The Journal of Microbiology, December 2007, p. 485-491
Vol. 45, No. 6

Biological Pretreatment of Softwood *Pinus densiflora* by Three White Rot Fungi

Jae-Won Lee[1], Ki-Seob Gwak[1], Jun-Yeong Park[2], Mi-Jin Park[1], Don-Ha Choi[3], Mi Kwon[4], and In-Gyu Choi[1]*

[1]*Department of Forest Sciences, College of Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Republic of Korea*
[2]*Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Republic of Korea*
[3]*Department of Wood Chemistry and Microbiology, Korea Forest Research Institute, Seoul 130-712, Republic of Korea*
[4]*Department of Forest Products, College of Forest Sciences, Kookmin University, Seoul 136-702, Republic of Korea*

(Received August 17, 2007 / Accepted November 21, 2007)

The effects of biological pretreatment on the Japanese red pine *Pinus densiflora*, was evaluated after exposure to three white rot fungi *Ceriporia lacerata*, *Stereum hirsutum*, and *Polyporus brumalis*. Change in chemical composition, structural modification, and their susceptibility to enzymatic saccharification in the degraded wood were analyzed. Of the three white rot fungi tested, *S. hirsutum* selectively degraded the lignin of this sortwood rather than the holocellulose component. After eight weeks of pretreatment with *S. hirsutum*, total weight loss was 10.7%, while lignin loss was the highest at 14.52% among the tested samples. However, holocellulose loss was lower at 7.81% compared to those of *C. lacerata* and *P. brumalis*. Extracelluar enzymes from *S. hirsutum* showed higher activity of ligninase and lower activity of cellulase than those from other white rot fungi. Thus, total weight loss and changes in chemical composition of the Japanese red pine was well correlated with the enzyme activities related with lignin- and cellulose degradation in these fungi. Based on the data obtained from analysis of physical characterization of degraded wood by X-ray Diffractometry (XRD) and pore size distribution, *S. hirsutum* was considered as an effective potential fungus for biological pretreatment. In particular, the increase of available pore size of over 120 nm in pretreated wood powder with *S. hirsutum* made enzymes accessible for further enzymatic saccharification. When Japanese red pine chips treated with *S. hirsutum* were enzymatically saccharified using commercial enzymes (Cellulclast 1.5 L and Novozyme 188), sugar yield was greatly increased (21.01%) compared to non-pretreated control samples, indicating that white rot fungus *S. hirsutum* provides an effective process in increasing sugar yield from woody biomass.

Keywords: biological pretreatment, white rot fungi, enzymatic saccharification, woody biomass, ligninase, available pore size

As energy consumption rises with the ever increasing global population, the demand on alternatives to fossil fuel resources will naturally also increase. In an attempt to address this issue, many researchers have tried to convert biomass into fuel ethanol (Cheung and Anderson, 1997; Nakamura *et al.*, 2001). In several technologically advanced countries such as the US and Japan, research and propagation policies support the promotion of alternative energy sources. Biomass has received special attention as a new source material for alternative energy. In particular, lignocellulosic material such as woody biomass is regarded as a promising energy source because it is renewable and consists of abundant carbohydrates (Sun and Cheng, 2002; Soderstrom *et al.*, 2003).

However, lignocellulosic biomass has complex structure containing mainly cellulose, hemicellulose and lignin. It is difficult to separate cellulose and hemicellulose for saccharification without the loss of carbohydrates. Therefore, for efficient enzymatic or acid saccharification from lignocellulosic biomass, pretreatment is a necessary process. Effective pretreatment is needed to reduce the lignin content and crystallinity of the cellulose, and to increase the surface area or pore size of materials. Since lignin makes the access of cellulolytic enzymes to cellulose difficult, its removal is especially necessary prior to the enzymatic hydrolysis (Sun and Cheng, 2002).

In pretreatment, various methods such as steam explosion (Nakamura *et al.*, 2001), steam explosion with dilute sulfuric acid (Yang *et al.*, 2002), organosolve extraction (Pan *et al.*, 2005), and biological treatment with white rot fungi (Ferraz *et al.*, 2000; Guerra *et al.*, 2003; Itoh *et al.*, 2003) have been extensively investigated. Recently, supercritical and subcritical water treatments have been of great interest because of their potential to induce the direct conversion of biomass resources into sugars without the need for a saccharification step (Sasaki *et al.*, 1998; Matsumura *et al.*, 2005).

Most pretreatments, except for biological pretreatment, require expensive instruments or equipments that have high energy requirements depending on the process. In particular, physical and thermochemical processes require abundant energy for biomass conversion. In the case of steam explosion, the glucose released from cellulose is degraded to 5-hydroxymethylfurfural (HMF), levulinic acid, and formic acid which are inhibitors of enzymatic saccharification and ethanol fermentation (Palmqvist *et al.*, 1999; Klinke *et al.*, 2003). Biological treatment using white rot fungi, a safe and environmently-friendly method is increasingly being ad-

* To whom correspondence should be addressed.
(Tel) 82-2-880-4785; (Fax) 82-2-873-2318
(E-mail) cingyu@snu.ac.kr

vocated as a process which does not require high energy for lignin removal from a lignocellulosic biomass in spite of long lignin degradation (Okano *et al.*, 2005). Lignin degradation by white rot fungi occurs through the action of lignin-degrading enzymes such as peroxidases and laccase. These enzymes are regulated by carbon and nitrogen sources.

