Medium-Temperature Pyrolysis of Corn Stover Improved by Biopretreatment with White-rot Fungi

Xuewei Yang,[a] Dhrubojyoti Dey Laskar,[b] Fuying Ma,[a] Xiaoyu Zhang,[a]* and Shulin Chen [b]*

This work investigated the ability of biopretreatment with different white-rot fungi to improve the medium-temperature pyrolysis of biomass. It was found that biopretreatment can significantly increase the production of phenols and glucopyranoside up to 2.82 and 2.94 fold, respectively. Biopretreatment can also decrease the content of carbon dioxide, propanol, and propanone, making the pyrolysis more efficient and product-oriented. Moreover, distinct bio-deconstruction mechanisms can result in different pyrolysis products. By deconstructing cellulose and modifying lignin with a minimum of demethoxyation, white-rot fungus *Irpex lacteus* CD2 can improve the production of acetaldehyde (up to 6.72%) and methoxyl substitutes such as dimethoxyphenyl (up to 21.59 folds). By decomposing carbohydrates, carbonxyl, and methoxyl groups, white-rot fungi *Pleurotus ostreatus* BP2 and *Echinodontium taxodii* 2538 can increase the production of D-allose (up to 3.09%) and formic acid (up to 6.98%), while decreasing the methoxyl substitutes such as 2-methoxy-4-vinylphenol (up to 70.08%).

Keywords: Bio-pretreatment; Biomass; White-rot fungi; Medium-temperature pyrolysis; Py-GC/MS; Solid state ^{13}C CP/MAS NMR

Contact information: a: Key Laboratory of Molecular Biophysics of MOE, Huazhong University of Science & Technology, Luoyu Road 1037, Wuhan, P.R. China; b: Biological Systems Engineering Department, Washington State University, WA 99164, USA;
** Corresponding author: paperimerhust@gmail.com*

INTRODUCTION

Recently, the serious problems of exhaustion of fossil energy resources and environment pollution have attracted great world-wide attention. Biomass, such as agriculture and forestry residues, due to its abundant availability and low price, is known as one of the most promising renewable energy sources (Liu *et al.* 2011). Higher-order structures in plants can contribute to biomass recalcitrance (Himmel *et al.* 2007), which makes biomass difficult to be converted as bioenergy. Thus pretreatment has been widely used in the technology for the biofuels industry (Alvira *et al.* 2010a).

Pyrolysis is considered a promising technology to convert biomass into valuable products and biofuels, such as solid products (charcoal), liquid products (wood tar, tar, oil, pyrolytic oil), and gaseous products (wood gas, pyrolytic gas) (Demiral and Ayan 2010). Due to the complex structure of lignocelluloses, it makes sense to use a pretreatment process before the pyrolysis of biomass (Ray *et al.* 2010). Pretreatment (Yu *et al.* 2009a) can deconstruct the biomass (Yang *et al.* 2010a), release the cellulose and hemicellulose, decrease the required reaction temperature, as well as increase the yields of products such as aromatic compounds and furans. Recently, four main types of pretreatment methods have been used before biomass pyrolysis, including physical

pretreatment such as chipping, pelletizing, pulverizing, and grinding (Zwart *et al.* 2006), chemical pretreatment such as the application of dilute acids (Jackson de Moraes Rocha *et al.* 2010), physical combined with chemical pretreatment (Yu *et al.* 2009c) such as steam explosion (Alvira *et al.* 2010b), and biological pretreatment (Chen *et al.* 2010; Hall *et al.* 2010; Ray *et al.* 2010; Yu *et al.* 2009a).

Although biological pretreatment needs heating (energy) and isolation (steam sterilization) to avoid exterior contamination and vice-versa, the energy consummation is still lower compared with physical pretreatment. However, biological pretreatment requires a long processing time. Compared with physical and chemical pretreatment, biological pretreatment requires less energy consumption and chemical addition, demands lower operating temperature, and provides more benefits with respect to environmental protection (Alvira *et al.* 2010b). Due to its great advantages for the sustainable development of bioenergy, biological pretreatment is attracting increasing interest (Alvira *et al.* 2010b). White-rot fungi (Hatakka 1994) are considered the most promising type of biopretreating microorganism, due to their great ability for the bio-deconstruction of lignocellulosic materials. Biological pretreatment with white-rot fungi can make the biofuel production more efficient with lower cost (Taniguchi *et al.* 2005). However, recently there has been little research dealing the mechanism and utilization of biopretreatment for biomass pyrolysis (Wu *et al.* 2011; Yang *et al.* 2010b). Also, little research has been carried out concerning the mechanism by which biopretreatment and biodeconstruction influence the pyrolysis products when using different white-rot fungi.

This study explored the mechanism of how the structure decomposition by different white-rot fungi influences the pyrolysis products at medium-temperature. The mechanisms of biopretreatment and their effect on the pyrolysis products were also investigated in the study, by using Py-GC/MS and Solid state ^{13}C CP/MAS NMR.

