoxified birch wood hydrolyzate.

At the OTR ranging 12.6 to 15.4 mmol-O_2 l^{-1} h^{-1}, xylitol production was greatly improved (30.0–28.2 g l^{-1}). Xylose in the detoxified hydrolyzate was successfully converted to xylitol (0.74–0.70 g xylitol g $xylose^{-1}$) with a volumetric productivity 1.0–1.3 g l^{-1} h^{-1}, and with more than 95 % xylose utilization. The fermentation time at the OTRs of 12.6 and 15.4 mmol O_2 l^{-1} h^{-1} was 50 and 42 h, respectively. Under these aeration conditions, acetic acid present in the hydrolyzate was almost completely consumed. It has been reported that 3.7 g l^{-1} of acetic acid in sugar cane hemicellulose hydrolyzate was completely consumed by *C. tropicalis* [37].

At the OTR ranging 17.5–22.3 mmol O_2 l^{-1} h^{-1}, the maximum xylitol concentration (26.2–25.5 g l^{-1}) occurred at a fermentation time of 31–28 h, although a slight decrease in the xylitol yield (0.64 g xylitol g $xylose^{-1}$) was observed, compared to the OTR of 15.4 mmol O_2 l^{-1} h^{-1}. Under aerobic conditions, xylose fermentable yeasts metabolize mainly xylose for energy production. Xylose absorbed into cells is first reduced to xylitol by a NADPH-dependent xylose reductase, and the xylitol formed is oxidized to xylulose by NAD^+-dependent xylitol dehydrogenase. After phosphorylation of xylulose, the resulting xylulose-5-phosphate can enter into either the pentosephosphate pathway or phosphoketolase bypass to give glycelaldehyde-3-phosphate (GAP). GAP is metabolized through glycolysis and tricarboxylic acid cycle for energy production. At the OTR ranging 9.6–22.3 mmol O_2 l^{-1} h^{-1}, a portion of the synthesized xylitol is secreted from the cell and the remainder is probably oxidized by NAD^+-dependent xylitol dehydrogenase for energy production. The best fermentative performance of *C. magnoliae* in the birch wood hydrolyzate (xylitol yield 0.74 g xylitol g $xylose^{-1}$; volumetric productivity 1.0 g l^{-1} h^{-1}) was obtained at the OTR of 12.6 mmol O_2 l^{-1} h^{-1}. Vandeska et al. [38] reported that an OTR of 14 mmol O_2 l^{-1} h^{-1} was optimal for xylitol production from a synthetic medium using *C. boidinii* (xylitol yield 0.48 g xylitol g $xylose^{-1}$; volumetric productivity 0.24 g l^{-1} h^{-1}).

Table 3 Production of xylitol from various lignocellulosic hydrolyzates by *Candida magnoliae*

Raw material	Sorbent for detoxification	OTR	S_o	P	$Y_{p/s}$	Q	Reference
Corn cob	Charcoal pellets	–[a]	24.9	18.7	0.75	0.525	[39]
Sasa senanensis culm	Steam-activated charcoal	14.1	35.6	17.2	0.60	0.82	[17]
Sasakurilensis culm	Steam-activated charcoal	11.2	31.0	13.2	0.62	0.55	[33]
Phyllostachypubescens culm	Steam-activated charcoal	9.6	37.4	23.9	0.67	0.48	[40]
Japanese white birch wood	Steam-activated charcoal, anion exchange resin	12.6	40.5	30.0	0.74	1.0	Present study

OTR oxygen transfer rate (mmol O_2 l^{-1} h^{-1}), S_o initial xylose concentration (g l^{-1}), P maximum xylitol concentration (g l^{-1}), $Y_{p/s}$ xylitol yield (g g^{-1}), Q xylitol volumetric productivity (g l^{-1} h^{-1})

[a] The value was not provided

Xylitol production from various lignocellulose hemicellulose hydrolyzates using *C. magnoliae* is summarized in Table 3. High xylitol yield and xylitol productivity were obtained by the fermentation of the detoxified birch wood hydrolyzate. Japanese white birch trees, especially nonmerchantable small-sized ones, are a promising source of xylitol.

Conclusion

A fermentable substrate with a relatively high xylose concentration (about 43 g l^{-1}) could beprepared from the wood of Japanese white birch by acid hydrolysis with 3 % sulfuric acid under mild hydrolysis conditions. Inhibitors, such as acetic acid, furfural, and low-molecular-weight phenols released from lignin were successfully removed by treatment with commercially available steam-activated charcoal followed by treatment with an anion exchange resin. The detoxified hydrolyzate could be successfully converted to xylitol by *C. magnoliae*. The best fermentative performance was obtained at the OTR 12.6 mmol O_2 l^{-1} h^{-1}. The two-stage oxygen supply control strategy permitted efficient microbial xylitol production from the birch wood hydrolyzate.

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