# BIOCONVERSION OF BAMBOO GRASS CULM HYDROLYZATE INTO XYLITOL BY YEAST *CANDIDA MAGNOLIAE*

MASAHIRO MIURA, NAOYA MATSUMOTO, KEISUKE SEINO, YASUTAKA SHIMOTORI, HISAYUKI NAKATANI and MASAKAZU AOYAMA

*Department of Biotechnology and Environmental Chemistry, Kitami Institute of Technology, Kitami 090-8507, Japan*

✉ *Corresponding author: Masahiro Miura, d1271300015@std.kitami-it.ac.jp*

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A sugar solution containing 32 g $dm^{-3}$ of xylose was prepared from the culm of *Sasa senanensis* by hydrolysis with 2% sulfuric acid with a liquid-to-solid ratio of 5 (g $g^{-1}$) at 120 °C for 1 h. During the acid hydrolysis, some byproducts were generated, such as acetic acid, furfural and low molecular weight phenols, which inhibit bioconversion of xylose to xylitol. Except for acetic acid, these inhibitors were successfully removed from the hydrolyzate by contacting with a steam-activated charcoal (25 g $dm^{-3}$ dose) for 1 h. 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 12.6 to 15.1 mmol-$O_2$ $dm^{-3}$ $h^{-1}$. The best fermentative performance of *C. magnoliae* in the culm hydrolyzate of *S. senanensis* (xylitol yield of 0.55–0.60 g-xylitol g-$xylose^{-1}$ and volumetric productivity of 0.82 g $dm^{-3}h^{-1}$) was obtained at the OTR ranging 14.1−15.1 mmol-$O_2$ $dm^{-3}$ $h^{-1}$.

***Keywords***: *Sasa senanensis*, bamboo grass, hemicellulose hydrolyzate, xylitol, *Candida magnoliae*

## INTRODUCTION

Non-merchantable forest biomass such as whole small-sized trees, forest residues and bamboos, is renewable, available in large quantities and an inexpensive source for fuels and chemical feedstuffs. Acid hydrolysis is the most commonly employed treatment to obtain sugar solutions from these lignocelluloses. The resulting sugar solutions can be used as fermentation substrates for the production of biofuels, protein and sugar derivatives.

Xylitol, a naturally occurring sugar alcohol, is of interest to the food and oral care industries because of its high sweetening power equivalent to sucrose, great negative heat of dissolution, and anticariogenic properties. It absorbs slowly from human digestive tract and enters the metabolic pathway independently of insulin. The glycemic index of xylitol (GI = 13) compares favorably with that of sucrose (GI = 65).[1] Therefore, xylitol is used clinically as a sucrose substituent for diabetics and for patients deficient of glucose-6-phosphate dehydrogenase (G6PD).

Xylitol is currently produced by a catalytic reduction of xylose present in hemicellulose hydrolyzates of hardwoods or agricultural wastes. The biotechnological production of xylitol from lignocellulose hydrolyzates is considered to be an alternative process because it requires neither pure xylose nor heavy metal catalysts for the xylose reduction. Although the biotechnological production of xylitol requires additional costs for the detoxification of hemicellulose hydrolyzates and purification of xylitol in the fermentation media, the additional costs for the environmentally 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.[2,3]

Bamboo grasses, perennial plants with woody culms, are mainly distributed in Japan. The hemicelluloses of *Sasa senanensis* are mainly composed of arabinoglucuronoxylan.[4] In this study, the microbial conversion of *S. senanensis* culm

hydrolyzate to xylitol has been examined. Before fermentation, the hydrolyzate was detoxified with a steam-activated charcoal. The microbial conversion was performed by the yeast *C. magnoliae* using a two-phase aeration process.[5-7] Ding and Xia[8] reported that the two-phase aeration is more effective than one-phase aeration for xylitol production. It is known that xylitol production is suppressed by glucose in the fermentation media.[9-12] The first step of the fermentation process was carried out under aerobic conditions to improve glucose consumption through cell proliferation in the medium.[12] The second step was performed under limited oxygen conditions and the intention is to increase the xylitol accumulation.[13]

## EXPERIMENTAL

### Hydrolysis

The ground culm of *S. senanensis* (P32 R82 mesh) is composed of 19.5% pentosan (including 18.1% xylan), 37.9% hexosan (including 36.5% glucan), 26.4% lignin (including 3.5% acid soluble lignin), and 2.8% ash. The ground culm (400 g) was hydrolyzed directly with 2% sulfuric acid (2000 g) at 120 °C for 1 h.

