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Short note

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Microbial xylitol production from culm of *Sasa kurilensis* using the yeast *Candida magnoliae*

Abstract: A sugar solution containing 31 g l^{-1} xylose was prepared from the culm of *Sasa kurilensis* by hydrolysis with 2% sulfuric acid with a liquor-to-solid ratio of 6 (g g^{-1}) at 121°C for 1 h. During acid hydrolysis, also some byproducts were generated, such as acetic acid, furfural, 5-hydroxymethylfurfral, and low molecular weight phenolics, which inhibit bioconversion of xylose to xylitol. Except for acetic acid, these inhibitors were successfully removed from the hydrolysate by contacting with a steam-activated charcoal (15 g l^{-1} dose) for 24 h. Bioconversion of the detoxified hydrolysate to xylitol by the yeast, *Candida magnoliae*, was investigated under various microaerobic conditions. The oxygen transfer rate (OTR) varied from 8.4 to 27.6 mmol-O_2 l^{-1} h^{-1}. The maximum xylitol yield (0.62 g-xylitol g-$xylose^{-1}$) was attained at the OTR of 1.2 mmol-O_2 l^{-1} h^{-1}. An additional increase in the OTR brought about cell growth, which consumed xylose. A proper control of the oxygen supply is necessary to produce efficiently xylitol from the culm hydrolysate.

Keywords: bamboo grass, *Candida magnoliae*, hemicellulose hydrolysate, *Sasa kurilensis*, xylitol

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Introduction

Recently, the utilization of hardwood hemicelluloses has received great attention from the viewpoint of biorefinery (Canettieri et al. 2007; Mendes et al. 2009; García et al. 2011; Poth et al. 2011; Rodríguez-López et al. 2012). The sugars obtained from the hemicelluloses by acid or enzymatic hydrolysis are potential sources of foods, fuels, and chemical feedstuffs.

Xylitol, a naturally occurring five-carbon sugar alcohol, is of interest to the food and oral care industries because of its sweetening power equivalent to sucrose and great negative heat of dissolution and anticariogenic properties. It is also used clinically as a sucrose substituent for diabetics and for patients deficient of glucose-6-phosphate dehydrogenase. Xylitol is currently produced by a catalytic reduction of xylose present in hemicellulose hydrolysates of hardwood or agricultural wastes. Biotechnological production of xylitol from hemicellulose hydrolysates is considered to be an alternative process because it requires neither pure xylose nor heavy metal catalysts for the xylose reduction. Although microbial production of xylitol requires additional costs for the detoxification of hemicellulose hydrolysates and purification of xylitol in the fermentation media, the additional costs for the environment-friendly process may be accepted by ecology-minded consumers. Among the microorganisms that can assimilate xylose, the yeasts belonging to the genus *Candida* are the best xylitol producers (Onishi and Suzuki 1966; Meyrial et al. 1991).

Bamboo grasses, perennial gramineous plants with woody culms, are mainly distributed in Japan. The hemicelluloses of bamboo grass (*Sasa senanensis*) are mainly composed of arabinoglucoronoxylan. Miura et al. (2010) have reported that xylose could be obtained in a 20% yield (corresponding to 83% of xylan in the raw material) by hydrolysis with 2% sulfuric acid from the culm of *Sasa kurilensis*. Bamboo grasses are, therefore, a potential source of xylose.

The microbial production of xylitol depends on the fermentation conditions employed (Silva et al. 1988). The oxygen transfer rate (OTR; mmol-O_2 l^{-1} h^{-1}) is the most important of all parameters that affect the microbial production of xylitol (Nolleau et al. 1995; Silva et al. 1997). Either a high degree of aeration rate or a high agitation rate promotes

cell propagation, but xylitol accumulation is repressed. In this work, the microbial conversion of hemicellulose hydrolysate has been examined, which was prepared from the culms of *S. kurilensis*. The conversion to xylitol was effectuated by the yeast *Candida magnoliae* by means of a two-phase aeration process (Saha and Bothast 1999; Preziosi-Belloy et al. 2000; Kastner et al. 2001). Ding and Xia (2006) reported that the two-phase aeration is more effective than one-phase aeration for xylitol production. Before fermentation, the hydrolysate was detoxified with steam-activated charcoal. It is known that xylitol production is suppressed by glucose in the fermentation media (Azuma et al. 2000; Ikeuchi et al. 2000; Kastner et al. 2001; Walthers et al. 2001a). The first step of the fermentation process was carried out under aerobic conditions to improve glucose consumption through cell propagation in the medium (Walther et al. 2001b). The second step was performed under limited oxygen conditions aiming at an elevated xylitol accumulation (Nolleau et al. 1995).