EXPERIMENTAL

Fungal Strain and Cultivation of Fungi

The white-rot fungi *Pleurotus ostreatus* BP2, *Echinodontium taxodii* 2538, and *Irpex lacteus* CD2 were obtained from Shennongjia Scenic Area in Hubei of China. The organisms were isolated by the authors in Key Laboratory of Molecular Biophysics of MOE, Huazhong University of Science & Technology. The organisms were maintained on potato extract agar slant cultures at 5 ^{o}C. Two discs cut from actively growing cultures on potato extract agar plates were used to inoculate 100 mL of 20% potato extract and 2% glucose medium (pH 5.5) in a 250 mL round flask incubated at 25 ^{o}C for 3 to 5 days on a reciprocal shaker. The contents of the flask were gently homogenized, and 10 mL was used to inoculate the second generation grown culture for 3 days under the same conditions.

Biopretreatment with Different White-rot Fungi for Corn Stover

Ten grams (dry mass) of chopped corn stover (40-50 mm long) collected from Henan in China was taken in a 500 mL Erlenmeyer flask, moistened with 25 mL of distilled water, autoclaved (150 kPa, 1 h), and inoculated with 10 mL of secondary generation homogenized seed culture of three different species of white-rot fungi (*P. ostreatus* BP2, *E. taxodii* 2538 and *I. lacteus* CD2). The concentration of these three fungi (*P. ostreatus* BP2, *E. taxodii* 2538, and *I. lacteus* CD2) inoculated in the corn

stover was 10 mL secondary generation seed culture for 10 g of corn stover (dry mass). The corn stover were pretreated with white-rot fungi at 28 ^{o}C. After 30 days of cultivation, the biopretreated corn stover was dried to remove water under vacuum at 60 ^{o}C. The cultivation time of 30 days was chosen based on the research about the biodegradation of white-rot fungi (Zhang *et al.* 2007b). Research showed that decomposition by white-rot fungi for 30 days can greatly degrade the structure of lignocellulose, which is important for the biomass biopretreatment (Yu *et al.* 2009b). After 30 days of biopretreatment, the weight loss of biomass was 15 to 20%, and the main components degraded in the biomass was the hemicellulose (Yang *et al.* 2010b). However, the lignin structure also was seriously decomposed during the biopretreatment (Xu *et al.* 2010), which could remove the biomass recalcitrance to improve the biomass conversion for producing biofuels (Zhang *et al.* 2007a).

Solid State ^{13}C CP/MAS NMR Analysis of Biopretreated Corn Stover

The carbon-13 cross-polarization (CP) magic angle spinning (MAS) (^{13}C CP/MAS) solid-state nuclear magnetic resonance (NMR) was carried out to address the compositional and chemical changes in the structure of fungi pretreated corn stover biomass without further degradation and/or isolation of its individual components. The finely ball milled tissues (250 mg) of the control and corresponding white-rot fungi biopretreated corn stover biomass tissues were homogeneously mixed with 100 μL of 3-(trimethylsilyl) propionic-2,2,3,4-*d4* acid (TSP) (20 mg/mL) individually and freeze dried. The resulting samples were individually packed in 5-mm pencil type rotors, and the spectra were recorded under identical acquisition parameters. The solid state ^{13}C CP/MAS analysis were carried out at 100 MHz with a Bruker Avance 400 spectrometer (NMR center, Washington State University), equipped with a Chemagnetics double resonance probe. A contact time of 0.5 ms, proton field *ca*. 40 kHz during CP and data acquisition, relaxation delay of 4 s, and spinning speed of 5 kHz were applied to obtain the ^{13}C CP/MAS spectra. All the corresponding ^{13}C CP/MAS spectra were derived from 17,500 scans, with the chemical shifts given in δ ppm. The integrals for each resonance and/or chemical shift values arising from the cell wall components of corn stover biomass tissues were normalized with reference to the internal standard for semi-quantitative analysis.

Py-GC/MS Analysis of Biopretreated Corn Stover

Pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS) is a method of chemical analysis in which the sample is heated to decomposition to produce smaller molecules that are separated by gas chromatography and detected using mass spectrometry. The samples had been dried under vacuum at 60 ^{o}C for 48 h. The pyrolysis temperature zone was from 25 °C to 410 °C. Prior to thermogravimetric experiments, samples were ground to small chips through a 0.15 x 0.2 mm screen. Around 5 mg of samples were pyrolyzed. The composition contents of the samples are shown below (Yang *et al.* 2010b): (1) 5 mg of original corn stover sample contained 11.09% lignin, 44.17% cellulose, and 25.48% hemicellulose; (2) 5 mg of sample pretreated by *P. ostreatus* BP2 contained 11.98% lignin, 43.48% cellulose, and 16.57% hemicellulose; (3) 5 mg of sample pretreated by *E. taxodii* 2538 contained 13.48% lignin, 44.44% cellulose, and 19.27% hemicellulose; (4) 5 mg of sample pretreated by *I. lacteus* CD2 contained 13.13% lignin, 46.93% cellulose, and 17.04% hemicellulose.