### Detoxification

The hydrolyzate was treated with a commercially available steam-activated charcoal (Shirasagi M, Japan EnviroChemicals, Ltd., Osaka, Japan) in a reciprocal shaker (160 strokes $min^{-1}$) at 30 °C for 1 h. The resulting sugar solution was filtered and neutralized with calcium carbonate, followed by centrifugation.

### Microorganism and inoculum

The strain of the yeast *C. magnoliae* TISTR5663 (deposited at the National Institute of Bioscience and Human-Technology, NIBH, Tsukuba, as FERM P-16522) was grown on an agar slant containing malt extract (3 g $dm^{-3}$), yeast extract (3 g $dm^{-3}$), peptone (5 g $dm^{-3}$), $_D$-glucose (10 g $dm^{-3}$) and agar (20 g $dm^{-3}$) at 4 °C for 3 d. A spoon of a slant culture was transferred to 5 $cm^3$ of the pre-culture medium containing $_D$-xylose (20 g $dm^{-3}$), casamino acids (1 g $dm^{-3}$), Difco yeast nitrogen base without amino acids and ammonium sulfate (1.7 g $dm^{-3}$) and urea (2.27 g $dm^{-3}$), 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.[14] A spoon of a slant culture was transferred to 5 $cm^3$ of the pre-culture medium containing increasing concentrations of the hydrolyzate (25%, 50%, 75% and 100%) supplemented with $_D$-xylose (0 to 6 g $dm^{-3}$), casamino acids (1.0 g $dm^{-3}$), yeast nitrogen base without amino acids and ammonium sulfate (1.7 g $dm^{-3}$) and urea (2.2 g $dm^{-3}$). 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 $dm^3$ of 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 13-15 h to promote the consumption of glucose, and then the aeration rate and agitation rate were reduced. In the second aeration phase, the agitation was set at 360–400 $min^{-1}$ and the aeration was fixed at the ratio air-to-medium of 0.67 (by volume $min^{-1}$).

The volumetric oxygen transfer coefficient ($K_La$) was determined by the dynamic method.[15] The dissolved oxygen concentration (DOC) of the medium was decreased to zero by nitrogen sparging and the $K_La$ was calculated from the rate of DOC increased during subsequent aeration. The oxygen transfer rate (OTR) was calculated as OTR = $K_La$ ($C^*$ - $C$), 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 and an Aminex HPX-87P column (300 × 7.8 mm, Bio-Rad, Richmond, VA); water as eluent (0.6 $cm^3$ $min^{-1}$), 85 °C. Furfural 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 $cm^3$ $min^{-1}$), 50 °C. The overall content of phenols in the hydrolyzate was evaluated by the absorbance at 280 nm ($A_{280}$) at pH 12.[16] Cell concentration was determined indirectly by correlation between the dry weight of the cell and the absorbance at 660 nm ($A_{660}$).[17]

## RESULTS AND DISCUSSION

The ground culms were hydrolyzed with 2% sulfuric acid with a liquid-to-solid ratio of 5 (g $g^{-1}$) at 120 °C for 1 h to afford a sugar solution containing 34.22 g $dm^{-3}$ xylose, 2.95 g $dm^{-3}$ arabinose, 9.51 g $dm^{-3}$ glucose, 1.2 g $dm^{-3}$ galactose, and 0.42 g $dm^{-3}$ mannose (Table 1). Besides neutral sugars, the original hydrolyzate also contained significant amounts of the fermentation inhibitors, such as acetic acid (7.92 g $dm^{-3}$), furfural (0.57 g $dm^{-3}$), 5-hydroxymethylfurfural (0.82 g $dm^{-3}$) and low molecular weight phenols generated during the hydrothermal treatment, as shown in Table 1.

Delgenes *et al*.[18] found that 0.5 g $dm^{-3}$ of furfural reduced the cell growth of the yeast, *Pichia stipitis* by 25%. Further, the $A_{280}$ value of the hydrolyzate (0.27) diluted 1:1000 with water at acidic pH was too high to perform microbial conversion of the sugar solution. Tada *et al*.[19] reported that successful xylitol production from corn cob hydrolyzates diluted 1:1000 with water by *C. magnoliae* required the $A_{280}$ value to be less than 0.02.