Materials and methods

Preparation of the hemicellulose hydrolysate

The ground culm of *S. kurilensis* (P32 R82 mesh) is composed of 17.5% pentosan (including 15.5% xylan), 37.9% hexosan (including 37.4% glucan), 24.4% lignin (including 2.7% acid soluble lignin), and 2.6% ash. The ground culms were hydrolyzed directly with 2% sulfuric acid with a liquid-to-solid ratio of 6 (g g^{-1}) at 121°C for 1 h to afford a sugar solution containing 31 g l^{-1} xylose. To remove toxic compounds generated during hydrolysis, the hydrolysate was treated with steam-activated charcoal (Shirasagi M, Japan EnviroChemicals, Ltd., Osaka, Japan) in a reciprocal shaker (160 strokes min^{-1}) at 30°C for 24 h. The resulting sugar solution was filtered and neutralized with $CaCO_3$ followed by centrifugation.

Microorganism and inoculum

Candida magnoliae TISTR5663 (deposited in the National Institute of Bioscience and Human-Technology, Tsukuba, Japan, 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 spoon of a slant culture was transferred to 5 ml of the pre-culture medium containing 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.

Experimental set-up

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 1.5 l medium. 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 14–21 h to promote the consumption of glucose; then, the aeration rate and agitation rate were reduced. In the second aeration phase, the agitation was set at 300, 350, 400, and 500 min^{-1} and the aeration was varied from the ratio air-to-medium of 0.33–0.67 (by volume min^{-1}).

The volumetric oxygen transfer coefficient ($K_L a$) was determined by the dynamic method (Taguchi and Humphrey 1966). 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 OTR was calculated as $OTR=K_L a\ (C^*-C)$, where C^* and C are saturated DOC and DOC, respectively.

The xylitol yield (Y_x) and volumetric productivity (P; g l^{-1} h^{-1}) were defined as Y_x=xylitol produced/xylose consumed (g g^{-1}).

P=maximum xylitol concentration/fermentation time (g l^{-1} h^{-1}).

Analytical methods

Neutral sugars, xylitol, and ethanol were determined by high-performance liquid chromatography (HPLC) equipped with RI detection and an Aminex HPX-87P column (300×7.8 mm; Bio-Rad, Richmond, VA); water as eluent (0.6 ml min^{-1}) at 85°C. Furfural, 5-hydroxymethylfurfural (5-HMF), and acetic acid were determined by HPLC with RI detection on a Shodex SH column (300×8 mm; Showa Denko, Tokyo, Japan) eluted with 0.01 M sulfuric acid (0.7 ml min^{-1}) at 50°C. Phenolics contents in the hydrolysates were estimated by at 280 nm (A_{280}) (Mussatto et al. 2004). The cell concentration was determined indirectly by correlation between the dry weight of the cell and A_{660} (Nakano et al. 2000).

Results and discussion

Fermentation of lignocellulose hydrolysates is 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 (Parajó et al. 1996; Mussatto and Roberto 2001). Recently, Gütsch and Sixta (2011) proposed a new high-temperature process for removal of lignin and lignin-derived phenolics with activated charcoal. In the present paper, the hydrolysate was treated with a commercially available steam-activated char at 30°C. Based on literature date (Parajó et al. 1996), 24 h contact time was set for all the sorption experiments.

Besides sugars, the original hydrolysate contained significant amounts of the inhibitors known from the literature (Palmqvist and Hahn-Hägerdal 2000), such as acetic acid (6.02 g l^{-1}), furfural (0.25 g l^{-1}), 5-HMF (0.36 g l^{-1}), and phenolics. The concentrations of furfural, 5-HMF, and low molecular weight phenolics (monitored as the A_{280}

