J Wood Sci (2002) 48:159–162

NOTE

Xin Li · Ryuichiro Kondo · Kokki Sakai

Biodegradation of sugarcane bagasse with marine fungus *Phlebia* sp. MG-60

Received: April 13, 2001 / Accepted: May 21, 2001

Abstract A marine fungus, *Phlebia* sp. MG-60, and three white-rot fungi were incubated with whole sugarcane bagasse (WSB). The residual lignin content and holocellulose content in the decayed WSB were determined, and based on their content delignification selection factors of the fungi were calculated. More than 50% of lignin in the WSB was degraded by *Phlebia* sp. MG-60, and less than 10% of the holocellulose was lost. The WSB was fractioned by sieving to separate long-fiber bagasse, short-fiber bagasse, and bagasse pith. When *Phlebia* sp. MG-60 was incubated with the three fractions, more efficient delignification activity on the bagasse pith was observed. As the most efficient utilization of bagasse, we predict that bagasse fiber might be employed as the raw material in the pulp and paper industry after biopulping with *Phlebia* sp. MG-60 and bagasse pith, or WSB might be used to produce animal feed after fermentation with the strain.

Key words Sugarcane bagasse · Biodegradation · White-rot fungi · Marine fungus · *Phlebia* sp. MG-60

Introduction

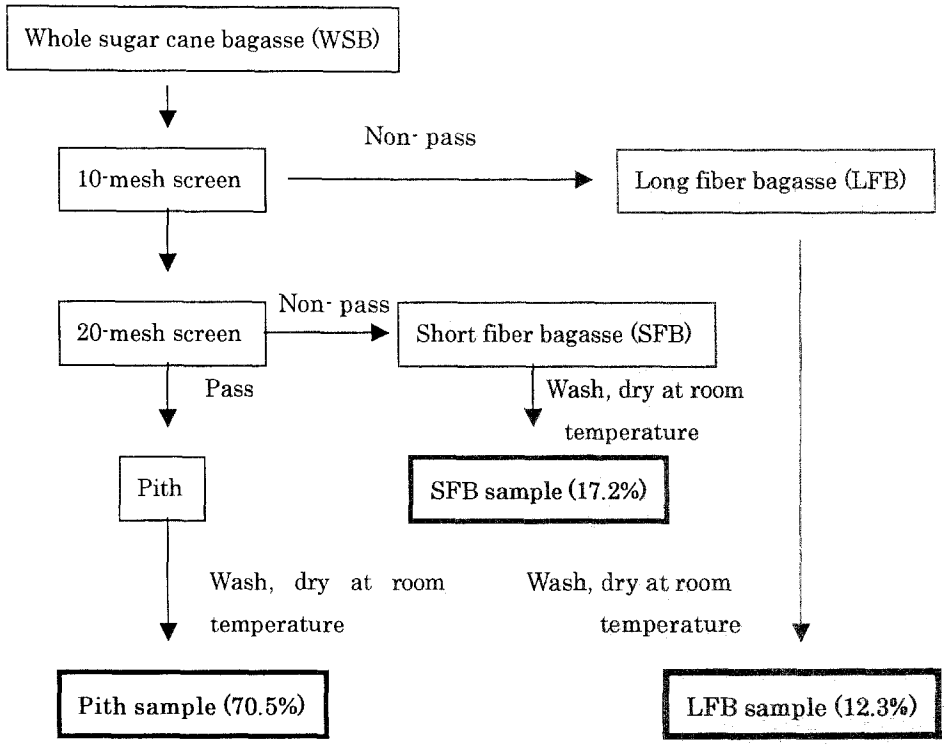
Sugarcane bagasse, generally called simply bagasse, is a by-product of the sugar industry. As a fibrous residue of cane stalks left over after crushing and extracting juice from sugar cane, more than 95% of the bagasse is used by the sugar factories themselves as fuel for the boiler after mixing it with heavy oil. More efficient utilization than as fuel has been under development in recent years. Bagasse has been employed for the production of enzymes, amino acids, drugs, ethanol, and single-cell protein as animal feed after cultivating it with a large number of microorganisms including bacteria, yeasts, and fungi, among which basidiomycetes are preferred.[1] However, such bioprocesses require only small quantities of bagasse and could not eventually solve the problem of surplus bagasse. Furthermore, the process of ethanol production from bagasse needs renewed consideration.[2] Hence, processes that can utilize large quantities of bagasse must be considered.