In this study, the biological pretreatment for enzymatic saccharification of the Japanese red pine *Pinus densiflora* was performed using three white rot fungi; *Stereum hirsutum, Polyporus brumalis,* and *Ceriporia lacerata.* These fungi were selected because of their effective degradation of lignin and lignin-like compounds such as endocrine disruptors or chlorine containing aromatic compounds (Lee *et al.*, 2005b; Lee *et al.*, 2006; Lee *et al.*, 2007; Yeo *et al.*, 2007). The quantitative and structural changes in the components of pretreated Japanese red pine and their susceptibility to enzymatic hydrolysis were investigated.

Materials and Methods

Woody biomass and white rot fungi

Softwood Japanese red pine of 20 years in age was used as woody biomass samples. The materials were cut in to 1×1×0.5 cm by a chipper, and stored at 4°C until further processing. *Ceriporia lacerata* (KUC 8090), was provided by the Wood Microbiology and Protection Laboratory of Korea University. *Stereum hirsutum* (KFRI 234) and *Polyporus brumalis* (KFRI 20912) were obtained from the Microbiology Chemistry Laboratory of the Korea Forest Research Institute (KFRI). The fungi were maintained on PDA (Potato Dextrose Agar) media at 30°C for 7 days, and subcultured in PDB (Potato Dextrose Broth) for liquid inoculations.

Biological pretreatment using white rot fungi

The cultivation bottle containing 50 g of wood chips and 80 ml of distilled water was autoclaved at 121°C for 30 min. It was inoculated with 0.1 g of dry weight mycelium respective white rot fungi, and cultivated at 30°C for 8 weeks. Dry weight of mycelium was defined as the oven-dried weight of PDB cultivation. After incubation, mycelium attached to wood chips were removed by distilled waster, oven-dried and weighed to calculate the weight loss. The wood chips were milled to 40 mesh powder for the analysis of the chemical components and the physical properties.

Chemical properties analysis of woody biomass

The chemical analysis of woody biomass was carried out using TAPPI test methods. Insoluble and soluble lignins (Klason lignin) were determined by acid treatment. Acid soluble lignin was determined by UV-spectrometry at 205 nm. Changes in moisture, holocellulose, ash, alkali extractives, and Klason lignin contents after the biological pretreatment were analyzed using TAPPI methods (T 207 om-88, TAPPI Useful Method 249, T 211 om-93, T 212 om-93, and T 222 om-88, respectively). Comparative statistical analysis was performed by analysis of variance (ANOVA).

Physical properties analysis of woody biomass

The measurement of crystallinity of woody biomass were carried out by powder High Resolution X-ray Diffractometry (HR-XRD, Bruker D8 DISCOVER, Germany) as described by Segal *et al.* (1959). Crystallinity (%) was defined as $[(I_{002}-I_{am})/I_{002}]\times100$, where I_{002} was the crystalline peak of the maximum intensity at 2θ between 22° and 23° for cellulose I, and I_{am} was minimum intensity at 2θ between 18° and 19° for cellulose I. The condition of measurement was 40 Kv, 40 mA, and 0.75/min of scanning speed.

The specific surface area and pore size of wood powders were estimated by nitrogen gas adsorption method. Nitrogen isotherms were measured using an adsorption apparatus (Micromeritics Instrument Corporation, Tristar 3000) at cold condition. Analysis condition were as follows; analysis bath temperature was 77.350 K and sample mass was 1 g, the equilibration interval was 10 sec, warm free space and cold free space were 9.6136 and 29.2568 cm^3, respectively, and total volumes were estimated on the basis of the nitrogen gas volume adsorbed at a relative pressure of 0.99.

Enzymatic assay of white rot fungi

After incubating the three fungi in Shallow Stationary Culture (SSC) medium with wood chips as a carbon source (Kirk *et al.*, 1986), ligninase activities were measured spectrophotometrically. Manganese peroxidase activity was measured by 2,6-dimethoxyphenol (2,6-DMP; ε_{469}=27,500/M/cm) oxidation. The reaction mixture contained 50 mM sodium malonate buffer (pH 4.5), 0.1 mM 2,6-DMP, 0.5 mM $MnSO_4 \cdot H_2O$, 0.1 mM H_2O_2 and extracellular enzyme solution. The reaction was started by adding H_2O_2 and monitored via absorbance at 469 nm for 3 min (Kirk *et al.*, 1986). Laccase activity was assayed using 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS; ε_{420}=36,000/M/cm) as substrate. The reaction mixture contained 2.55 ml of 0.2 M lactate buffer (pH 4.5), 150 μl of ABTS, and 300 μl extracellular enzyme solution, and the increase in absorbance was monitored at 420 nm for 3 min (Shin and Lee, 2000).