With the application of Py-GC/MS, researchers can study pyrolysis and pyrolysis products in detail (Azeez *et al.* 2010). The pyrolysis process was performed with a CDS 5000 pyrolysis autosampler attached to a Thermo Trace GC 6890N/MSD 5975B gas chromatography/mass spectrometry system (Agilent Technologies, Inc., Bellevue, WA, USA). The samples were first pretreated at 210 °C for 3 min, and then pyrolyzed at a temperature of 410 °C for 1 min. There was only one injection in the GC. After the pyrolysis process, the volatile products were injected into the GC/MS. The volatile products were separated on a 30 m×0.25 μm inner diameter (5% phenyl)-methylpoly-siloxane non-polar column with helium as carrier gas (17.3 mL/min). The pyrolysis interface was kept at 210 °C, and the GC/MS interface was kept at 280 °C. The GC/MS device was programmed for a temperature rise from 40 °C (1 min) to 280 °C (15 min) at a rate of 6 °C /min. The mass spectrometer was operated in EI mode (70 eV) at a source temperature of 230 °C. The eluted pyrolysis products were identified by their EI mass spectra using the NIST MS Search 107 2.0 electronic libraries.

RESULTS AND DISCUSSION

Structure Decomposition Analysis of Biopretreated Corn Stover

Previous research by the authors showed that biopretreatment is seldom able to decrease the content of lignin and cellulose from the corn stover; however, biopretreatment can greatly influence the thermogravimetric characteristics of the corn stover (Yang *et al.* 2010b). Thus, the present work further explores the structural decomposition mechanism of biopretreatment with solid state ^{13}C CP/MAS NMR. Solid-state NMR analysis deals with the characteristic resonance chemical shift values corresponding to individual cell wall components of biomass, such as cellulose, hemicellulose, and lignin. Herein, results of a solid-state NMR study of control and corresponding fungal-treated (*P. ostreatus* BP2, *E. taxodii* 2538, and *I. lacteus* CD2) corn stover biomass tissues using the conventional CP/MAS (cross polarization-magic angle spinning) method were obtained. The resulting normalized ^{13}C CP/MAS NMR spectra are depicted in Fig. 1 (Almendros *et al.* 1992; Gilardi *et al.* 1995; Sun *et al.* 2005; Zimbardi *et al.* 1999). As can be seen, all of the acquired spectra were comprised mainly of chemical resonance values analogous to the carbohydrate region (60 to 100 ppm) and aromatic region (100 to 162 ppm). In addition, the chemical shift resonances of carbonyl and carboxyl groups (162 to 200 ppm), methoxyl group (52 to 55 ppm), and carbon in etherified and/or non-etherified forms (132 to 152 ppm) were also observed. Direct comparison of the ^{13}C CP/MAS NMR spectra of the fungal-pretreated samples with the control revealed significant differences in the relative intensities of chemical resonances predominantly in the lignin-derived region (Fig. 1).

The ^{13}C CP/MAS NMR analysis also showed reduced intensity in the ascribed carbonyl and carboxyl group resonances (160 to 200 ppm) for the fungal-pretreated corn stover samples. This gave strong evidence for the removal of hemicelluloses and side chain alterations in lignin macromolecular assembly that resulted from cell wall deconstruction during the fungi pretreatment process. In addition, the slight decrease in the intensity at chemical shift values of 80 to 90 ppm in the fungal-pretreated samples (most prominent in *I. lacteus* CD2) in comparison to the control were indicative of a partial breakdown of β(1– 4) linkages in cellulose structure, thereby accounting for a lesser extent of cellulose degradation as compared to lignin and hemicellulose.

