ARTICLE

by Jurnal Rudi Amirta_1

Submission date: 27-Oct-2018 08:16AM (UTC+0700)

Submission ID: 1027662122

File name: 1. Methane fermentation of Japanese cedar wood pretreated with a white rot fungus, Ceriporiopsis subvermispora.pdf

Word count: 4023

Character count: 20887

Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Biotechnology 123 (2006) 71–77
Journal of BIOTECHNOLOGY
www.elsevier.com/locate/jbiotec

Methane fermentation of Japanese cedar wood pretreated with a white rot fungus, *Ceriporiopsis subvermispora*

Rudianto Amirta [a], Toshiaki Tanabe [a], Takahito Watanab [a], Yoichi Honda [a], Masaaki Kuwahara [b], Takashi Watanabe [a,*]

[a] *Research Institute for Sustainable Humanosphere, Kyoto University, Gokasho, Uji, Kyoto, Japan*
[b] *Institute of Wood Technology, Akita Prefectural University, Noshiro, Akita, Japan*

Received 12 July 2005; received in revised form 16 September 2005; accepted 6 October 2005

Abstract

Methane fermentation of Japanese cedar wood was carried out after pretreatment with four strains of white rot fungi, *Ceriporiopsis subvermispora* ATCC 90467, CZ-3, CBS 347.63 and *Pleurocybella porrigens* K-2855. These fungi were cultivated on wood chip media with and without wheat bran for 4–8 weeks. The pretreated wood chip was fermented anaerobically with sludge from a sewage treatment plant. Pretreatments with *C. subvermispora* ATCC 90467, CZ-3 and CBS 347.63 in the presence of wheat bran for 8 weeks decreased 74–76% of β-O-4 aryl ether linkages in the lignin to accelerate production of methane. After fungal treatments with *C. subvermispora* ATCC 90467 and subsequent 30-days methane fermentation, the methane yield reached 35 and 25% of the theoretical yield based on the holocellulose contents of the decayed and original wood, respectively. In contrast, treatment with the three strains of *C. subvermispora* without wheat bran cleaved 15–26% of the linkage and produced 6–9% of methane. There were no significant accelerating effects in wood chips treated with *P. porrigens* which has a lower ability to decompose the lignin. Thus, it was found that *C. subvermispora*, with a high ability to decompose aryl ether bonds of lignin, promoted methane fermentation of softwood in the presence of wheat bran.

Keywords: White rot fungi; Methane fermentation; Lignin; Softwood; Japanese cedar

1. Introduction

The forestry and wood industries supply renewable biomass that provides electrical/heat energy, transport fuel or chemical feedstock. Residual wood in the forest area, and wood waste are abundant lignocellulosic materials that could be fermented to methane, ethanol and other chemical products (Delgenes et al., 1996; Kaygusuz and Türker, 2002; Palm and Zacchi, 2003). Generation of methane in landfills is facilitated by microbial decomposition of organic compounds under anaerobic condition, with carbon partitioned into

* Corresponding author. Tel.: +81 774 38 3640; fax: +81 774 38 3643.
E-mail address: twatanab@rish.kyoto-u.ac.jp (T. Watanabe).

0168-1656/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jbiotec.2005.10.004

approximately equal amounts of methane and carbon dioxide (Bingemer and Crutzen, 1988; Nozhevnikova et al., 1993). In general, fermentation of lignocellulosic material requires several steps: firstly, delignification to liberate cellulose and hemicellulose from their complex with lignin; secondly, depolymerization of the carbohydrate polymers (cellulose and hemicelluloses) to monosaccharides and fermentation (Aristidou and Penttilä, 2000). Lignin is the major factor determining the extent of organic substrate degradation in anaerobic conditions (Chandler et al., 1980). Due to the heterogeneity, lignin is resistant to biological attack by many kinds of microorganisms. However, basidiomycetes called white rot fungi are known as an aggressive lignin degrader (Kirk and Farrell, 1987).

Pretreatments of wood include mechanical size reduction, steaming, steam explosion, autohydrolysis, alkali treatments, chemical pulping, solvent extraction, fungal degradation and their combinations. The pretreatment is an essential step to decrease the amount of lignin, maximize subsequent bioconversion yields and minimize the formation of inhibitory compounds (Chynoweth and Jerger, 1985; Young and Frazer, 1987). The pretreatment increases the specific surface area of cell wall polysaccharides to accelerate enzymatic hydrolysis (Gharpuray et al., 1983).