Endo-1,4-β-glucanase activity was assayed using ostazin brilliant red-hydroxyl cellulose (Sigma Chemical Co., USA). The reaction mixture (750 μl) contained 25 μl of 2.5 mg OBR/ml and 10 μl enzyme solution in 50 mM citrate-phosphate buffer (pH 4.8). After incubation for 15 min at 40°C, the reaction was terminated by the addition of three volumes of ethanol/acetone (2:1, v/v). Release of the chromogenic product from the polymeric substrate was monitored by the increase in absorbance at 550 nm (Guedon *et al.*, 2002). Cellobiohydrogenase activity on a chromogenic substrate was measured as follows. The reaction mixture consisted of 0.4 ml of 5 mM p-nitrophenyl β-D-lactoside (Sigma Chemical Co., USA), 0.4 ml of enzyme solution and 0.8 ml of 50 mM sodium acetate buffer (pH 5.0). The mixture was incubated at 40°C for 60 min and then, 2.0 ml of 1% (w/v) sodium carbonate solution was added to the reaction mixture. The *p*-nitrophenol released from substrate was estimated colorimetrically at 420 nm (Bothwell *et al.*, 1993). β-Glucosidase activity was assayed by monitoring the release of *p*-nitrophenol from *p*-nitrophenyl-β-D-glucopyranoside (Sigma Chemical Co., USA). The enzyme solution (100 μl) was incubated for 5 min at 30°C with 1 mM *p*-nitrophenyl-β-D-glucopyranoside in 10 mM sodium acetate buffer (pH 5.0). The enzymatic reaction was terminated by the addition of 100 μl of 2 M sodium carbonate. The amount of *p*-nitro-

phenol released by β-glucosidase was determined by the same method as that of cellobiohydrolase (Kawai *et al.*, 2003). Xylanase activity was determined using RBB-xylan (Sigma Chemical Co., USA) as substrate. To measure activity, 50 μl of an RBB-xylan solution (10 mg/ml) was added to 100 μl of enzyme solution and 150 μl of 50 mM sodium acetate buffer (pH 4.5). After incubation for 30 min at 30°C, the reaction was stopped by the addition of two volumes of 95% ethanol. The samples were spun down, and the resulting supernatant containing the soluble digested xylan fraction was measured at 620 nm (Lee *et al.*, 2005a).

Enzymatic saccharification

For the comparison of sugar yield between the white rot fungi used during the pretreatment process, Celluclast 1.5 L (Novo Co., Denmark), and Novozyme 188 (Novo Co., Denmark) were used as cellulase prepared by culture filtrates of *Trichoderma reesei* and β-glucosidase, respectively.

A biologically pretreated woody biomass of 0.25 g was transferred to a 100 ml Erlenmeyer flask, and 20 ml of 50 mM sodium acetate buffer (pH 5.0) was added. Next, appropriate amounts of cellulase (80 EGU/g) and β-glucosidase (72 IU/g) were added. The flask was placed in a shaking incubator at 50°C and 150 rpm and incubated for 24, 48, and 72 h. After hydrolysis, incubation supernatants of 2 ml were centrifuged and filtered with a 0.45 μm filter, and the solution was analyzed for monosaccharides by high-performance anionic exchange chromatography (HPAEC) (Dionex Bio-LC50 system; Dionex, USA). The Carbo Pac PA10 Column (4×250 mm) and ED 50 pulsed amperometic detector (PAD) were used. Analysis was performed with 3 mM NaOH as mobile phase by 0.8 ml/min for 45 min. Arabinose, galactose, glucose, xylose, and mannose as standard monosaccharides were used.

Results and Discussion

Chemical components analysis of pretreated woody biomass

The analysis of wood components after the 8-week biological pretreatment process are presented on Table 1 and 2. Weight losses of suffered by the samples as a result of treatment with *S. hirsutum* and *P. brumalis* were 10.7% and 9.9%, respectively. Moreover, weight losses of holocellulose by *S. hirsutum* and *P. brumalis* were 7.8% and 10.6%, respectively, with *C. lacerata* inducing similar levels of holocellulose loss as of *S. hirsutum*. Low weight loss of holocellulose for *C. lacerata* and *S. hirsutum* implied that it was difficult for the fungi to degrade the holocellulose using their secreted hydrolytic enzyme system. According to statistical analysis, the lignin degradation rate had difference between each fungus. However, weight and holocellulose loss from treatment with of *C. lacerata* and *S. hirsutum* had no differences also exhibited no statistical difference ($P_{0.05}$).

The lignin degradation caused by *S. hirsutum* was higher than those induced by other fungi. Acid insoluble lignin content probably decreased due to the degradation of the lignin to low molecular phenolic compounds by ligninases of the white rot fungi, while acid soluble lignin slightly increased across treatment with all fungi (Table 2). According to the results of weight loss of lignin and holocellulose, only *S. hirsutum* selectively degraded lignin rather than holocellulose. This result was also confirmed by the contents of alkali extractives. Contents of alkali extractives increased from 15.74% for the control sample to 18.85%, 16.52%, and 17.16% after the cultivation of *S. hirsutum*, *P. brumalis*, and *C. lacerata*, respectively, implying that low molecular weight carbohydrates and lignin degradation products were produced by the extracellular enzymes of the these fungi during cultivation. Based on statistical comparative analysis, the chemical components were statistically significantly different compared to the control ($P_{0.05}$). Conversely, no statistical significance was notice between the control and the three fungi in acid soluble lignin and ash contents.