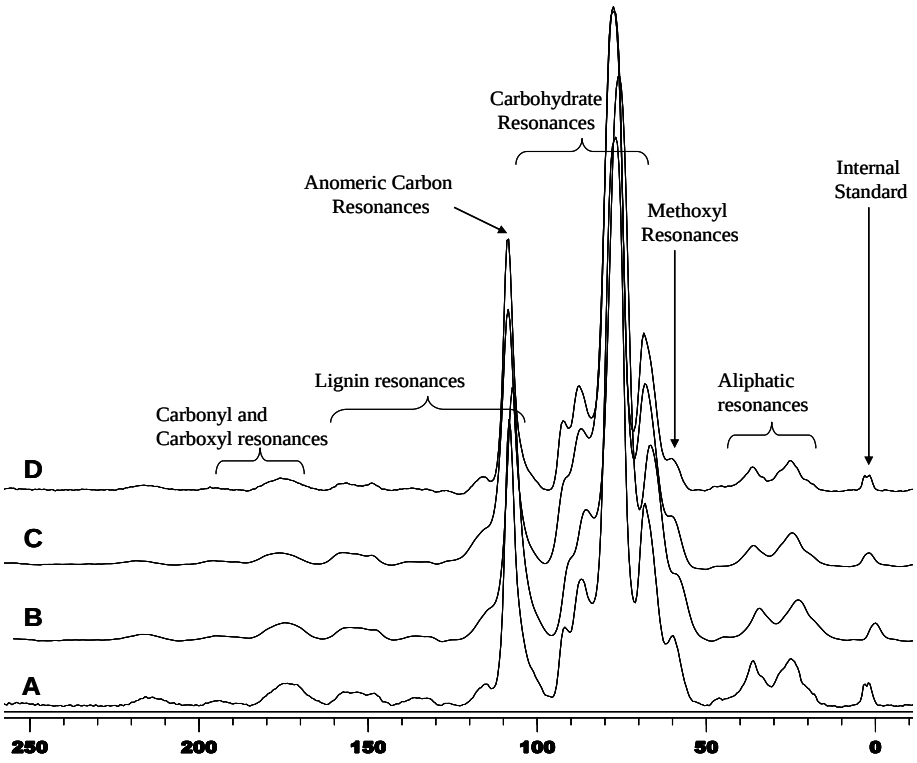

Fig. 1. Solid state ^{13}C CPMAS NMR analysis of corn stover samples; (A) control corn stover, (B) corn stover biopretreated with *I. lacteus* CD2 for 30 days, (C) corn stover biopretreated with *E. taxodii* 2538 for 30 days, and (D) corn stover biopretreated with *P. ostreatus* BP2 for 30 days

The quantification of resonances with reference to the internal standard (TSP) (0-2 ppm) of the ^{13}C CP/MAS NMR spectra of control and fungi treated samples were also carried out under normalized condition to determine the contents (in %) of cell wall components in the corresponding samples (Table 1). It is clear from Table 1 that the carbohydrate/aromatic ratio was increased for the fungal-pretreated sample, which can be attributed to the decrease of aromatic content, demonstrated for lignin deconstruction in comparison to its control.

Table 1. Semi-quantitative Contents Analysis of Cell Wall Components in Control and Fungal-treated Corn Stover by ^{13}C CPMAS NMR Analysis

Sample	Carbohydrate content (%)	Aromatic content (%)	Methoxyl group (%)	Carbonyl and Carboxyl group (%)	Carbohydrate/Aromatic ratio
Control[a]	65.87	16.88	3.23	2.68	3.90
E. taxodii 2538[b]	64.08	14.37	2.76	1.46	4.46
P. ostreatus BP2[c]	64.39	13.98	2.45	1.81	4.60
I. lacteus CD2[d]	63.21	15.62	2.98	2.36	4.05

Moreover, bio-deconstruction by different white-rot fungi was able to decrease the methoxyl group content compared with the control sample, which indicated that

biopretreatment can remove the methoxyl groups from biomass. Compared with *I. lacteus* CD2, the other two white-rot fungi showed greater ability for deconstructing aromatic, methoxyl, and carbonyl groups.

Py-GC/MS Analysis of Biopretreated Corn Stover at Medium-Temperature

Comparison study between original and biopretreated corn stover

Significant differences were found in the 410 °C pyrolysis results between the biopretreated and original corn stover, as shown in Fig. 2 and Table 2.

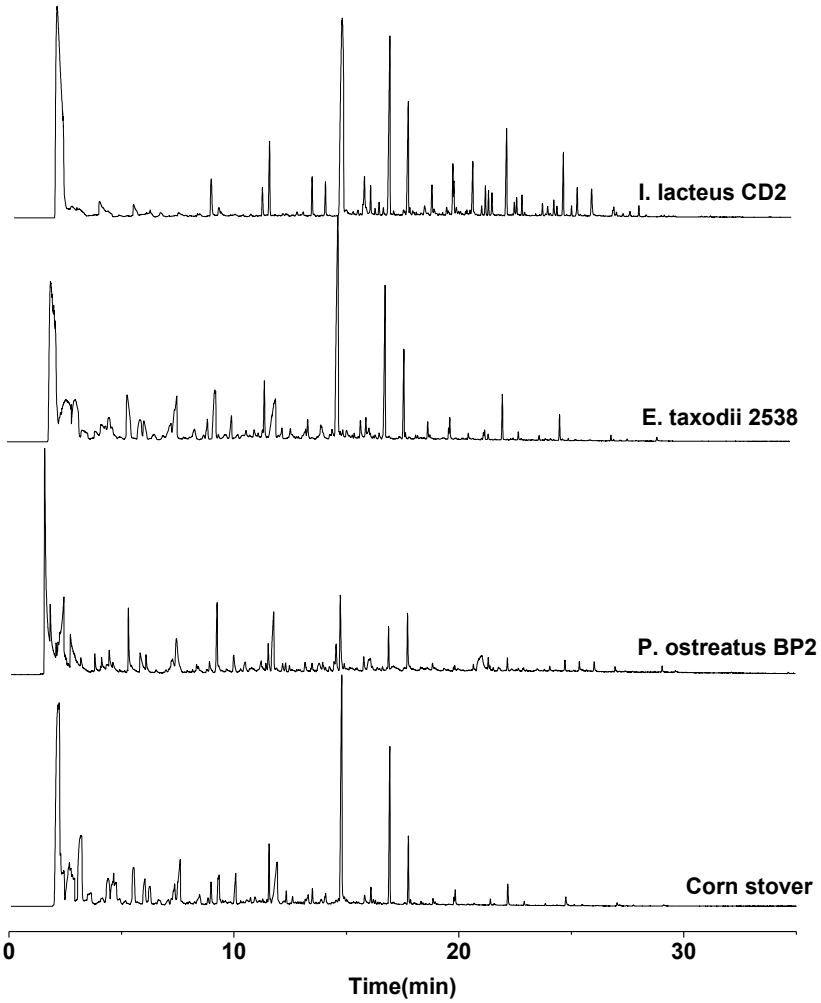

Fig. 2. Effect of biopretreatment by white-rot fungi on the main pyrolysis products at 410℃ (*P. ostreatus* BP2 = corn stover pretreated by *P. ostreatus* BP2 for 30 days, *E. taxodii* 2538 = corn stover pretreated by *E. taxodii* 2538 for 30 days, *I. lacteus* CD2 = corn stover pretreated by *I. lacteus* CD2 for 30 days)*.*