value) greatly decreased by sorption on activated charcoal, whereas the monosaccharide concentrations stayed almost constant (Table 1). When 20 ml of the hydrolysate were treated with 0.3 g activated charcoal (15 g l^{-1} carbon dose), the furfural concentration decreased from 0.52 to 0.06 g l^{-1}. Delgenes et al. (1996) found that 0.5, 1.0, and 2.0 g l^{-1} furfural reduced the cell growth of *Pichia stipitis* by 25%, 47%, and 99%, respectively. On the contrary, Roberto et al. (1991) reported about a certain activating effect of furfural on the cell growth of the same yeast species at concentrations up to 0.5 g l^{-1}. Tran and Chambers (1986) found little effect on fermentation caused by 1.3 g l^{-1} furfural. Pessoa et al. (1996) reported that furfural and 5-HMF, in concentrations of 2.0 and 0.08 g l^{-1}, respectively, did not inhibit the cellular metabolism of *Candida tropicalis*. Although the tolerance of yeasts to these furan derivatives and low molecular weight phenolics depends on the yeast species, at least 15 g l^{-1} activated charcoal was necessary to eliminate large parts of these inhibitors as indicated in Table 1.

The inhibitor acetic acid – originating from xylan – could not be removed after treatment with the activated charcoal (Table 1). At the optimal pH range of fermentation (pH 4 and 5), acetic acid occurs largely in an undissociated form. After its diffusion into cell cytoplasm, it dissociates and uncouples the energy production and transportation of nutrients. The sensitivity to acetic acid is yeast species dependent. Pessoa et al. (1996) reported that 3.7 g l^{-1} acetic acid in sugar cane hemicellulose hydrolysate was completely consumed by *C. tropicalis*. *Candida guilliermondii* was also able to assimilate significant amounts of acetic acid in hemicellulose hydrolysates of eucalyptus wood and sugar cane bagasse in amounts of 40% and 50%, respectively (Felipe et al. 1996, 1997).

The proper conditions for oxygen supply of *C. magnoliae* were investigated under strictly controlled conditions. Figure 1 and Table 2 show the effects of the OTR within the range of 8.4–27.6 mmol-O_2 l^{-1} h^{-1} on xylitol production. Glucose in the fermentation media (~5 g l^{-1}) was completely consumed during the first 14–18 h under aerobic conditions. A slow rate of xylose consumption was

Table 1 Concentration of components in the *S. kurilensis* culm hydrolysates treated with activated char.[a]

Component	Concentration of carbon dose (g l^{-1})			
	Original	(10 g l^{-1})[b]	(15 g l^{-1})[b]	(20 g l^{-1})[b]
Arabinose	1.94	1.96	2.00	2.03
Xylose	31.09	31.08	30.54	31.08
Glucose	6.67	7.03	7.09	7.18
Mannose	0.53	0.48	0.44	0.53
Acetic acid	5.39	5.64	5.87	5.42
Furfural	0.52	0.28	0.06	0.07
HMF	0.40	0.26	0.07	0.08
Phenolics[c]	0.14	0.02	0.01	0.01

[a]The ground culm of *S. kurilensis* was hydrolyzed with 2% sulfuric acid with a liquid-to-solid ratio of 6 at 121°C for 1 h.

[b]The hydrolysate was treated with a steam-activated char (Shirasagi M, Japan EnviroChemicals, Ltd.) in a reciprocal shaker (160 strokes min^{-1}) at 30°C for 24 h.

[c]Absorbance at 280 nm.

OTR (mmol O_2 l^{-1} h^{-1})	Agitation rate(rpm)	Aeration rate (wm)
□ 8.4 (k_La=35.7 h^{-1})	300	0.67
• 8.8 (k_La=37.3 h^{-1})	350	0.33
× 11.2 (k_La=47.6 h^{-1})	350	0.67
■ 15.0 (k_La=63.7 h^{-1})	400	0.67
○ 27.6 (k_La=117.3 h^{-1})	500	0.67