Enhanced production of methane on pretreatment of *Eucalyptus globulus* wood chip by using steam, NaOH and steam explosion was reported (Nakamura and Godliving, 2003). The amount of methane gas produced depended on a decrease in Klason lignin of the treated wood chip (Nakamura and Godliving, 2003). However, there have been few reports demonstrating effectiveness of fungal pretreatments of wood for methane fermentation. We were interested to apply white rot fungi to the pretreatments for methane fermentation of wood. Methane fermentation is advantageous for on-site energy supply. Methane gas can be converted to electricity using fuel cells or turbine systems, or combusted directly. Development of bioconversion system from wood to methane should accelerate the establishment of bioenergy-based societies that use wood and forestry wastes to make electricity, heat and fuels.

In the fungal pretreatments of woody biomass for enzymatic saccharification and microbial fermentation, the network of lignin must be degraded with minimum loss of cell wall polysaccharides. Among the numerous wood rot fungi so far isolated, a white rot fungus, *Ceriporiopsis subvermispora*, is known as the best biopulping fungus that can degrade lignin without intensive damage of cellulose (Akhtar et al., 1992, 1998; Messner and Srebotnik, 1994). This fungus is unique in its ability to cleave β-O-4 aryl ether linkages between lignin units without intensive weight loss of cellulose in early stage of wood decay (Guerra et al., 2002). In the present paper, fungal pretreatments with several strains of *C. subvermispora* and *Pleurocybella porrigens* on methane fermentation of Japanese cedar (*Cryptomeria japonica* (Lf) D.Don) were studied. Japanese cedar is the most important softwood species in the Japanese forest industry, but it is difficult to decompose its cell wall structures by chemical and biological treatments. We herein report that pretreatment of Japanese cedar wood with a selective white rot fungus, *C. subvermispora*, in the presence of wheat bran increased production of methane. Correlation between the fungal pretreatment effects and changes in wood components including cleavage of β-O-4 aryl ether linkages in lignin is discussed.

2. Materials and methods

2.1. Reagents

Acetic anhydrate, tetracosane and pyridine were obtained from Nacalai Tesque (Kyoto, Japan). Acetyl bromide (AcBr), zinc dust and *trans*-cinnamyl alcohol were purchased from Wako Chemical Industries (Tokyo, Japan). All of the reagents used were of analytical grade.

2.2. Fungal pretreatments

C. subvermispora ATCC 90467, CZ-3 (ATCC 96608), CBS 347.63 and *P. porrigens* K-2855 (a strain obtained from Takara Shuzo Co. Ltd., Kyoto, Japan) were cultured on a PDA plate at 28 °C for 1 week. Four pellets of inocula from the precultures were added to 10 g of Japanese cedar wood chip media containing 30 ml Milli-Q^{TM} water and 1 g of wheat bran (Nisshin Flour Milling Inc., Tokyo) in a 300-ml Erlenmeyer flask. The flasks were incubated at 28 °C with 70% relative humidity for 4–8 weeks. The wood chips were used without pre-extraction. Wood media without the

wheat bran were also cultured. After the incubation, the cultures were autoclaved and subjected to methane fermentation. For component analysis, the cultures were air-dried, and milled to 40–60 mesh by an Iwatani portable miller IFM-170G.

2.3. Determination of wood component

Moisture content, weight decrease, content of holocellulose, α-cellulose and Klason lignin were analyzed as described (Itoh et al., 2003).

2.4. Determination of β-O-4 aryl ether linkage by the derivative followed by reductive and cleavage method (DFRC)

Cleavage of β-O-4 aryl ether linkages of lignin in Japanese cedar by fungal pretreatments was determined by DFRC method (Lu and Ralph, 1997, 1998) (Fig. 1). Japanese cedar wood meal (20 mg) was reacted with an AcBr reagent (AcBr/acetic acid mixture, 2:8, v/v, 2.5 ml) at 50 °C for 3 h. Acetic acid was removed after reaction. The brominated wood meal was dissolved in an acidic reduction solution (dioxane/acetic acid/water mixture, 5:4:1, v/v, 2.5 ml). Then, 50 mg zinc dust was added and the mixture was stirred at room temperature for 30 min. The reaction mixture was transferred to a separatory funnel, and partitioned between saturated NaCl (10.0 ml) and CH_2Cl_2 (10.0 ml) containing an internal standard (0.2 mg tetracosane). The water phase was extracted twice with additional CH_2Cl_2 (2× 5.0 ml). The organic layer was dried over Na_2SO_4 and evaporated. The sample was acetylated, and subjected to GC–MS. Tetracosane was used as an internal standard.