Table 2. Effect of Biopretreatment by Different White-rot fungi on the Main Pyrolysis Products at 410 °C (A = corn stover, B = corn stover pretreated by *P. ostreatus* BP2 for 30 days, C = corn stover pretreated by *E. taxodii* 2538 for 30 days, D= corn stover pretreated by *I. lacteus* CD2 for 30 days)

Compound	Formula	MW	Sample Area (%)			
			A	B	C	D
Carbon dioxide	CO_2	44	26.07	18.71	22.97	14.21
Formic acid, ethenyl ester	$C_3H_4O_2$	72		6.98		
Acetaldehyde	C_2H_4O	44				6.72
Butanoic acid, 2-oxo-	$C_4H_6O_3$	102				2.46
2-Propanone, 1,3-dihydroxy-	$C_3H_6O_3$	90			1.52	
1-Propanol, 2-amino-	C_3H_9NO	75		1.75		
1-Propen-2-ol, acetate	$C_5H_8O_2$	100	2.80			
2-Propanone, 1-hydroxy-	$C_3H_6O_2$	74	8.39	6.36	5.75	1.33
2,2'-Bioxirane, (R*,R*)-(ñ)-	$C_4H_6O_2$	86		0.80		
1,2-Epoxy-3-propyl acetate	$C_5H_8O_3$	116		1.27	1.42	
2H-Pyran, 3,4-dihydro-	C_5H_8O	84		1.98	1.06	1.82
Butanedial	$C_4H_6O_2$	86	4.91	0.71	0.69	
3-Amino-2-oxazolidinone	$C_3H_6N_2O_2$	102		1.47	1.75	1.56
Propanoic acid, 2-oxo-, methyl ester	$C_4H_6O_3$	102	1.49			
Furfural	$C_5H_4O_2$	96	2.53	2.61	3.67	3.28
2-Furanmethanol	$C_5H_6O_2$	98	1.60	1.99	1.73	1.54
2-Decenal, (E)-	$C_{10}H_{18}O$	154				1.03
Diallylethylamine	$C_8H_{15}N$	125	0.29			
Phenol	C_6H_6O	94	0.84	0.72	0.75	0.68
Oxazolidine, 2,2-diethyl-3-methyl-1,3-	$C_8H_{17}NO$	143	1.69	3.55	3.11	5.52
4-Heptanone	$C_7H_{14}O$	114			0.20	
N-Methyl-3-hydroxymethylpyrrolidin-2-one	$C_6H_{11}NO_2$	129			0.49	
1-Amino-2,6-dimethylpiperidine	$C_7H_{16}N_2$	128			0.37	
3-Pyrazolidinone, 1,4-dimethyl	$C_5H_{10}N_2O$	114		0.89		
Acetic acid, 2-methyl-6-oxo-heptyl ester	$C_{10}H_{18}O_3$	186			0.51	
1,2,6-Hexanetriol	$C_6H_{14}O_3$	134		0.48		
Tetrahydro[2,2']bifuranyl-5-one	$C_8H_{12}O_3$	156		1.10		
Butanoic acid, 2-ethyl-2-methyl-	$C_7H_{14}O$	108	0.39			
Methyl 2-furoate	$C_6H_6O_3$	126		0.32		
Phenol, 4-methoxy-	C_7H_8O	108	0.16		0.22	1.26
2,5-Dimethyl-4-hydroxy-3(2H)-furanone	$C_6H_8O_3$	128			0.36	
Phenol, 2-methoxy-	$C_7H_8O_2$	124	1.72	1.21	1.53	
Cyclopropyl carbinol	C_4H_8O	72	3.78	4.10	3.85	4.96
Maltol	$C_6H_6O_3$	126		0.47		
2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	$C_7H_{10}O_2$	126	0.37	0.36	0.45	
2H-Pyran-3(4H)-one, dihydro-6-methyl-	$C_6H_{10}O_2$	114		0.22		
2,4(3H,5H)-Furandione, 3-methyl-	$C_5H_6O_3$	114	0.26		0.71	0.18