S. hirsutum used in this study had lower loss of holocellulose and higher loss of lignin than those of other reported white rot fungi for softwoods. *Ceriporiopsis subvermispora* grown on *Pinus radiata* simultaneously degraded both cellulose and lignin. After solid-state incubation for 45 days, lignin and holocellulose were degraded by 20 and 15%, respectively (Ferraz *et al.*, 2001; Machuca and Ferraz, 2001;

Table 1. Weight loss and changes in chemical components of Japanese red pine after biological pretreatment with white rot fungi

White rot fungi	Weight loss (%)	Lignin loss (%)	Holocellulose loss (%)
C. lacerata	9.5±0.5	13.1±0.4	8.0±0.5
P. brumalis	9.9±0.4	11.6±0.3	10.6±0.3
S. hirsutum	10.7±0.7	14.5±0.4	7.8±0.3

Values are expressed as percent decrease based on the oven-dried weight of original wood

Table 2. Change in the chemical components after biological pretreatment with white rot fungi

Biological pretreatment	Acid soluble lignin[a]	Alkali extractive (%)[a]	Ash (%)[a]
Control	0.68	15.74	0.45
C. lacerata	0.95	17.16	0.32
P. brumalis	0.91	16.52	0.32
S. hirsutum	0.95	18.85	0.33

Values are expressed as percent decrease based on the oven-dried weight of original wood
[a] The averages from triplicate experiments with each condition

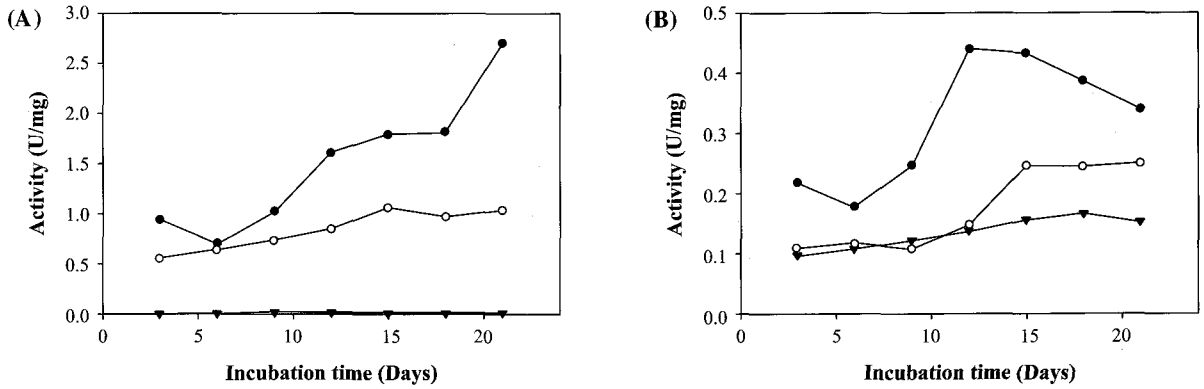

Fig. 1. Change in the ligninase activities of white rot fungi during cultivation on Japanese red pine wood powder as a carbon source. (A) laccase, (B) manganese peroxidase, (•) *S. hirsutum*, (○) *P. brumalis*, (▼) *C. lacerata*.