Due to increasing restraints on forest harvesting, the use of agro-based residues for pulp and paper production has been steadily increasing in recent years.[3] In addition, bagasse offers numerous advantages compared with other agro-based residues such as rice straw and wheat straw because of its lower ash content. The advantages of using fungal treatment prior to mechanically refining wood have been confirmed, and the benefits of biopulping agro-based materials with white-rot fungi have been researched.[4,5]

It is generally acknowledged that microorganisms degrade untreated bagasse slowly; therefore, isolation of efficient strains is regarded as an important research area for lignin degradation in bagasse. Breccia et al. screened several white-rot fungi to degrade long-fiber bagasse aiming at biopulping and found that about 16% of the lignin was removed.[6] A marine fungus, *Phlebia* sp. MG-60, which has been screened from mangrove stands, proved to have excellent lignin degrade ability and selectivity.[7] In this research, its ability to degrade bagasse was investigated and compared with that of several white-rot fungi: *Phanerochaete chrysosporium* ME-466, *Phanerochaete sordida* YK-624, and *Ceriporia* sp. MZ-340. Whole sugarcane bagasse (WSB) was fractioned to long-fiber bagasse (LFB), short-fiber bagasse (SFB), and pith; and they were respectively degraded with *Phlebia* sp. MG-60. Based on the investigation of lignin degradation in WSB and its fractions by *Phlebia* sp. MG-60, their efficient utilization in industry is proposed.

X. Li · R. Kondo (✉) · K. Sakai
Graduate School of Bioresources and Bioenvironmental Sciences, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan
Tel. +81-92-642-2811; Fax +81-92-642-2811
e-mail: kondo@brs.kyushu-u.ac.jp

Fig. 1. Schema for fractionation



Materials and methods

Fungal strains

Phlebia sp. MG-60 isolated from mangrove stands in Okinawa, Japan, *P. chrysosporium* ME-466, *P. raside* YK-624,[8] and *Ceriporia* sp. MZ-340[9] were maintained in potato dextrose agar (Difco Laboratories) slants at 4°C before use.

Raw material and its preparation

Bagasse was placed in a plastic bag and frozen to prevent the growth of contaminating microorganisms. Before being treated with white-rot fungi, bagasse was washed with running water for 12 h and dried at room temperature.[10] Bagasse was sieved with 10- and 20-mesh screens as shown in Fig. 1.

Analytical methods

Klason lignin and holocellulose were analyzed with TAPPI standard methods.[11,12]

Inoculum preparation

Phlebia sp. MG-60, *P. chrysosporium* ME-466, *P. sordida* YK-624, and *Ceriporia* sp. MZ-340 were, respectively, inoculated in potato dextrose broth (PDB) (Difco Laboratories) liquid medium, and then incubated at 30°C for 5 days. In each case the mycelium was separated from PDB liquid medium, washed in sterilized water, and homogenized with 8 ml of sterilized water. WSB or bagasse fractions of 4.0 g were mixed with 8 ml of sterilized water or Kirk medium,[13] which contained glucose 10 g/l, ammonium tartrate 0.221 g/l, sodium acetate 1.64 g/l, Tween 80 1.0 g/l, Kirk's salts solution and Kirk's trace element solution; they were then sterilized and inoculated with the homogenized fungal mycelium. The inoculated WSB and its fractions were incubated without shaking at 30°C for 30 days.

Evaluation of biodegradation ability of fungi

The lignin-degrading selection factor (SF) of every strain was calculated as follows:

SF = lignin loss/holocellulose loss

in which holocellulose loss was calculated as follows:

Holocellulose loss = weight loss − lignin loss

The weight loss, residual lignin content, and SF are employed to evaluate the delignification ability of the strains.[8]

Results

Composition of bagasse

The compositions of WSB and its fractions before treatment are tabulated in Table 1. The untreated WSB contained 29.4% Klason lignin and 62.9% holocellulose. The lignin and holocellulose contents of LFB, SFB, and pith were different. The holocellulose content in LFB (71%)

Table 1. Composition of bagasse

Substance	Moisture (%)	Ash content (%)	Klason lignin (%)	Holocellulose (%)
WSB	10.1	1.9	29.4	62.9
LFB	4.0	1.9	21.5	71.0
SFB	5.5	1.9	29.0	63.9
Pith	6.7	2.5	37.3	53.2

WSB, whole sugarcane bagasse; LFB, long-fiber bagasse; SFB, short-fiber bagasse

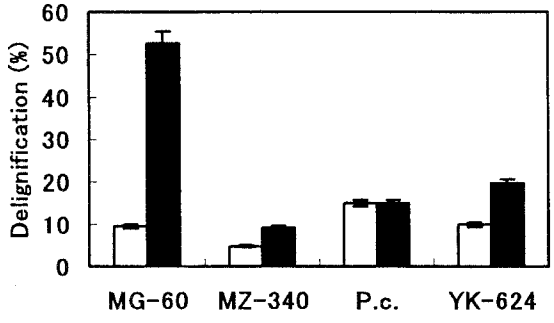


Fig. 2. Lignin decrease in whole sugarcane bagasse (WSB) after treatment with white-rot fungi in water medium (*open bars*) and Kirk medium (*filled bars*) (incubation time 30 days)