Glycylsarcosine	$C_5H_{10}N_2O_3$	146	0.53	0.53		
1-Methyl-8-nitroisoxazolizidine	$C_6H_{10}N_2O_4$	174			0.47	
Bicyclo[4.1.0]octane, 7,7-dichloro-1-methyl-	$C_8H_{12}Cl_2$	178		0.46		
Phenol, 4-ethyl-	$C_8H_{10}O$	122	0.53			0.52
à-D-Glucopyranoside, O-à-D-glucopyranosyl-(1.fwdarw.3)-á-D-fructofuranosyl	$C_{18}H_{32}O_{16}$	504	0.31	0.90	0.76	0.62
2-Deoxy-D-galactose2	$C_6H_{12}O_5$	164				1.07
Phenol, 2-methoxy-4-methyl-	$C_8H_{10}O_2$	138	0.55		0.51	
Dodecanoic acid, 3-hydroxy-	$C_{12}H_{24}O_3$	216			0.57	
5-Hydroxymethyldihydrofuran-2-one	$C_5H_8O_3$	116		0.32		
1,2-Benzenediol	$C_6H_6O_2$	110		0.47	0.44	0.45
1,4:3,6-Dianhydro-à-d-glucopyranose	$C_6H_8O_4$	144		1.38		
Cyclohexanone, 2-(hydroxymethyl)-	$C_7H_{12}O_2$	128	0.12			
Benzofuran, 2,3-dihydro-	C_8H_8O	120	10.20	3.53	10.83	17.43
2-Methyl-4,5-tetramethylene-5-ethyl-2-oxazoline	$C_{10}H_{17}NO$	167			0.37	
2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126			0.42	
1,2-Benzenediol, 3-methoxy-	$C_7H_8O_3$	140	0.45	0.64	0.68	0.68
1-[3-Hydroxy-4-(3,4,5-trihydroxy-6-hydroxymethyltetrahydropyran-2-yloxy)phenyl]ethanone	$C_{14}H_{18}O_8$	314				1.09
Phenol,4-ethyl-2-methoxy-	$C_9H_{12}O_2$	152	0.50	1.15	0.55	0.59
trans-2-undecenoic acid	$C_{11}H_{20}O_2$	184			0.47	
2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	4.82	1.41		7.01
Phenol, 2,6-dimethoxy-	$C_8H_{10}O_3$	154	1.76	2.55	2.07	2.04
Phenol,2-methoxy-5-(1-propenyl)-, (E)-	$C_{10}H_{12}O_2$	164	0.13		0.15	
Benzenemethanol, 3-hydroxy-5-methoxy-	$C_8H_{10}O_3$	154			0.11	0.37
Vanillin lactoside	$C_{20}H_{28}O_{13}$	476		0.20		
Vanillin	$C_8H_8O_3$	152			0.39	
Propenylguaethol	$C_8H_8O_3$	152	0.15			
2,5-Dimethoxybenzyl alcohol	$C_9H_{12}O_3$	168	0.17		0.19	0.16
Phenol, 2-methoxy-4-(1-propenyl)-, (E)-	$C_{10}H_{12}O_2$	164	0.41	0.17	0.59	0.56
1,4-Dimethoxy-2,3-dimethylbenzeneBenzene,	$C_{10}H_{14}O_2$	166			0.16	
D-Allose	$C_6H_{12}O_6$	180		3.09		
Trimethoxyamphetamine, 2,3,5-	$C_{12}H_{19}NO_3$	225	0.17			
2H-1-Benzopyran-3,4-diol, 2-(3,4-dimethoxyphenyl)	$C_{18}H_{20}O_5$	316	0.61	0.84	0.14	2.29
Benzene, 1,2,3-trimethoxy-5-methyl-	$C_{10}H_{14}O_3$	182			0.19	
Phenol,2,6-dimethoxy-4-(2-propenyl)-	$C_{11}H_{14}O_3$	194	0.09	0.42	0.23	1.86
Ethanone,1-(4-hydroxy-3,5-	$C_{10}H_{12}O_4$	196		0.32		

dimethoxyphenyl)-3',5'-Dimethoxyacetophenone	$C_{10}H_{12}O_3$	180		0.86	
4-Hydroxy-3-methoxycinnamaldehyde	$C_{10}H_{10}O_3$	178			0.79
4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenolSyringin	$C_{10}H_{12}O_3$	180			0.47
3,5-Dimethoxy-4-hydroxyphenylacetic acid	$C_{10}H_{12}O_5$	212	0.38		
Desaspidinol	$C_{11}H_{14}O_4$	210			0.34
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296		0.10	

Biopretreatment with white-rot fungi can increase the production of furfural (up to 1.30 times), α-D-Glucopyranoside (up to 2.94 times), 3-methoxy-1,2-benzenediol (up to 1.52 times), 4-ethyl-2-methoxy-phenol (up to 2.82 times), and 2,6-dimethoxy-phenol (up to 1.45 times). The white-rot fungus *P. ostreatus* BP2 showed the greatest ability to increase the phenol production, compared with other white-rot fungi. Moreover, pretreated corn stover was able to release up to 1.98% 3,4-dihydro-2H-pyran and 0.47% 1,2-benzenediol during the medium-temperature pyrolysis, which can hardly be detected in the pyrolysis of original corn stover.

On the other side, biopretreatment could decrease the release of carbon dioxide from 26.07% to 14.21%, with the production of butanedial declining from 4.91% to 0.12%. The content of 2-amino-1-propanol, 1-propen-2-ol, 1-hydroxy-2-propanone, and propanoic acid dropped significantly for biopretreated samples. Corn stover pretreated with *I. lacteus* CD2 seldom produced 2-propanone derivatives that accounted for only 1.33%, compared with that of 8.39% in the control sample.