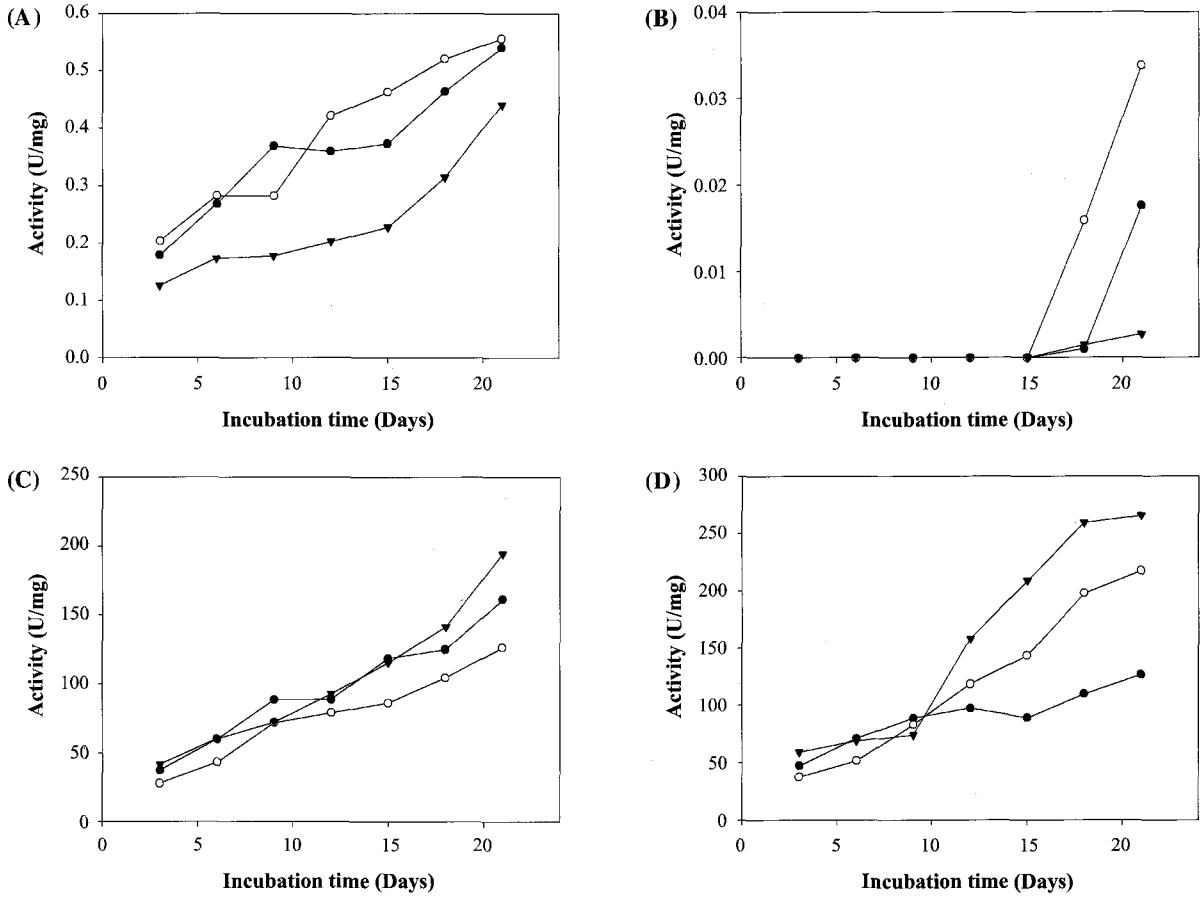

Fig. 2. Change in the cellulolytic enzyme activities of white rot fungi during cultivation on Japanese red pine wood powder as a carbon source. (•) *S. hirsutum*, (○) *P. brumalis*, (▼) *C. lacerata*, (A) β-glucosidase, (B) cellobiohydrolase, (C) endoglucanase, (D) xylanae. Values shown are the averages from triplicate experiments with each condition.

Itoh *et al.*, 2003).

For the enzymatic saccharification, fungi should be selected based on their ability to degrade lignin with minimal effects on cellulose. As the results indicate, *S. hirsutum* was considered as a more suitable fungus for the biological pretreatment process compared with *P. brumalis* and *C. lacerata*.

Enzymatic assay of white rot fungi

The highest laccase and manganese peroxidase activities were observed in *S. hirsutum* throughout the incubation period (Fig. 1A and B). However, laccase activity was not detected in *C. lacerata* as predicted based on previous results which found that *C. lacerata* did not secrete laccase and lignin peroxidase on the liquid culture (Ruttimann *et al.*, 1993;

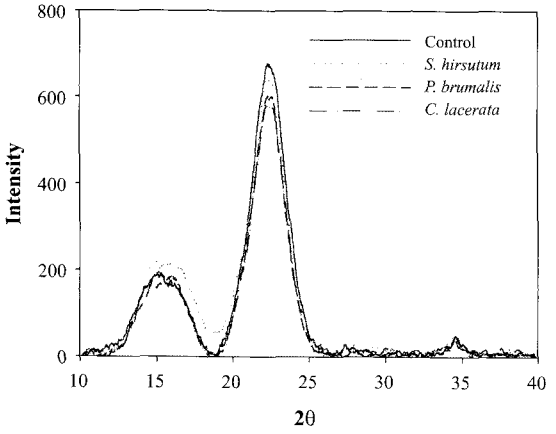

Fig. 3. Change in the crystallinty value of lignocellulosic biomass by biological pretreatment.

Srebotnik *et al.*, 1994). As previously reported (Lee *et al.*, 2005b, 2006), *P. brumalis* also shows both laccase and peroxidase activities but not as much as *S. hirsutum*. Therefore, highest ligninase activity in *S. hirsutum* (Fig. 1) may contribute the most significant loss of lignin (Table 1).

The activities of various cellulolytic enzymes produced by the three white rot fungi during cultivation are shown in Fig. 2. The activities of β-glucosidase and endoglucanase gradually increased with incubation time (Fig. 2A and C), consistent with the results of chemical changes shown Table 1. The activities of endoglucanase and xylanase, which randomly degraded the main chain of cellulose and hemicellulose, were significantly higher than β-glucosidase and cellobiohydrolase (Fig. 2). On the other hand, cellobiohydrolase was either very low or not detected until 15 days of incubation in all three fungi. At 20 days of incubation, cellobiohydrolase activity was slightly detected in *P. brumalis* and *S. hirsutum* but still very negligible in *C. lacerata* (Fig. 2B). It has been previously reported that cellobiohydrolase was not detected in a shaking culture of *C. lacerata* (Sethuraman *et al.*, 1998). However, the xylanase activity was 2-fold higher in *C. lacerata* than in *S. hirsutum* (Fig. 2D). *C. lacerata* has been known to be the fungus which produces xylanase with a higher activity than any other enzymes under the same condition (Guerra *et al.*, 2003). Although *C. lacerata* possessed highest activities of xylanase and endoglucanase (Fig. 2), the fungus was not effectively able to cause holocellulose degradation (Table 1). Instead, *P. brumalis* exhibited the highest activities of β-glucosidase and cellobiohydrolase with a fair level of endoglucanase and xylanase activities, resulting most significant holocellulose loss compared to others (Table 1 and Fig. 2).