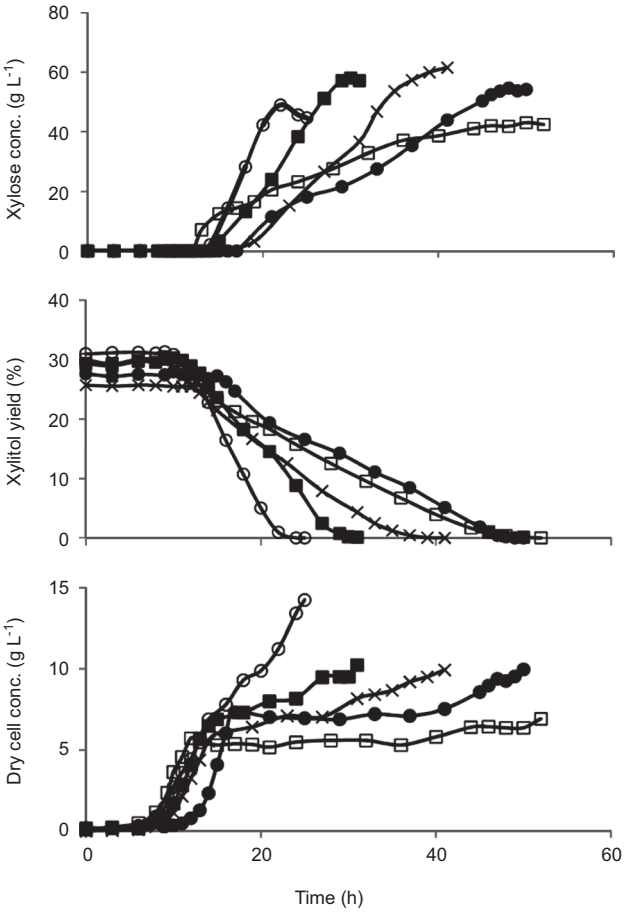

Figure 1 Time course of xylitol yield, xylose, and dry cell concentrations in batch xylitol fermentations by *C. magnoliae* from hydrolysate of *S. kurilensis* obtained from detoxified culm hemicellulose under various microaerobic conditions.

Table 2 Effects of OTR on xylitol production from culm *S. kurilensis* hemicellulose hydrolysates by *C. magnoliae*.

OTR (mmol O_2 l^{-1} h^{-1})	Xylose consumed (%)	Final concentration of		Xylitol yield (Y_x; g-xylitol g-xylose^{-1})	Volumetric productivity (P, g l^{-1} h^{-1})
		Dry cell (g l^{-1})	Xylitol (g l^{-1})		
27.6	97	11.2	12.7	0.49	1.41
15.0	100	9.5	14.6	0.58	0.92
11.2	100	9.5	13.2	0.62	0.55
8.8	100	9.2	13.5	0.55	0.43
8.4	100	6.4	11.0	0.43	0.30

observed before the glucose was completely assimilated. Then, *C. magnoliae* metabolized xylose at a higher rate. At the OTR of 8.4 mmol-O_2 l^{-1} h^{-1}, 12.7 g l^{-1} xylitol (Y_x=0.43 g g^{-1}) was produced, whereas the xylitol volumetric productivity was relatively low (P=0.30 g l^{-1} h^{-1}) and cell growth stayed almost constant. At the OTR of 11.2 mmol-O_2 l^{-1} h^{-1}, xylose in the hydrolysate was successfully converted to xylitol (Y_x=0.62 g g^{-1}) and cell growth was gradually increased. At OTR of 27.6 mmol-O_2 l^{-1} h^{-1}, both xylitol yield and xylitol concentration decreased compared with OTR of 11.2 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 an NADP-dependent xylose reductase. At OTR of 27.6 mmol-O_2 l^{-1} h^{-1}, it is clear that a portion of the synthesized xylitol is secreted from the cell and the remainder is oxidized by an NAD-dependent xylitol dehydrogenase. Under aerobic conditions, xylulose generated through the latter process is used as a carbon source for energy production. At OTR of 27.6 mmol-O_2 l^{-1} h^{-1}, dry cell concentration was increased until complete xylose consumption, indicating that the oxygen supply conditions are undesirable for xylitol production.

At OTR of 8.4 mmol-O_2 l^{-1} h^{-1}, dry cell concentration was almost constant during the microaerobic phase. After complete glucose consumption, ethanol was detected in the fermentation medium. Pampulha and Loureiro (1989) observed that acetic acid is more toxic in the presence of ethanol in the fermentation media. The anaerobic condition (OTR of 8.4 mmol-O_2 l^{-1} h^{-1}) is unsuitable for xylitol production. The best fermentative performance of *C. magnoliae* in a culm hemicellulose hydrolysate of *S. kurilensis* (Y_x=0.62; P=0.55 g l^{-1} h^{-1}) was obtained at the OTR of 11.2 mmol-O_2 l^{-1} h^{-1}. Vandeska et al. (1995) 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* (Y_x=0.48; P=0.24 g l^{-1} h^{-1}).