Medium-temperature pyrolysis differences between different white-rot fungi

Significant differences were observed in the pyrolysis of biopretreated samples with different white-rot fungi. It was found that corn stover pretreated with the white-rot fungus *P. ostreatus* BP2 was able to uniquely produce formic acid and D-allose up to 6.98% and 3.09%. Since formic acid is formed from the cleaving of carboxylic groups (Demirbas 2007), results showed that by decomposing the structure of carboxyl group (shown in the solid state CP-MAS analysis Table 1), pretreatment with *P. ostreatus* BP2 can make the biomass easier to remove carboxyl groups and greatly produce formic acid.

On the other hand, *I. lacteus* CD2 pretreated sample was able to produce acetaldehyde and 2-oxo-butanoic acid up to 6.72% and 2.46%, and these products are seldom released for medium-temperature pyrolysis. Acetaldehyde can be formed from the thermal decomposition of glucose, fructose and sucrose (Baker *et al.* 1984; Miller and Saunders 1987), which means that decomposing the β(1– 4) linkages in cellulose structure of (shown in Table 1), *I. lacteus* CD2 rendered the cellulose of corn stover more susceptible to thermal decomposition and production of acetaldehyde.

Pretreatment with *I. lacteus* CD2 was able to significantly increase the production of dimethoxyphenyl derivatives such as 2,6-dimethoxy-4-(2-propenyl)-phenol and 2-(3,4-dimethoxyphenyl) by up to 21.59 and 3.77 times, respectively. Since the aromatic group content decreased in *I. lacteus* CD2 treated samples, the rise in production of dimethoxyphenyl derivatives might be attributed to the lignin modification and less demethoxyation compared with other white-rot fungi (shown in Table 1). Due to the high

demethoxyation, 2-methoxy-4-vinylphenol declined by 70.08% for other white-rot fungi pretreatment, while that of *I. lacteus* CD2 pretreated sample increased 1.46 times.

CONCLUSIONS

1. Biopretreatment with different white-rot fungi can greatly improve the medium-temperature pyrolysis of corn stover, by increasing production of phenols and glucopyranoside, as well as helping release pyran and benzenediol, which usually can only be produced in high-temperature pyrolysis.
2. Biopretreatment can also reduce the production of carbon dioxide, propanol, and propanone, making the pyrolysis more efficient and product-oriented.
3. Distinct bio-deconstruction mechanisms can result in different pyrolysis products.
4. By deconstructing cellulose and modifying lignin with minimal demethoxyation, white-rot fungus *I. lacteus* CD2 can increase acetaldehyde and methoxyl substitutes.
5. By decomposing carbohydrates and carbonxyl, white-rot fungus *P. ostreatus* BP2 can increase the production of D-allose.

ACKNOWLEDGMENTS

This project was supported by the National Science Foundation of China for Distinguished Young Scholars (Grant No. 21206047). The authors also appreciated the work of the Centre of Analysis and Test of Huazhong University of Science and Technology.

REFERENCES CITED

Almendros, G., Martinez, A. T., Gonzalez, A. E., Gonzalez-Vila, F. J., Fruend, R., and Luedemann, H. D. (1992). "CPMAS carbon-13 NMR study of lignin preparations from wheat straw transformed by five lignocellulose-degrading fungi," *Journal of Agricultural and Food Chemistry* 40(7), 1297-1302.

Alvira, P., Tomás-Pejó, E., Ballesteros, M., and Negro, M. (2010a). "Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review," *Bioresource Technology* 101(13), 4851-4861.

Alvira, P., Tomas-Pejo, E., Ballesteros, M., and Negro, M. (2010b). "Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review," *Bioresource Technology* 101(13), 4851-4861.

Azeez, A., Meier, D., Odermatt, J., and Willner, T. (2010). "Fast pyrolysis of african and european lignocellulosic biomasses using py-GC/MS and fluidized bed reactor," *Energy & Fuels* 24(3), 2078-2085.

Baker, R., Dymond, H., and Shillabeer, P. (1984). "Pyrolysis. Determination of α, β-unsaturated compounds formed by a burning cigarette," *Anal. Proc.* 135-137.

Chen, S., Zhang, X., Singh, D., Yu, H., and Yang, X. (2010). "Biological pretreatment of lignocellulosics: Potential, progress and challenges," *Biofuels* 1(1), 177-199.

Demiral, and Ayan, E. A. (2010). "Pyrolysis of grape bagasse: Effect of pyrolysis conditions on the product yields and characterization of the liquid product," *Bioresource Technology* 102(4), 3946-3951.

Demirbas, A. (2007). "The influence of temperature on the yields of compounds existing in bio-oils obtained from biomass samples via pyrolysis," *Fuel Processing Technology* 88(6), 591-597.

Gilardi, G., Abis, L., and Cass, A. E. G. (1995). "Carbon-13 CP/MAS solid-state NMR and FT-IR spectroscopy of wood cell wall biodegradation," *Enzyme and Microbial Technology* 17(3), 268-275.

Hall, M., Bansal, P., Lee, J. H., Realff, M. J., and Bommarius, A. S. (2010). "Biological pretreatment of cellulose: Enhancing enzymatic hydrolysis rate using cellulose-binding domains from cellulases," *Bioresource Technology* 102(3), 2910-2915.