GC–MS analysis was performed on a Shimadzu QP-5050A mass spectrometer with a SPB™-5 capillary column (30 m × 0.20 mm i.d., film thickness 0.2 μm; Supelco, PA, USA). Electron impact mass spectra (EIMS) were recorded at an ionization energy of 70 eV. The column oven temperature was maintained at 160 °C for 1 min and raised to 300 °C at 3 °C min^{-1} and maintained at 300 °C for 15 min.

2.5. Methane fermentation

Methane fermentation of Japanese cedar was carried out in a 500 ml Erlenmeyer flask. The fermentation system consists of 12 g of pretreated and untreated Japanese cedar wood chips and 400 ml of digested sludge (SS: 2.2%) from the Noshiro sewage treatment plant (Noshiro, Akita Pref, Japan). The fermentation flasks were purged with argon gas and incubated at 35 °C for 60 days. Biogas produced was collected in a measuring cylinder submerged under saturated sodium chloride. The volume of gas produced was measured periodically. Composition of the gas produced was analyzed by gas chromatography. Gas production from flasks containing the sludge without wood chips and wheat bran was measured. Anaerobic fermentation of the wheat bran (25 g) without wood chips was also carried out using the sludge.

GC was performed on a Shimadzu GC-14A equipped with a TCD detector. Separation was performed on a stainless column, Porapak Q (2.0 m × 3.0 mm i.d., 80/100 mesh; Water Associates Inc., MA,

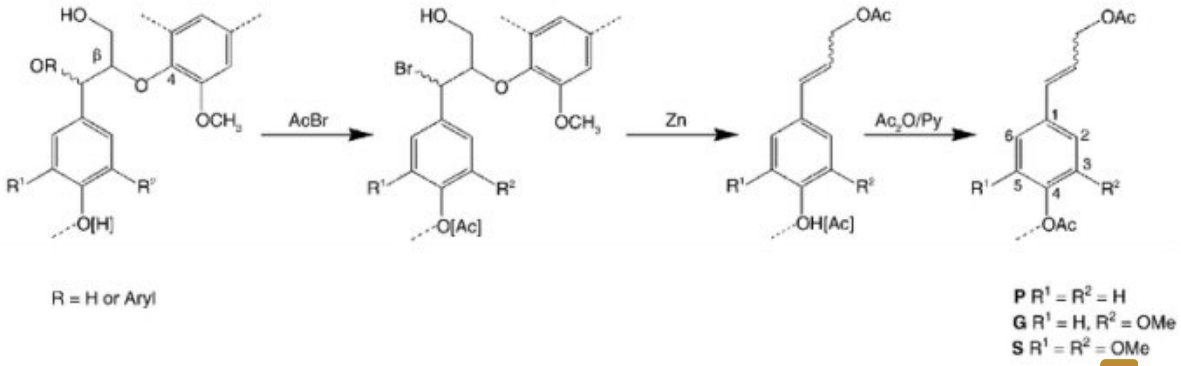

Fig. 1. Selective cleavage of aryl ether bonds in lignin by DFRC method, releasing diagnostic monomers 4-acetocinnamyl acetate (P), coniferyl diacetate (G) and sinapyl diacetate (S) monomers from *p*-hydroxylphenyl, guaiacyl and syringyl units (Lu and Ralph, 1997, 1998).

USA) using Ar as a carrier gas at a flow rate of 30 ml min^{-1}. Temperatures of the column, injector and detector were 30 °C. Conversion yield of hollocellulose to methane was calculated based on the theoretical yield from cellulose and hemicellulose to methane reported by Kobayashi et al. (2004).