Activated charcoal treatment is an efficient method for reducing the concentrations of furan derivatives and low molecular weight phenols.[20,21] In this study, the hydrolyzate was treated with a commercially available steam-activated charcoal at 30 ºC for 1 h. When 20 mL of the hydrolyzate was treated with 0.5 g of activated charcoal (25 g $dm^{-3}$ of carbon dose), the concentrations of furfural and 5-hydroxymethylfurfural decreased from 0.57 g $dm^{-3}$ to 0.03 g $dm^{-3}$ and from 0.82 $dm^{-3}$ to 0.07 $dm^{-3}$, respectively. The $A_{280}$ value of the hydrolyzate also decreased from 0.24 to 0.02. The results strongly suggested that 25 g $dm^{-3}$ of the sorbent could eliminate large parts of furan derivatives and low molecular weight phenols from the hydrolyzate. In contrast, the concentrations of each neutral sugar and acetic acid (originating from xylan) stayed almost constant after treatment with the activated charcoal. At the optimal pH range of fermentation (pH 4-5), acetic acid occurs largely in an undissociated form. After its diffusion into cell cytoplasm, it dissociates, uncouples the energy production and impairs transport of nutrients. However, the sensitivity to acetic acid is yeast species dependent. Pessoa *et al*.[22] reported that 3.7 g $dm^{-3}$ of acetic acid in sugar cane hemicellulose hydrolyzate was completely consumed by *C. tropicalis*. *C. guilliermondii* was also able to assimilate significant amounts of acetic acid in hemicellulose hydrolyzates of eucalyptus wood and sugar cane bagasse in amounts of 40% and 50%, respectively.[23,24] Ferrari *et al*.[25] reported that acetic acid in the *Eucalyptus* wood hydrolyzate (10.1–10.4 g $dm^{-3}$) was partially consumed by *P. stipitis*. *C. magnoliae* also consumed large parts of acetic acid present in the fermentation media (7.44–7.81 g $dm^{-3}$) as a carbon source under microaerobic conditions, as shown in Figure 1 (d).

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 oxygen transfer rate (OTR) within the range of 12.6–15.1 mmol-$O_2$ $dm^{-3}$ $h^{-1}$ on xylitol production. Glucose in the fermentation media (about 9.7 g $dm^{-3}$) was completely consumed during the first 13–15 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 1
Chemical composition of raw and detoxified *Sasa senanensis* culm hydrolyzate

| Components | Concentration (g $dm^{-3}$) | |
|---|---|---|
| | Original hydrolyzate[1] | Detoxified hydrolyzate[2] |
| Arabinose | 2.95 | 2.16 |
| Xylose | 34.22 | 32.73 |
| Galactose | 1.15 | 1.14 |
| Glucose | 9.51 | 9.34 |
| Mannose | 0.42 | 0.42 |
| Acetic acid | 7.92 | 7.05 |
| Furfural | 0.57 | 0.03 |
| 5-Hydroxymethylfurfural | 0.82 | 0.07 |
| Phenolics[3] | 0.24 | 0.02 |

[1] The ground culm of *Sasa senanensis* was hydrolyzed with 2% sulfuric acid with a liquid-to-solid ratio of 5 (g $g^{-1}$) at 120ºC for 1 h; [2] The original hydrolyzate was treated with a steam-activated charcoal (25 g $dm^{-3}$) in a reciprocal shaker (160 strokes $min^{-1}$) at 30 ºC for 1 h; [3] The overall content of phenols was evaluated by the absorbance at 280 nm ($A_{280}$) at pH 12