Hatakka, A. (1994). "Lignin-modifying enzymes from selected white-rot fungi: production and role from in lignin degradation," *FEMS Microbiology Reviews* 13(2-3), 125-135.

Himmel, M. E., Ding, S.-Y., Johnson, D. K., Adney, W. S., Nimlos, M. R., Brady, J. W., and Foust, T. D. (2007). "Biomass recalcitrance: Engineering plants and enzymes for biofuels production," *Science* 315(5813), 804-807.

Jackson de Moraes Rocha, G., Martin, C., Soares, I. B., Souto Maior, A. M., Baudel, H. M., and Moraes de Abreu, C. A. (2010). "Dilute mixed-acid pretreatment of sugarcane bagasse for ethanol production," *Biomass and Bioenergy* 35(1), 663-670.

Liu, S., Abrahamson, L. P., and Scott, G. M. (2011). "Biorefinery: Ensuring biomass as a sustainable renewable source of chemicals, materials, and energy," *Biomass and Bioenergy* 39, 1-4.

Miller, I. J., and Saunders, E. R. (1987). "Reactions of acetaldehyde, acrolein, acetol, and related condensed compounds under cellulose liquefaction conditions," *Fuel* 66(1), 130-135.

Ray, M. J., Leak, D. J., Spanu, P. D., and Murphy, R. J. (2010). "Brown rot fungal early stage decay mechanism as a biological pretreatment for softwood biomass in biofuel production," *Biomass and Bioenergy* 34(8), 1257-1262.

Sun, X. F., Sun, R. C., Fowler, P., and Baird, M. S. (2005). "Extraction and characterization of original lignin and hemicelluloses from wheat straw," *Journal of Agricultural and Food Chemistry* 53(4), 860-870.

Taniguchi, M., Suzuki, H., Watanabe, D., Sakai, K., Hoshino, K., and Tanaka, T. (2005). "Evaluation of pretreatment with *Pleurotus ostreatus* for enzymatic hydrolysis of rice straw," *Journal of Bioscience and Bioengineering* 100(6), 637-643.

Wu, J., Gao, S., Wan, J., Zeng, Y., Ma, F., and Zhang, X. (2011). "Thermogravimetric kinetics of corn stalk pretreated by oleaginous fungi *Cunninghamella echinulata*," *Bioresource Technology* 102(8), 5255-5258.

Xu, C., Ma, F., Zhang, X., and Chen, S. (2010). "Biological pretreatment of corn stover by Irpex lacteus for enzymatic hydrolysis," *Journal of Agricultural and Food Chemistry* 58(20), 10893-10898.

Yang, X., Ma, F., Yu, H., Zhang, X., and Chen, S. (2010a). "Effects of biopretreatment of corn stover with white rot fungus on low-temperature pyrolysis products," *Bioresource Technology* 102(3), 3498-3503.

Yang, X., Zeng, Y., Ma, F., Zhang, X., and Yu, H. (2010b). "Effect of biopretreatment on thermogravimetric and chemical characteristics of corn stover by different white-rot fungi," *Bioresource Technology* 101(14), 5475-5479.

Yu, H., Guo, G., Zhang, X., Yan, K., and Xu, C. (2009a). "The effect of biological pretreatment with the selective white-rot fungus *Echinodontium taxodii* on enzymatic hydrolysis of softwoods and hardwoods," *Bioresource Technology* 100(21), 5170-5175.

Yu, H., Guo, G., Zhang, X., Yan, K., and Xu, C. (2009b). "The effect of biological pretreatment with the selective white-rot fungus *Echinodontium taxodii* on enzymatic hydrolysis of softwoods and hardwoods," *Bioresource Technology* 100(21), 5170-5175.

Yu, J., Zhang, J., He, J., Liu, Z., and Yu, Z. (2009c). "Combinations of mild physical or chemical pretreatment with biological pretreatment for enzymatic hydrolysis of rice hull," *Bioresource Technology* 100(2), 903-908.

Zhang, X., Xu, C., and Wang, H. (2007a). "Pretreatment of bamboo residues with *Coriolus versicolor* for enzymatic hydrolysis," *Journal of Bioscience and Bioengineering* 104(2), 149-151.

Zhang, X., Yu, H., Huang, H., and Liu, Y. (2007b). "Evaluation of biological pretreatment with white rot fungi for the enzymatic hydrolysis of bamboo culms," *International Biodeterioration & Biodegradation* 60(3), 159-164.

Zimbardi, F., Viggiano, D., Nanna, F., Demichele, M., Cuna, D., and Cardinale, G. (1999). "Steam explosion of straw in batch and continuous systems," *Applied Biochemistry and Biotechnology* 77(1), 117-125.

Zwart, R. W., Boerrigter, H., and van der Drift, A. (2006). "The impact of biomass pretreatment on the feasibility of overseas biomass conversion to Fischer-Tropsch products," *Energy & Fuels* 20(5), 2192-2197.

Article submitted: April 9, 2013; Peer review completed: August 21, 2013; Revised version received: October 18, 2013; Accepted: October 19, 2013; Published: October 24, 2013.