Conclusion

A fermentable substrate with a relatively high xylose concentration (31 g l^{-1}) could be prepared from the culm of *S. kurilensis* by acid hydrolysis with 2% sulfuric acid under mild hydrolysis conditions. Inhibitors such as dehydration products of solubilized sugars and low molecular weight phenolics released from lignin were successfully removed by treatment with a steam-activated charcoal. The detoxified hydrolysate could be successfully converted to xylitol by *C. magnoliae*. The two-stage oxygen supply control strategy permitted efficient microbial xylitol production from bamboo grass hemicellulose hydrolysates.

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References

Azuma, M., Ikeuchi, T., Kiritani, R., Kato, J., Ooshima, H. (2000) Increase in xylitol production by *Candida tropicalis* upon addition of salt. Biomass Bioeng. 19:129–135.

Canettieri, E.V., Rocha, G.J.M., Carvalho, J.A., Silva, J.B.A. (2007) Optimization of acid hydrolysis from the hemicellulosic fraction of *Eucalyptus grandis* residue using response surface methodology. Biores. Technol. 98: 422–428.

Delgenes, J.P., Moletta, R., Navarro, J.M. (1996) Effects of lignocellulose degradation products on ethanol fermentations of glucose and xylose by *Saccharomyces cerevisiae*, *Zymomonas mobilis*, *Pichia stipites*, and *Candida shehatae*. Enzyme Microb. Technol. 19:220–225.

Ding, X., Xia, L. (2006) Effect of aeration rate on production of xylitol from corncob hemicellulose hydrolysate. Appl. Biochem. Biotechnol. 133:263–270.

Felipe, M.G.A., Alves, L.A., Silva, S.S., Roberto, I.C., Mancilha, I.M., Almeida e Silva, J.B. (1996) Fermentation of eucalyptus hemicellulosic hydrolysate to xylitol by *Candida guilliermondii*. Biores. Technol. 56:281–283.

Felipe, M.G.A., Vitolo, M., Mancilha, I.M., Silva, S.S. (1997) Environmental parameters affecting xylitol production from

sugar cane bagasse hemicellulosic hydrolyzate by *Candida guilliermondii*. J. Ind. Microbiol. Biotechnol. 18:251–254.

García, J.F., Sánchez, S., Bravo, V., Cuevas, M., Rigal, L., Gaset, A. (2011) Xylitol production from olive-pruning debris by sulphuric acid hydrolysis and fermentation with *Candida tropicalis*. Holzforschung 65:59–65.

Gütsch, J.S., Sixta, H. (2011) Purification of *Eucalyptus globulus* water prehydrolyzates using the HiTAC (high-temperature adsorption on activated charcoal). Holzforschung 65:511–518.

Ikeuchi, T., Kiritani, R., Azuma, M., Ooshima, H. (2000) Effect of D-glucose of xylose reductase and xylitol dehydrogenase in *Candida tropicalis* in the presence of NaCl. J. Basic Microbiol. 40:167–175.

Kastner, J.R., Eiteman, M.E., Lee, S.A. (2001) Glucose repression of xylitol production in *Candida tropicalis* mixed-sugar fermentations. Biotechnol. Lett. 23:1663–1667.

Mendes, C.V.T., Baptista, C.M.S.G., Rocha, J.M.S., Carvalho, M.G.V.S. (2009) Prehydrolysis of *Eucalyptus globulus* Labill. hemicelluloses prior to pulping and fermentation of the hydrolysates with the yeast *Pichia stipitis*. Holzforschung 63:737–743.

Meyrial, V., Delgenes, J.P., Moletta, R., Navarro, J.M. (1991) Xylitol production from D-xylose by *Candida guilliermondii*: fermentation behaviour. Biotechnol. Lett. 13:281–286.

Miura, M., Yokono, K., Miyamoto, H., Aoyama, M., Tada, K., Horiuchi, J., Kojima, Y., Sakai, C., Nakahara, M. (2010) Prehydrolysis of xylan in culm of *Sasa kurilensis* with dilute sulphuric acid. Eur. J. Wood Prod. 68:139–142.

Mussatto, S.I., Roberto, I.C. (2001) Hydrolysate detoxification with activated charcoal for xylitol production by *Candida guilliermondii*. Biotechnol. Lett. 23:1681–1684.