3. Results and discussion

3.1. Changes in wood components during fungal pretreatments

Effects of fungal pretreatments of Japanese cedar on production of methane were investigated. A number of fungal strains have been screened for biodelignification prior to pulping. *C. subvermispora* is characterized as one of the best biopulping fungi (Akhtar et al., 1992; Messner and Srebotnik, 1994). Effectiveness of the fungus in the pretreatments of beech wood for ethanol fermentation was demonstrated (Itoh et al., 2003). This attracted our interest in the use of this fungus for the pretreatment of wood for methane fermentation. In the present study, three strains of *C. subvermispora* ATCC 90467, CZ-3 and CBS 347.63 were used, together with a white rot fungus *P. porrigens* which is known as a saprophytic fungus for Japanese cedar wood.

Weight loss of the Japanese cedar wood after cultivation of *C. subvermispora* ATCC 90467, CZ-3, CBS 347.63 and *P. porrigens* K-2855 on the Japanese cedar/wheat bran media for 8 weeks were 15.9, 15.1, 13.2 and 10.3%, respectively (Table 1). Weight loss of the Japanese cedar wood cultured with wheat bran by *C. subvermispora* for 8 weeks was two to four times higher than that found in the wood cultures without wheat bran. In addition, the weight of lignin decreased much faster by addition of wheat bran for all the fungi examined. After 4 weeks of cultivation, lignin content decreased by 0.8–2.1% without wheat bran but addition of wheat bran in the wood medium decreased the lignin content by 9.6–15.9%. No significant differences were found for decrease in lignin content among the three strains of *C. subvermispora* (Table 2).

3.2. Cleavage of β-O-4 aryl ether linkages

Effects of fungal pretreatments on cleavage of β-O-4 aryl ether bonds in Japanese cedar lignin were investigated. From the analysis of DFRC monomeric products, it was found that aryl ether linkages of Japanese cedar lignin were cleaved during the fungal pretreatments (Fig. 2). Decreased values in the β-O-4 aryl ether bonds by cultivation of *C. subvermispora* without

Table 1
Decrease in total weight of Japanese cedar wood after fungal treatment[a]

Fungal strain	4 weeks		8 weeks	
	Wood	Wood + wheat bran	Wood	Wood + wheat bran
C. subvermispora ATCC 90467	0.9 ± 0.1	7.3 ± 0.1	2.6 ± 0.1	15.9 ± 1.0
C. subvermispora CZ-3	1.1 ± 0.0	8.6 ± 0.0	2.8 ± 0.0	15.1 ± 0.3
C. subvermispora CBS 347.63	0.8 ± 0.0	6.1 ± 0.6	3.1 ± 0.6	13.2 ± 0.1
P. porrigens K-2855	0.1 ± 0.0	5.2 ± 0.6	0.4 ± 0.6	10.3 ± 0.1

[a] Values are expressed as percent decrease based on the weight of original wood.

Table 2
Decrease in Klason lignin content of Japanese cedar wood after fungal treatment[a]

Fungal strain	4 weeks		8 weeks	
	Wood	Wood + wheat bran	Wood	Wood + wheat bran
C. subvermispora ATCC 90467	0.8 ± 1.1	13.7 ± 0.7	9.9 ± 0.2	28.2 ± 0.7
C. subvermispora CZ-3	1.6 ± 0.6	15.9 ± 0.8	11.1 ± 0.2	28.2 ± 0.0
C. subvermispora CBS 347.63	2.1 ± 1.1	14.2 ± 0.6	11.2 ± 0.0	28.2 ± 0.2
P. porrigens K-2855	0.8 ± 1.0	9.6 ± 1.7	6.8 ± 0.0	16.7 ± 0.3

[a] Values are expressed as percent decrease based on the weight of lignin in original wood.

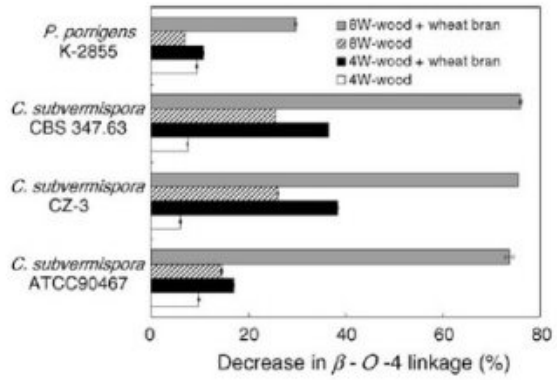

Fig. 2. Decrease in β-O-4 aryl ether linkages in lignin of Japanese cedar wood after fungal pretreatments.

and with wheat bran for 4 weeks were 6.0 and 38.4%, respectively.