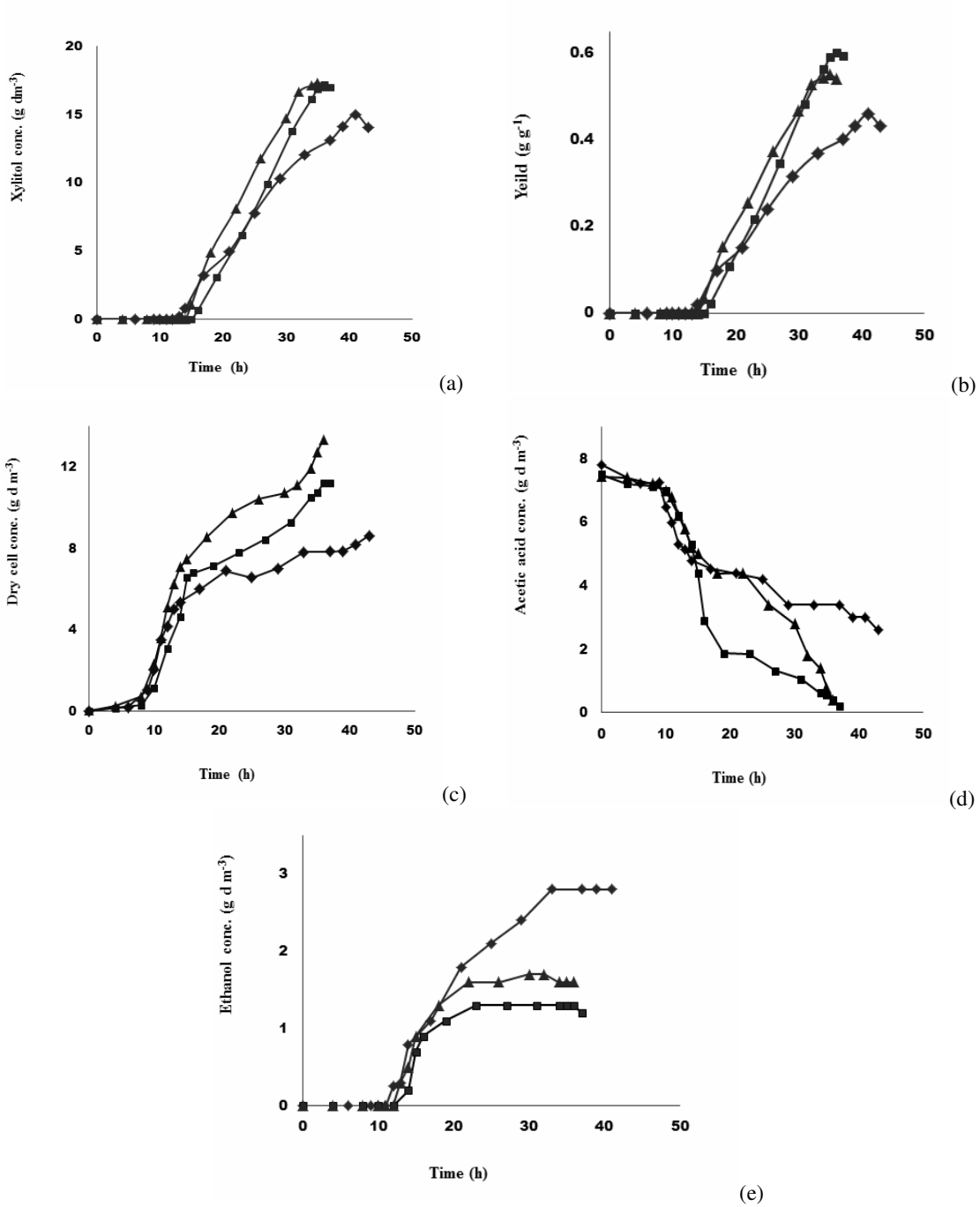


Figure 1: Time course of xylitol concentration (a), xylitol yield (b), dry cell concentration (c), acetic acid concentration (d) and ethanol production (e) in batch xylitol fermentations by *Candida magnoliae* from detoxified culm hydrolyzate of *Sasa senanensis* under different microaerobic conditions. Symbols: ◆ OTR = 12.6 mmol $dm^{-3}$ $h^{-1}$ (agitation: 360 rpm; aeration: 0.67 vvm); ■ OTR = 14.1 mmol $dm^{-3}$ $h^{-1}$ (agitation: 390 rpm; airation: 0.67 vvm); ▲ OTR = 15.1 mmol $dm^{-3}$ $h^{-1}$ (agitation: 400 rpm; aeration: 0.67 vvm)

Table 2
Effects of oxygen transfer rate (OTR) on xylitol production from *S. senanensis* culm hydrolyzate by *Candida magnoliae*

| | Oxygen transfer rate (mmol-$O_2$ $dm^{-3}h^{-1}$) | | |
|---|---|---|---|
| | 12.6 | 14.1 | 15.1 |
| Initial xylose concentration (g $dm^{-3}$) | 32.7 | 35.6 | 31.6 |
| Xylose consumed (%) | 92.2 | 94.7 | 93.0 |
| Maximum xylitol concentration (g $dm^{-3}$) | 15.0 | 17.2 | 17.4 |
| Xylitol yield (g-xylitol g-$xylose^{-1}$) | 0.46 | 0.60 | 0.55 |
| Volumetric productivity (g $dm^{-3}$ $h^{-1}$) | 0.54 | 0.82 | 0.82 |
| Final dry cell concentration (g $dm^{-3}$) | 9.7 | 12.2 | 14.3 |