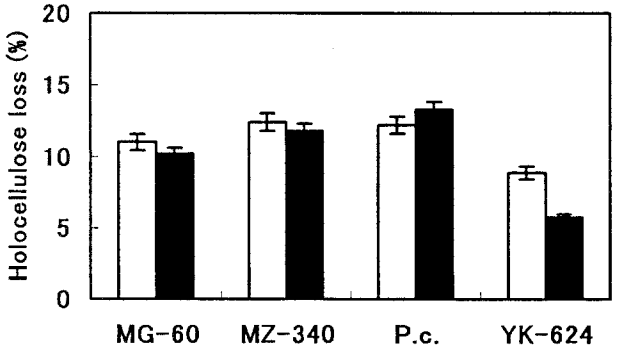


Fig. 3. Holocellulose loss in WSB after treatment with white-rot fungi in water medium (*open bars*) and Kirk medium (*filled bars*) (incubation time 30 days)

was the highest, and its lignin content (21.5%) was the lowest in the three fractions. The lignin content in pith was almost twice that in LFB. The different components in the three fractions appear to lend themselves to different industrial uses.

Degradation of WSB by four fungal strains

WSB was treated with four white-rot fungi for 4 weeks with or without Kirk medium added to the culture. The residual lignin content and holocellulose content were estimated (Figs. 2, 3), and SF was calculated for each strain (Fig. 4). Without Kirk medium addition, *Phlebia* sp. MG-60 did not show higher delignification ability or better delignification selectivity than the other white-rot fungi. However, when Kirk medium was added to the culture instead of sterilized water, outstanding delignification capability and excellent

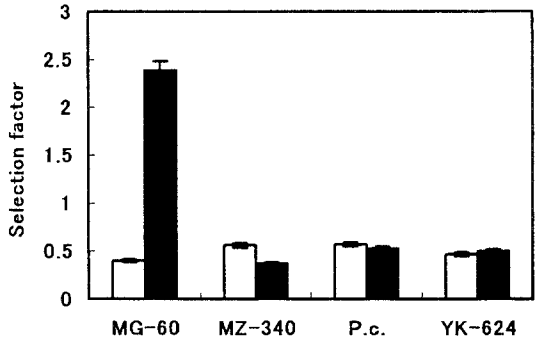


Fig. 4. Selective properties to delignify WSB during treatment with white-rot fungi in water medium (*open bars*) and Kirk medium (*filled bars*) (incubation time 30 days)

selective property to delignify sugarcane bagasse were observed. Thus, with proper addition of a nutrient such as Kirk medium, *Phlebia* sp. MG-60 could efficiently degrade lignin in sugarcane bagasse while holocellulose was scarcely damaged.

Degradation of bagasse fibers with *Phlebia* sp. MG-60

The white-rot fungus *Phlebia* sp. MG-60 was employed to treat the bagasse fractions with addition of Kirk medium to the incubation culture. The delignification and holocellulose loss of LFB and SFB were evaluated, and the results are shown in Table 2. The delignification efficiency of LFB and SFB was almost the same, although the holocellulose loss in LFB was slightly higher than that in SFB; that is, there was no significant difference for *Phlebia* sp. MG-60 to degrade LFB and SFB selectively.

Degradation of bagasse pith by *Phlebia* sp. MG-60

Although about 33% of lignin in bagasse pith was degraded by *Phlebia* sp. MG-60, only 6% of the holocellulose was damaged (Table 2). Therefore, it was concluded that the strain could selectively degrade lignin in bagasse pith.

Discussion

Lignin and holocellulose contents have been reported to be almost the same in bagasse fiber and pith,[14] but, a different result for bagasse composition was observed in our investi-

Table 2. Delignification and holocellulose loss of bagasse fractions treated with *Phlebia* sp. MG-60 for 30 days

Parameter	WSB (n = 3)	Mixt. (n = 3)	LFB (n = 3)	SFB (n = 3)	Pith (n = 3)
Delignification (%)[a]	52.7 ± 1.3	45.8 ± 3.3	21.9 ± 1.2	22.9 ± 1.1	33.0 ± 1.2
Holocellulose loss (%)	10.2	10.6	9.2	7.7	6.2
Selection factor	2.4	2.1	0.7	1.4	3.7

Mixt., mixture of LFB, SFB, and pith
[a] Results are mean ± SD

gation. As shown in Table 1, the holocellulose content in bagasse fibers, especially LFB, was higher than that in pith, whereas the lignin content in fibers was lower than that in pith.