The highest loss of aryl ether was found in the sample treated by *C. subvermispora* in the presence of wheat bran after pretreatment for 8 weeks. In this case, cleavage of β-O-4 aryl ether linkages reached 74–76% after 60 days (based on lignin content). The present results indicate that *C. subvermispora* decomposed the lignin accompanied by cleavage of β-O-4 linkages in lignin.

Lignin contains a variety of linkages such as β-O-4, β-β, β-5, 5-1 and 5-5 (Guerra et al., 2002, 2004). Among the C–C and C–O–C bonds, β-O-4 aryl ether linkage is the most frequent bond inter-linking the phenylpropan units. *C. subvermispora* is effective to decompose the principal linkages of lignin even in early stage of wood decay when weight loss of the lignin is not prominent. This observation is consistent with previous reports by Guerra et al. (2002, 2004). They concluded that β-O-4 aryl ether cleavage was a significant route for lignin degradation during the solid-state fermentation of *P. taeda* by *C. subvermispora*.

3.3. Effects of fungal pretreatments on methane fermentation

The effects of fungal pretreatments of Japanese cedar on methane fermentation were evaluated. Pretreatments were carried out to increase susceptibility of polysaccharides to microbial degradation by decomposing a network of lignin surrounding the polysaccharides. Fungal pretreatments with a selective lignin

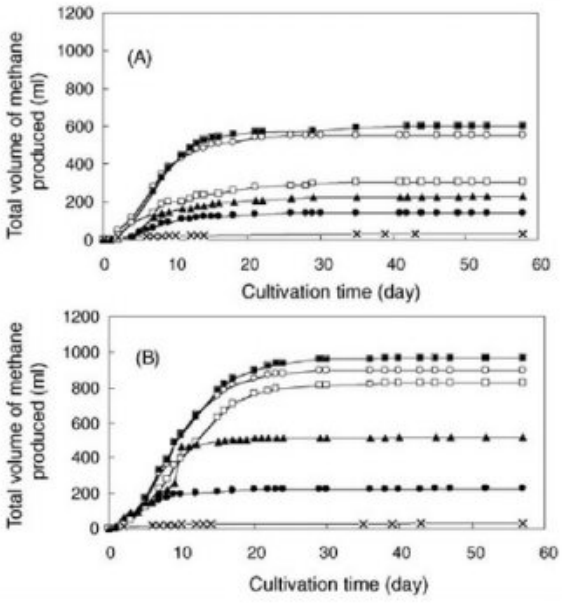

Fig. 3. Methane production from Japanese cedar wood after fungal pretreatments in the presence of wheat bran, and subsequent methane fermentation for 60 days. Cultivation of white rot fungi was carried out on wood/wheat bran media for: (A) 4 weeks and (B) 8 weeks. *C. subvermispora* ATCC 90467 (■), CZ-3 (◯), CBS 347.63 (□), *P. porrigens* K-2855 (▲), Control 1 (untreated wood) (●) and Control 2 (without wood and wheat bran) (×).

degrading fungus, *C. subvermispora*, significantly increased production of biogas and methane from the wood (Figs. 3 and 4). Methane production increased with increase in the cultivation time and reached plateau after 30 days. The wood cultures with wheat bran produced a higher amount of methane (Fig. 3) than those without wheat bran (Fig. 4). The yield of methane from the wood chips pretreated with *C. subvermispora* in the presence of wheat bran for 8 weeks reached 21–25% of the theoretical yield based on holocellulose content of the original wood (Fig. 5). However, production of methane after fungal treatments with *C. subvermispora* in the absence of wheat bran was 6–9%. The maximum yield, 25%, was obtained by the treatment with *C. subvermispora* ATCC 90467 in the presence of wheat bran for 8 weeks. This corresponds to 35% of the theoretical yield based on holocellulose in the decayed wood. Production of methane from the sludge in the absence of wood and wheat bran was negligible. Without inoculation of white rot fungi, around