At the OTR of 12.6 mmol-$O_2$ $dm^{-3}$ $h^{-1}$, xylitol concentration of 15.0 g $dm^{-3}$ was obtained after 41 h of fermentation, which corresponds to a xylitol volumetric productivity of 0.54 g $dm^{-3}$ $h^{-1}$, and a xylitol yield of 0.46 g-xylitol g-$xylose^{-1}$. After complete glucose consumption, ethanol also accumulated gradually in the fermentation medium, and reached the maximum concentration (2.75 g $dm^{-3}$) after a fermentation time of 32 h. Pampulha and Loureiro[26] observed that acetic acid is more toxic in the presence of ethanol in the fermentation media. Therefore, the OTR of 12.6 mmol-$O_2$ $dm^{-3}$ $h^{-1}$ is slightly anaerobic for xylitol production from the hydrolyzate. At the OTR of 14.1 mmol-$O_2$ $dm^{-3}$ $h^{-1}$, xylitol production was greatly improved. Xylose in the detoxified hydrolyzate was successfully converted to xylitol (0.60 g-xylitol g-$xylose^{-1}$) with a volumetric productivity 0.82 g $dm^{-3}h^{-1}$, and with about 95% xylose utilization. At the OTR of 15.1 mmol-$O_2$ $dm^{-3}$ $h^{-1}$, the maximum xylitol concentration (17.4 g $dm^{-3}$) occurred at a fermentation time of 34 h, although a slight decrease in the xylitol yield (0.55 g $g^{-1}$) was observed, compared to the OTR of 14.1 mmol-$O_2$ $dm^{-3}$ $h^{-1}$. Under aerobic conditions, xylose fermentable yeasts metabolize mainly xylose for energy production. Xylose absorbed into cells is first reduced to xylitol by a NADP-dependent xylose reductase, and then 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 pentose phosphate pathway or phosphoketolase bypass to afford glycelaldehyde-3-phosphate. Glycelaldehyde-3-phosphate is metabolized through the Embden-Meyerhoff-Parnas passway and tricarboxylic acid cycle for energy production. At the OTR ranging between 12.6–15.1 mmol-$O_2$ $dm^{-3}$ $h^{-1}$, it is clear that a portion of the synthesized xylitol is secreted from cell and the remainder is oxidized by NAD-dependent xylitol dehydrogenase for energy production, as shown in Figure 1 (a) and (c). The best fermentative performance of *C. magnoliae* in a culm hemicellulose hydrolyzate of *S. senanensis* (xylitol yield of 0.55–0.60 g-xylitol g-$xylose^{-1}$ and volumetric productivity of 0.82 g $dm^{-3}$ $h^{-1}$) was obtained at the OTRs of 14.1 and 15.1 mmol-$O_2$ $dm^{-3}$ $h^{-1}$. Vandeska *et al.*[27] reported that an OTR of 14 mmol–$O_2$ $dm^{-3}$ $h^{-1}$ was optimal for xylitol production from a synthetic medium using *C. boidinii* (xylitol yield of 0.48 g-xylitol g-$xylose^{-1}$ and volumetric productivity of 0.24 g $dm^{-3}$ $h^{-1}$). Recently, we have reported that the best fermentative performance of *C. magnoliae* in a clum hemisellulose hydrolyzate of *S. kurilensis* (xylitol yield = 0.62; xylitol volumetric productivity = 0.55 g $dm^{-3}$ $h^{-1}$) was obtained at the OTR of 11.2 mmol-$O_2$ $dm^{-3}$ $h^{-1}$.[28] It means that the same fermentation performance is attained from a mixture of two different representative species of bamboo grasses.

## CONCLUSION

A fermentable substrate with a relatively high xylose concentration (32 g $dm^{-3}$) could be prepared from the culm of *S. senanensis* by acid hydrolysis with 2% sulfuric acid under mild hydrolysis conditions. Inhibitors, such as dehydration products of solubilized sugars and low molecular weight phenols released from lignin were successfully removed by treatment with a commercially available steam-activated charcoal. The detoxified hydrolyzate could be successfully converted to xylitol by *C. magnoliae*. The best fermentative performance was obtained at the OTR ranging between 14.1–15.1 mmol-$O_2$ $dm^{-3}$ $h^{-1}$. When the recovery losses during the hydrolysis and detoxification processes are considered, 73 g

of xylitol can be obtained from 1 kg of bamboo grass culm. The two-stage oxygen supply control strategy permitted efficient microbial xylitol production from bamboo grass culm hydrolyzates.