Mussatto, S.I., Santos, J.C., Roberto, I.C. (2004) Effect of pH and activated charcoal adsorption on hemicellulose hydrolysate detoxification for xylitol production. J. Chem. Technol. Biotechnol. 79:590–596.

Nakano, K., Katsu, R., Tada, K, Matsumura, M. (2000) Production of highly concentrated xylitol by *Candida magnoliae* under a microaerobic condition maintained by simple fuzzy control. J. Biosci. Bioeng. 89:372–376.

Nolleau, V., Preziosi-Belloy, L., Navarro, J.M. (1995) The reduction of xylose to xylitol by *Candida guilliermondii* and *Candida parapsilosis*: incidence of oxygen and pH. Biotechnol. Lett. 17:417–422.

Onishi, H., Suzuki, T. (1966) The production of xylitol, L-arabitol and ribitol by yeasts. Agric. Biol. Chem. 30:1139–1144.

Palmqvist, E., Hahn-Hägerdal, B. (2000) Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. Biores. Technol. 74:25–33.

Pampulha, M.E., Loureiro, V. (1989) Interaction of the effects of acetic acid and ethanol on inhibition of fermentation in *Saccharomyces cerevisiae*. Biotechnol. Lett. 11:269–274.

Parajó, J.C., Domínguez, H., Domínguez, J.M. (1996) Charcoal adsorption of wood hydrolysates for improving their fermentability: influence of the operational conditions. Biores. Technol. 57:179–185.

Pessoa, A., Jr., Mancilha, I.M., Sato, S. (1996) Cultivation of *Candida tropicalis* in sugar cane hemicellulosic hydrolyzate for microbial protein production. J. Biotechnol. 51:83–88.

Poth, S., Monzon, M., Tippkötter, N., Ulber, R. (2011) Lignocellulosic biorefinery: process integrated of hydrolysis and fermentation (SSF process). Holzforschung 65:633–637.

Preziosi-Belloy, L., Nolleau, V., Navarro, J.M. (2000) Xylitol production from aspenwood hemicellulose hydrolysate by *Candida guilliermondii*. Biotechnol. Lett. 22:239–243.

Roberto, I.C., Lacis, L.S., Barbosa, M.F.S., de Mancilha, I.M. (1991) Utilization of sugar cane bagasse hemicellulosic hydrolysate by *Pichia stipitis* for the production of ethanol. Process Biochem. 26:15–21.

Rodríguez-López, J., Romaní, A., González-Muñoz, M.J., Garrote, G., Parajó, J.C. (2012) Extracting value-added products before pulping: hemicellulosic ethanol from *Eucalyptus globulus* wood. Holzforschung 66:591–599.

Saha, B.S., Bothast, R.J. (1999) Production of xylitol by *Candida peltata*. J. Ind. Microbiol. Biotechnol. 22:633–636.

Silva, S.S., Felipe, M.G.A., Mancilha, I.M. (1988) Factors that affect the biosynthesis of xylitol by xylose-fermenting yeasts. A review. Appl. Biochem. Biotechnol. 70–72:331–339.

Silva, S.S., Riberio, J.D., Felipe, M.G.A., Vitolo, M. (1997) Maximizing the xylitol production from sugar cane bagasse hydrolysate by controlling the aeration rate. Appl. Biochem. Biotechnol. 63–65:557–564.

Taguchi, H., Humphrey, A.E. (1966) Dynamic measurement of the volumetric oxygen transfer coefficient in fermentation systems. J. Ferment. Technol. 44:881–889.

Tran, A.V., Chambers, R.P. (1986) Ethanol fermentation of red oak acid hydrolysate by the yeast *Pichia stipitis* CBS 5776. Enzyme Microb. Technol. 8:439–444.

Walther, T., Hensirisak, P., Agblevor, F.A. (2001a) The influence of aeration and hemicellulosic sugars on xylitol production by *Candida tropicalis*. Biores. Technol. 76:213–220.

Walthers, T., Hensirisak, P., Agblevor, F.A. (2001b) Model compound studies. Appl. Biochem. Biotechnol. 91–93:423–435.

Vandeska, E., Kuzmanova, S. Jeffries, T.W. (1995) Xylitol formation and key enzyme activities in *Candida boidinii* under different oxygen transfer rates. J. Ferment. Bioeng. 80:513–516.