It has been demonstrated that the use of white-rot fungi helps remove lignin from lignocellulosic materials.[15–17] When several white-rot fungi, including *Phlebia* sp. MVHC 5535, were employed to degrade LFB by Breccia et al., they produced LFB with the lowest lignin content, but the highest delignification efficiency was less than 17% after 30 days of incubation.[6] *Phlebia* sp. MG-60 removed more than 21.9% of lignin in LFB, so it can be regarded as an effective strain for biopulping or biodegradation of lignocellulosic residues. Although pulp property of the decayed bagasse was not analyzed to evaluate its pulping possibility, it should be helpful during mechanical or chemical pulp production by selectively removing as much lignin as possible during the fungal pretreatment process.

Alternative utilization of WSB is for animal feed production. When lignocellulosic residues are employed to produce animal feed, the key process is to degrade lignin to improve their digestibility for ruminant animals such as cattle. After fermentation by *Phlebia* sp. MG-60, more than 50% of the lignin in WSB is removed; therefore, its digestibility as animal feed is significantly improved and the protein enriched. One of the potential applications of *Phlebia* sp. MG-60 is for animal feed production by fermentation with WSB.

An interesting result was obtained in our investigation. Lignin was less effectively degraded in the bagasse fractions than in WSB. To demonstrate this, the fractions were mixed according to the ratio of fractions in the WSB (Fig. 1) and then incubated under the same conditions. After treatment with MG-60, the weight loss and lignin decrease were determined. The lignin decrease is shown in Table 2. Although lignin reduction from the mixture of the three fractions was lower than that from the WSB, the lignin reduction of the mixture was much higher than that of each fraction. The results demonstrate that WSB was more effectively degraded with MG-60 than its fractions under the same incubation conditions. When the WSB was fermented, bagasse pith or metal ions in pith is likely to affect the delignification ability of MG-60, but this must be further investigated.

Acknowledgment We thank Tomoe Nyugyo Company, Japan, which provided the sugarcane bagasse samples for all experiments.

References

1. Eriksson KE (1990) Biotechnology in the pulp and paper industry. Wood Sci Technol 24:79–101
2. Pandey A, Soccol CR, Nigam P, Soccol VT (2000) Biotechnological potential of agro-industrial residues. I. Sugarcane bagasse. Bioresource Technol 74:69–80
3. Bustamante P, Ramos J, Zúúiga V, Sabharwal HS, Young RA (1999) Biomechanical pulping of bagasse with the white rot fungi *Ceriporiopsis subvermispora* and *Pleurotus ostreatus*. TAPPI J 82(6):123–128
4. Akhtar M, Attridge MC, Myers G (1992) Biomechanical pulping of loblolly pine with different strains of the white-rot fungus *Ceriporiopsis subvermispora*. TAPPI J 75:105–109
5. Sabharwal HS, Akhtar M, Blanchette RA (1994) Biomechanical pulping of kenaf. TAPPI J 77:105–112
6. Breccia JD, Bettucci L, Piaggio M, Sineriz F (1997) Degradation of sugar cane bagasse by several white-rot fungi. Acta Biotechnol 17:177–184
7. Li Xin, Kondo R, Sakai K (2002) Studies on hypersaline-tolerant white-rot fungi. I. Screening of lignin-degrading fungi in hypersaline conditions. J Wood Sci 48:147–152
8. Hirai H, Kondo R, Sakai K (1994) Screening of lignin-degrading fungi and their enzyme activities during biological bleaching of kraft pulp. Mokuzai Gakkaishi 40:980–986
9. Suhara H, Kondo R, Sakai K (2000) Identification of valuable white rot fungi by 18SrDNA sequences. In: Proceedings of the 45th Lignin Symposium, Matsuyama, Japan, pp 187–188
10. Raghukumar C, D'souza TM, Thorn RG, Reddy CA (1999) Lignin-modify enzymes of *Flavodon flavus*, a basidiomycete isolated from a coastal marine environment. Appl Environ Microbiol 65:2103–2111
11. TAPPI (1987) TAPPI T9M-54 and ASTM D1104-56
12. TAPPI (1989) TAPPI T222OS-74
13. Tien M, Kirk T (1988) Lignin peroxidase of *Phanerochaete chrysosporium*. Methods Enzymol 161:238–249
14. Knapp SB, Watt RA, Wethern JD (1957) Sugarcane bagasse as a fibrous papermaking material. I. Chemical composition of Hawaiian bagasse. TAPPI J 40:595–597
15. Ortega GM, Martinez EO, González PC, Betancourt D, Otero MA (1993) Enzyme activities and substrata degradation during white rot fungi growth on sugarcane straw in a solid state fermentation. World J Microbiol Biotechnol 9:210–212
16. Prasad DY, Rajesh KS, Praburaj T, Mohan-Rao NR, Joyce TW (1996) Enzyme-assisted bleaching of bagasse. Cell Chem Technol 30:463–472
17. Gao Y, Wang S, Lin L, Chen JX (1997) Studies on bagasse lignin biodegradation by white rot fungi. Hunan Ligong Daxue Xuebao, Ziran Kexueban 24:37–43