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**REFERENCES**

[1] G. Livesey, *Nut. Res. Rev.*, **16**, 163 (2003).
[2] H. Onishi and T. Suzuki, *Agric. Biol. Chem.*, **30**, 1139 (1966).
[3] V. Meyrial, J. P. Delgenes, R. Moletta and J. M. Navarro, *Biotechnol. Lett.*, **13**, 281 (1991).
[4] S. Yoshida, A. Kuno, N. Saito, M. Aoyama and I. Kusakabe, *J. Wood Sc*i., **44**, 457 (1998).
[5] B. S. Saha and R. J. Bothast, *J. Ind. Microbiol. Biotechnol.*, **22**, 633 (1999).
[6] L. Preziosi-Belloy, V. Nolleau and J. M. Navarro, *Biotechnol. Lett.*, **22**, 239 (2000).
[7] J. R. Kastner, M. E. Eiteman and S. A. Lee, *Biotechnol. Lett.*, **23**, 1663 (2001).
[8] X. Ding and L. Xia, *Appl. Biochem. Biotechnol.*, **133**, 263 (2006).
[9] M. Azuma, T. Ikeuchi, R. Kiritani, J. Kato and H. Ooshima, *Biomass Bioeng.*, **19**, 129 (2000).
[10] T. Ikeuchi, R. Kiritani, M. Azuma and H. Ooshima, *J. Basic Microbiol.*, **40**, 167 (2000).
[11] T. Walthers, P. Hensirisak and F. A. Agblevor, *Appl. Biochem. Biotechnol.*, **91-93**, 423 (2001).
[12] T. Walther, P. Hensirisak and F. A. Agblevor, *Bioresour. Technol.*, **76**, 213 (2001).
[13] V. Nolleau, L. Preziosi-Belloy and J. M. Navarro, *Biotechnol. Lett.*, **17**, 417 (1995).
[14] S. Amartey and T. Jeffries, *World J. Microbiol. Biotechnol.*, **12**, 281 (1996).
[15] H. Taguchi and A. E. Humphrey, *J. Ferment. Technol.*, **44**, 881 (1966).
[16] S. I. Mussatto, J. C. Santos and I. C. Roberto, *J. Chem. Technol. Biotechnol.*, **79**, 590 (2004).
[17] K. Nakano, R. Katsu, K. Tada and M. Matsumura, *J. Biosci. Bioeng.*, **89**, 372 (2000).
[18] J. P. Delgenes, R. Moletta and J. M. Navarro, *Enzyme Microb. Technol.*, **19**, 220 (1996).
[19] K. Tada, J. Horiuchi, T. Kanno and M. Kobayashi, *J. Biosci. Bioeng.*, **98**, 228 (2004).
[20] J. C. Parajó, H. Domínguez and J. M. Domínguez, *Bioresour. Technol.*, **57**, 179 (1996).
[21] S. I. Mussatto and I. C. Roberto, *Biotechnol. Lett.*, **23**, 1681 (2001).
[22] A. Pessoa, Jr., I. M. Mancilha and S. Sato, *J. Biotechnol.*, **51**, 83 (1996).
[23] M. G. A. Felipe, L. A. Alves, S. S. Silva, I. C. Roberto, I. M. Mancilha *et al*., *Bioresour. Technol*., **56**, 281 (1996).
[24] M. G. A. Felipe, M. Vitolo, I. M. Mancilha and S. S. Silva, *J. Ind. Microbiol. Biotechnol.*, **18**, 251 (1997).
[25] M. D. Ferrari, E. Neirotti, C. Albornoz, and E. Saucedo, *Biotechnol. Bioeng.*, **40**, 753 (1992).
[26] M. E. Pampulha and V. Loureiro, *Biotechnol. Lett.*, **11**, 269 (1989).
[27] E. Vandeska, S. Kuzmanova and T. W. Jeffries, *J. Ferment. Bioeng.*, **80**, 513 (1995).
[28] M. Miura, T. Seo, Y. Shimotori, M. Aoyama, H. Nakatani *et al*., *Holzforschung*, **67**, 881 (2013).