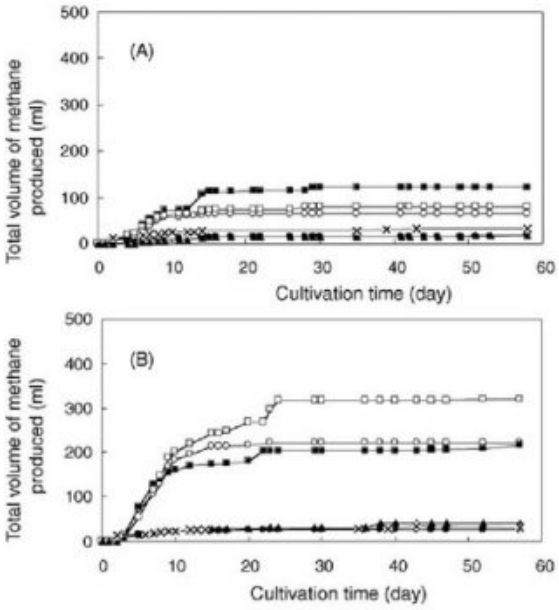

Fig. 4. Methane production from Japanese cedar wood after fungal pretreatments in the absence of wheat bran, and subsequent methane fermentation for 60 days. Cultivation of white rot fungi were carried out on wood media for: (A) 4 weeks and (B) 8 weeks. *C. subvermispora* ATCC 90467 (■), CZ-3 (◯), CBS 347.63 (□), *P. porrigens* K-2855 (▲), Control 1 (untreated wood) (●) and Control 2 (without wood) (×).

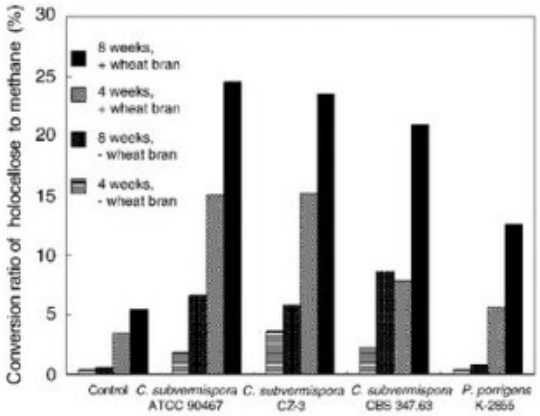

Fig. 5. Conversion ratio of holocellulose of Japanese cedar wood to methane and after fungal pretreatments for 8 weeks and subsequent methane fermentation for 30 days. Values are expressed as percentage based on the weight of holocellulose in the original wood.

200 ml of methane was produced from a mixture of the intact Japanese cedar wood chips, wheat bran and sludge. This volume was ascribed to production of methane from the wheat bran. Wheat bran is known as a good substrate for various white rot fungi including *C. subvermispora* (Fukushima and Kirk, 1995). It promotes cell growth and production of hemicellulolytic and ligninolytic enzymes such as xylanase, laccase and manganese peroxidase. In the present study, addition of wheat bran accelerated the degradation of Japanese cedar wood as found in the weight loss, Klason lignin content and cleavage of β-O-4 aryl ether linkages in the lignin (Tables 1 and 2; Fig. 2).

The enhancement of methane production by the effective fungal pretreatments is explained by the breakdown of cell wall structure. The lignin degradation increased the surface area of exposed cellulose to increase its susceptibility to microbes and their enzymes (Komilis and Ham, 2003). The present results indicate that cleavage of β-O-4 aryl ether linkages evaluated by the DFRC method in the lignin of Japanese cedar has a correlation with the biogas production. This suggests that extent of β-O-4 aryl ether bond cleavage is a good parameter to estimate the pretreatment effects of wood by white rot fungi for methane fermentation. The accelerating effects of *C. subvermispora* for methane fermentation are in accordance with our recent finding that *C. subvermispora* ATCC 90467 was the best white rot fungus to increase in vitro ruminal digestibility of Japanese cedar wood (Okano et al., 2003).