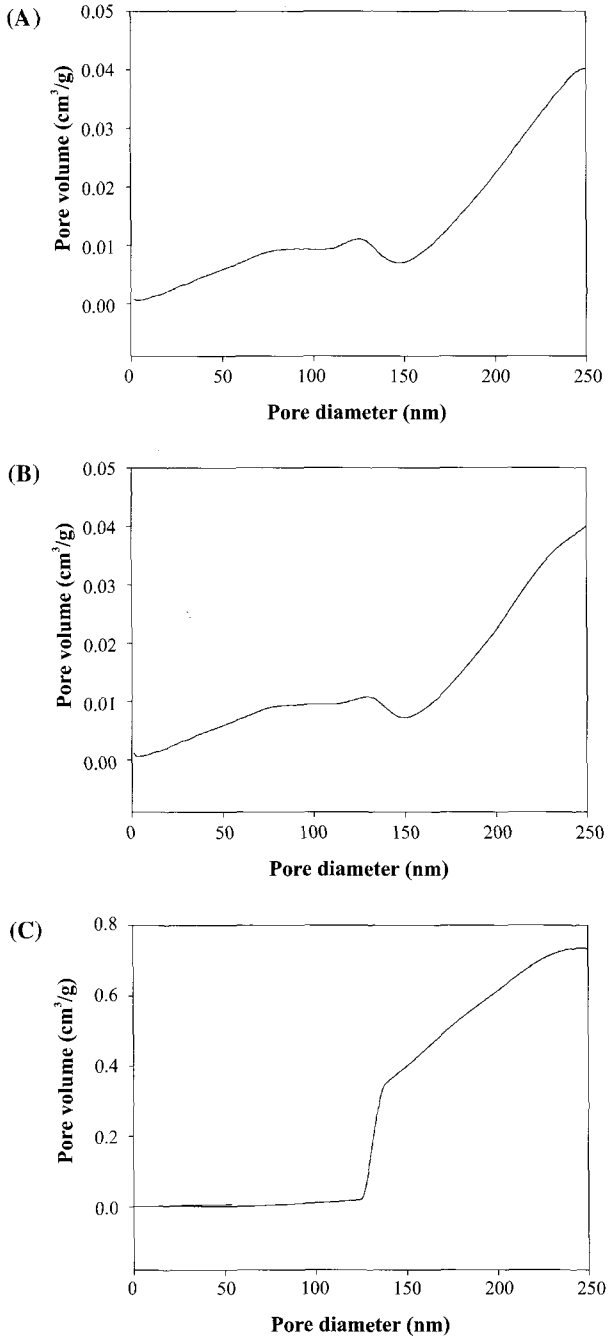

Fig. 4. Pore size distribution for non-treated and pretreated wood powders. (A) non-treated wood powder, (B) pretreated wood powder with *P. brumalis*, (C) pretreated wood powder with *S. hirsutum*.

Physical properties of pretreated woody biomass

The structures of non-treated and pretreated wood powders were examined by X-ray Diffractometry (XRD). The crystallinity value of non-treated wood was 68.4%, while it ranged between 64~65.9% in the pretreated wood powder (Fig. 3). Therefore, biological pretreatment by three white rot fungi did not show a dramatic effect on crystallinity (Fig. 3). Crystallinity has previously been shown to be affected by various cellulases, especially cellobiohydroase (Woodward, 1991). Low cellobiohydrolase activity of the three white rot fungi used in this study (Fig. 2B) may be the reason for the minimum decrease of crystallinity (Fig. 3).

In general, pore size and surface area are important to adsorption and accessibility of enzymes. Lee and Fan (1982) suggested that protein adsorption correlates positively with the available surface area of substrate. The pore size distribution for non-treated and pretreated wood powders by white rot fungi is shown in Fig. 4. Nitrogen gas adsorption

Table 3. Monosaccharides and total sugar yields of non-treated and pretreated woody biomasses by commercial enzymes

Carbon source	Time (h)	Monomeric sugars (%)					Sugar yield (%)
		Arabinose	Galactose	Glucose	Xylose	Mannose	
CON	24	0.20	0.08	1.68	0.29	0.14	2.38
CON	48	0.87	0.44	6.70	1.18	1.15	10.34
CON	72	1.11	0.62	8.83	2.11	1.93	14.60
STH	24	0.38	0.13	7.67	1.48	0.42	10.07
STH	48	0.77	0.27	11.83	2.68	1.71	17.26
STH	72	1.01	0.41	13.56	3.26	2.77	21.01
POB	24	0.45	0.14	6.23	1.01	0.33	8.16
POB	48	0.71	0.26	8.47	1.61	1.15	12.21
POB	72	0.95	0.43	9.68	1.96	1.90	14.91
CEL	24	0.46	0.18	8.94	1.44	0.65	11.67
CEL	48	0.53	0.25	9.91	1.70	1.22	13.61
CEL	72	0.56	0.28	10.78	1.82	1.56	15.03

Enzymatic saccharification was performed for 24, 48, and 72 h at 50°C using commercial enzymes [% from dry weight of biomass, CON, non-treated Japanese red pine (JRP); STH, JRP pretreated by *S. hirsutum*; POB, JRP pretreated by *P. brumalis*; CEL, JRP pretreated by *C. lacerata*]. Values shown are the averages from triplicate experiments with each condition.

and desorption values were 1.044 and 1.1948 m^2/g, respectively. Similar values of adsorption and desorption are indicators in the reliability of experimental data obtained in this study. The results showed a different distribution curve compared with the pretreated wood powder by acid (Thompson and Chen, 1992). The size of cellulose, with a spherical diameter, was reported to be 24~74 nm (Cowling and Kirk, 1976). An available pore size of over 120 nm for enzyme access sharply increased in the pretreated wood powder with *S. hirsutum* than that of non-treated wood powder. The result implied that efficiency of saccharification may be promoted by increase of available pore size and surface area as predicted based on suggestions by Stone and Scallan (1968). Stone and Scallan (1968) indicated that enzymatic hydrolysis and available surface area were closely connected. Therefore, the increase of available pore size in degraded wood by *P. brumalis* and *S.hirsutum* may be caused by the degradation of lignin and a small portion of hemicellulose by the secreted enzymes from these fungi.