In conclusion, fungal pretreatments by *C. subvermispora* ATCC 90467, CZ-3 and CBS 347.63 are useful not only for biopulping but also for pretreatments of softwood for methane fermentation. Wheat bran is a good accelerator for the fungal delignification and subsequent methane fermentation. Because yields of enzymatic saccharification and fermentation increased by the combination of white rot fungi with steam explosion (Sawada et al., 1995) or ethanolysis (Itoh et al., 2003), combination of *C. subvermispora* and thermochemical pretreatments is attractive to increase the methane yields.

References

Akhtar, M., Blanchette, R.A., Myers, G., Kirk, T.K., 1998. An overview of biomechanical pulping research. In: Young, R.A.,

Akhtar, M. (Eds.), Environmental Friendly Technologies for the Pulp and Paper Industry. Wiley, New York, pp. 309–340.

Akhtar, M., Attridge, M.C., Blanchette, R.A., Myers, G.C., Wall, M.B., Sykes, M.S., Koning Jr., J.W., Burgess, R.R., Wegner, T.H., Kirk, T.K., 1992. The white-rot fungus *Ceriporiopsis subvermispora* saves electrical energy and improves strength properties during biomechanical pulping of wood. In: Kuwahara, M., Shimada, M. (Eds.), Biotechnology in the Pulp and Paper Industry, Proceedings of the 5th International Conference on Biotechnology in the Pulp and Paper Industry. Uni Publishers, Tokyo, pp. 3–8.

Aristidou, A., Penttilä, M., 2000. Metabolic engineering applications to renewable resource utilization. Curr. Opin. Biotechnol. 11, 187–198.

Bingemer, H.G., Crutzen, P.J., 1988. The production of methane from solid wastes. J. Geophys. Res. 92, 2181–2187.

Chandler, J.A., Jewell, W.J., Gossett, J.M., Van Soest, P.J., Roberston, J.B., 1980. Predicting methane fermentation biodegradability. In: Biotechnology and Bioengineering Symposium No. 10. John Wiley & Sons, New York, pp. 93–107.

Chynoweth, D.P., Jerger, D.E., 1985. Anaerobic digestion of woody biomass. Dev. Ind. Microbiol. 26, 235–246.

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

Fukushima, Y., Kirk, T.K., 1995. Laccase component of the *Ceriporiopsis subvermispora*. Appl. Environ. Microbiol. 61, 872–876.

Gharpuray, M.M., Lee, Y.H., Fan, L.T., 1983. Structural modification of lignocellulosics by pretreatments to enhance enzymatic hydrolysis. Biotechnol. Bioeng. 25, 157–172.

Guerra, A., Mendonça, R., Ferraz, A., 2002. Characterization of the residual lignins in *Pinus taeda* biodegraded by *Ceriporiopsis subvermispora* by using in situ CuO oxidation and DFRC methods. Holzforschung 56, 157–160.

Guerra, A., Mendonça, R., Ferraz, A., Lu, F., Ralph, J., 2004. Structural characterization of lignin during *Pinus taeda* wood treatment with *Ceriporiopsis subvermispora*. Appl. Environ. Microbiol. 70, 4073–4078.

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

Kaygusuz, K., Türker, M.E., 2002. Biomass energy potential in Turkey. Renew. Energy 26, 661–678.

Kirk, T.K., Farrell, R.L., 1987. Enzymatic combustion: the microbial degradation of lignin. Annu. Rev. Microbiol. 41, 465–505.

Kobayashi, F., Take, H., Asada, C., Nakamura, Y., 2004. Methane production from steam-exploded bamboo. J. Biosci. Bioeng. 97, 426–428.

Komilis, D.P., Ham, R.K., 2003. The effect of lignin and sugars to the aerobic decomposition of solid wastes. Waste Manage. 23, 419–423.

Lu, F., Ralph, J.J., 1997. Derivatization followed by reductive cleavage (DFRC method), a new method for lignin analysis: protocol for analysis of DFRC monomers. J. Agric. Food Chem. 45, 2590–2592.

Lu, F., Ralph, J.J., 1998. The DFRC method for lignin analysis. 2. Monomers from isolated lignins. J. Agric. Food Chem. 46, 547–552.

Messner, K., Srebotnik, E., 1994. Biopoulping: an overview of developments in an environmentally safe paper-making technology. FEMS Microbiol. Rev. 13, 351–364.

Nakamura, Y., Godliving, M., 2003. Anaerobic fermentation of woody biomass treated by various methods. Biotechnol. Bioproc. Eng. 8, 179–182.

Nozhevnikova, A.N., Lifshitz, A.B., Lebedev, V.S., Zavarzin, G.A., 1993. Emission of methane into the atmosphere from landfills in the former USSR. Chemosphere 26, 401–417.

Okano, K., Kitagawa, M., Sasaki, Y., Watanabe, T., 2003. Conversion of Japanese red cedar (*Cryptomeria japonica*) into a feed for ruminants by white-rot basidiomycetes. Anim. Feed Sci. Technol. 120, 235–243.

Palm, M., Zacchi, G., 2003. Extraction of hemicellulosic oligosaccharides from spruce using microwave oven or steam treatment. Biomacromolecules 4, 617–623.

Sawada, T., Nakamura, Y., Kobayashi, F., Kuwahara, M., Watanabe, T., 1995. Effects of fungal pretreatment and steam explosion pretreatment on enzymatic saccharification of plant biomass. Biotechnol. Bioeng. 48, 719–724.

Young, L.Y., Frazer, A.C., 1987. The fate of lignin and lignin derived compounds in anaerobic environment. Geomicrobiology 5, 261–293.

ARTICLE

ORIGINALITY REPORT

10%	5%	8%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Chima Ngumah, Jude Ogbuli, Justina Orji, Ekperechi Amadi, Chris Nweke, Jude Allino. "Optimizing biomethanation of a lignocellulosic biomass using indigenous microbial-cellulases systems", BioTechnologia, 2017 Publication	1%
2	www.agmrc.org Internet Source	1%
3	Aristidou, A.. "Metabolic engineering applications to renewable resource utilization", Current Opinion in Biotechnology, 20000401 Publication	1%
4	www.karger.com Internet Source	1%
5	Qing Yang. "Structural characterization of lignin from wheat straw", Wood Science and Technology, 05/15/2010 Publication	1%
6	www.wakenyaku.co.jp Internet Source	1%

7	Yoshito Ohtani. "On the Determination of β-Aryl Ether Units in Wood Lignin by an Ozonation Method", Journal of Wood Chemistry and Technology, 1/1/2003 Publication	<1%
8	André Aguiar, André Ferraz. "Effects of exogenous calcium or oxalic acid on Pinus taeda treatment with the white-rot fungus Ceriporiopsis subvermispora", International Biodeterioration & Biodegradation, 2012 Publication	<1%
9	Moon, S.J.. "Characterization of lignin-rich residues remaining after continuous super-critical water hydrolysis of poplar wood (Populus albaglandulosa) for conversion to fermentable sugars", Bioresource Technology, 201105 Publication	<1%
10	Submitted to South Bank University Student Paper	<1%
11	docplayer.net Internet Source	<1%
12	Amen-Chen, C.. "Production of monomeric phenols by thermochemical conversion of biomass: a review", Bioresource Technology, 200109 Publication	<1%
13	coste53.net	

	Internet Source	<1%
14	Kiyohiko Nakasaki, Sang Hagk Kwon, Yuji Takemoto. "An interesting correlation between methane production rates and archaea cell density during anaerobic digestion with increasing organic loading", Biomass and Bioenergy, 2015 Publication	<1%
15	www.cazv.cz Internet Source	<1%
16	Submitted to University of Malaya Student Paper	<1%
17	www.mustafafehmiturker.com.tr Internet Source	<1%
18	"Handbook of Industrial Chemistry and Biotechnology", Springer Nature, 2017 Publication	<1%
19	Kurata, A.. "Asymmetric reduction of 2-chloroacrylic acid to (S)-2-chloropropionic acid by a novel reductase from Burkholderia sp. WS", Tetrahedron: Asymmetry, 20040920 Publication	<1%
20	Fang Chen. "Evidence for a novel biosynthetic pathway that regulates the ratio of syringyl to guaiacyl residues in lignin in the differentiating xylem of Magnolia kobus DC", Planta, 02/17/1999	<1%

Publication

21	Komilis, D.P.. "The effect of lignin and sugars to the aerobic decomposition of solid wastes", Waste Management, 2003 Publication	<1%
22	www.cheric.org Internet Source	<1%

Exclude quotes	Off	Exclude matches	< 10 words
Exclude bibliography	On		