Sugar yield

Yield of glucose and other monosaccharides were assayed after enzymatic saccharification with commercial enzymes (Celluclase 1.5 L and Novozyme 188) (Table 3). The saccharification of pretreated wood gave rise to different yields of glucose and monosaccharides compared to the untreated control. The highest sugar yield (21.01%) was obtained when saccharification was performed with pretreated woody biomass by *S. hirsutum* for 72 h at 50°C. On the other hand, when pretreated woody biomass by *P. brumalis* and *C. lacerata* were used at enzymatic saccharification, sugar yield were 14.91 and 15.03% under the same condition, respectively. Glucose was most abundantly generated in all the cases. Yield of xylose and mannose which are derived from glucomannan and arabinoglucuronoxylan as main component of hemicellulose of coniferous woody biomass. The high yield of obtained xylose and mannose from pretreated woody biomass by enzymatic hydrolysis indicated that hemicelluloses were converted to easily degradable structure by white rot fungi. In particular, *S. hirsutum* was a suitable fungus for biological pretreatment.

Acknowledgments

We are grateful for the graduate fellowship provided by the Ministry of Education through the Brain Korea 21 Project. This work was also supported by Korea Forest Research Institute.

References

Bothwell, M.K., L.P. Walker, K.B. Wilson, D.C. Irwin, and M. Price. 1993. Synergism between pure *Thermomonospora fusca* and *Trichoderma reesei* cellulases. *Biomass Bioenergy* 4, 293-299.

Cheung, S.W. and B.C. Anderson. 1997. Laboratory investigation of ethanol production from municipal primary waste water solids. *Bioresour. Technol.* 59, 81-96.

Cowling, E.B. and T.K. Kirk. 1976. Properties of cellulose and lignocellulosic materials as substrates for enzymatic conversion processes. *Biotechnol. Bioeng. Symp.* 6, 95-123.

Ferraz, A., A.M. Cordova, and A. Machuca. 2003. Wood biodegradation and enzyme production by *Ceriporiopsis subvermispora* during solid-state fermentation of *Eucalyptus grandis*. *Enzyme Microb. Technol.* 32, 59-65.

Ferraz, A., R. Mendonca, and F.T. Silva. 2000. Organosolv delignification of white-and brown-rotted *Eucalyptus grandis* hardwood. *J. Chem. Technol. Biotechnol.* 75, 18-24.

Ferraz, A., J. Rodriguez, J. Freer, and J. Baeza. 2001. Biodegradation of *Pinus radiata* soft wood by white- and brown-rot fungi. *World J. Microbiol. Biotechnol.* 17, 31-34.

Guedon, E., M. Desvaux, and H. Petitdemange. 2002. Improvement of cellulolytic properties of *Clostridium cellulolyticum* by metabolic engineering. *Appl. Environ. Microbiol.* 68, 53-58.

Guerra, A., R. Mendonca, and A. Ferraz. 2003. Molecular weight

distribution of wood components extracted from *Pinus taeda* biotreated by *Ceriporiopsis subvermispora*. *Enzyme Microb. Technol.* 33, 12-18.

Itoh, H., M. Wada, Y. Honda, M. Kuwahara, and T. Watanabe. 2003. Bioorganosolve pretreatments for simultaneous saccharification and fermentation of beech wood by ethanolysis and white rot fungi. *J. Biotechnol.* 103, 273-280.

Kawai, R., M. Yoshida, T. Tani, K. Igarashi, T. Ohira, H. Nagasawa, and M. Samejima. 2003. Production and characterization of recombinant *Phanerochaete chrysosporium* β-glucosidase in the mthylotrophic yeast *Picha pastoris*. *Biosci. Biotechnol. Biochem.* 67, 1-7.

Kirk, T.K., S. Croan, M. Tien, K.E. Murtagh, and R.L. Farrell. 1986. Production of multiple ligninases by *Phanerochaete chrysosporium*: effect of selected growth condition and use of a mutant strain. *Enzyme Microb. Technol.* 8, 27-32.

Klinke, H.B., L. Olssomn, A.B. Thomsen, and B.K. Ahring. 2003. Potential inhibitors from wet oxidation of wheat straw and their effect on ethanol production of *Saccharomyces cerevisiae*: wet oxidation and fermentation by yeast. *Biotechnol. Bioeng.* 81, 738-747.

Lee, C.C., D.W.S. Wong, and G.H. Robertson. 2005a. Cloning and characterization of the Xyn11A gene from *Lentinula edodes*. *Protein J.* 24, 21-26.

Lee, S.M., B.W. Koo, J.W. Choi, D.H. Choi, B.S An, E.B. Jeung, and I.G. Choi. 2005b. Degradation of bisphenol A by white rot fungi, *Stereum hirsutum* and *Heterobasidium insulare*, and reduction of its estrogenic activity. *Bio. Pharm. Bull.* 28, 201-207.

Lee, J.W., S.M. Lee, E.J. Hong, E.B. Jeung, H.Y. Kang, M.K. Kim, and I.G. Choi. 2006. Estrogenic reduction of styrene monomer degraded by *Phanerochaete chrysosporium* KFRI 20724. *J. Microbiol.* 44, 177-184.

Lee, S.M., J.W. Lee, B.W. Koo, M.K. Kim, D.H. Choi, and I.G. Choi. 2007. Dibutyl phthalate biodegradation by the white rot fungus, *Polyporus brumalis*. *Biotechnol. Bioeng.* 97, 1516-1522.

Lee, Y.H. and L. Fan. 1982. Kinetic studies of enzymatic hydrolysis of insoluble cellulose: analysis of the initial rates. *Biotechnol. Bioeng.* 24, 2383-2406.

Machuca, A. and A. Ferraz. 2001. Hydrolytic and oxidative enzymes produced by white- and brown-rot fungi during *Eucalyptus grandis* decay in solid medium. *Enzyme Microb. Technol.* 29, 386-391.

Matsumura, Y., M. Sasaki, K. Okuda, S. Takami, S. Ohara, M. Umetsu, and T. Adschiri. 2005. Supercritical water treatment of biomass for energy and material recovery. *Combust. Sci. Tech.* 178, 509-536.

Nakamura, Y., T. Sawada, and E. Inoue. 2001. Enhanced ethanol production from enzymatic treated steam exploded rice straw using extractive fermentation. *J. Chem. Tech. Biotechnol.* 76, 879-884.

Okano, K., M. Kitagaw, Y. Sasaki, and T. Watanabe. 2005. Conversion of Japanese red ceder (*Cryptomeria japonica*) into a feed for ruminants by white-rot basidiomycetes. *Animal Feed Sci. Technol.* 120, 235-243.

Palmqvist, E., H. Grage, N.Q. Meinander, and B. Hahn-Hagderdal. 1999. Main and interaction effects of acetic acid, furfural, and p-hydroxybenzoic acid on growth and ethanol productivity of yeasts. *Biotechnol. Bioeng.* 63, 46-55.

Pan, X., C. Arato, N. Gilkes, D. Gregg, W. Mabee, K. Pye, Z. Xiao, X. Zhang, and J. Saddler. 2005. Biorefining of softwoods using ethanol olganosolve pulping: reliminary evaluation of process streams for manufacture of fuel-grade ethanol and co-products. *Biotechnol. Bioeng.* 90, 473-481.

Ruttimann, J.C., L. Salas, R. Vicuna, and T.K. Kirk. 1993. Extracellular enzyme-production and synthetic lignin mineralization by *Ceriporiopsis subvermispora*. *Appl. Environ. Microbiol.* 59, 1792-1797.

Sasaki, M., B. Kabyemela, R. Malaluan, S. Hirose, N. Takeda, T. Adschiri, and K. Arai. 1998. Cellulose hydrolysis in subcritical and supercritical water. *J. Supercrit. Fluids* 13, 261-268.

Segal, L., J.J. Creely, A.E. Martin, Jr, and C.M. Conrad. 1959. An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractomerter. *Text. Res. J.* 786-794.

Sethuraman, A., D.E. Akin, and K.E.L. Eriksson. 1998. Plant-cell-wall-degrading enzymes produced by the white-rot fungus *Ceriporiopsis subvermispora*. *Biotechnol. Appl. Biochem.* 27, 37-47.

Shin, K.S. and Y.J. Lee. 2000. Purification and characterization of a new member of the laccase family from the white-rot basidiomycete *Coriolus hirsutus*. *Arch. Biochem. Biophys.* 384, 109-115.

Soderstrom, J., L. Pilcher, M. Galbe, and G. Zacchi. 2003. Two-step steam pretreatment of softwood by dilute H_2SO_4 impregnation for ethanol production. *Biomass Bioenergy* 24, 475-486.

Srebotnik, E., K.A. Jensen, and K.E. Hammel. 1994. Fungal degradation of recalcitrant nonphenolic lignin structures without lignin peroxidase. *Proc. Natl. Acad. Sci. USA* 91, 4222-4231.

Stone, J.E. and A.M. Scallen. 1968. A structure model for the cell wall of water-swollen wood pulp fibers based on their accessibility to macromolecules. *Cellulose Chem. Technol.* 2, 343-358.

Sun, Y. and J. Cheng. 2002. Hydrolysis of lignocellulosic materials for ethanol production: review. *Bioresour. Technol.* 83, 1-11.

Thompson, D.N. and H.C. Chen. 1992. Comparison of pretreatment methods on the basic of available surface area. *Bioresour. Technol.* 39, 155-163.

Woodward, J. 1991. Synergism in cellulose systems. *Bioresour. Technol.* 36, 67-75.

Yang, B., A. Boussaid, S.D. Mansfield, D.J. Gregg, and J.N. Saddler. 2002. Fast and efficient alkaline peroxide treatment to enhance the enzymatic digestibility of stream-exploded softwood substrate. *Biotechnol. Bioeng.* 76, 678-684.

Yeo, S., N. Park, H.G. Song, and H.T. Choi. 2007. Generation of a transformant showing higher manganese peroxidase (Mnp) activity by overexpression of Mnp Gene in *Trametes versicolor*. *J. Microbiol.* 45, 213-218.