WHITE ROT FUNGUS (*Marasmius* sp.) DELIGNIFICATION ON SUGARCANE BAGASSE FOR BIOETHANOL PRODUCTION

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Abstract

Sugarcane bagasse is one of the potential lignocellulose materials for alternative energy especially on the development of bioethanol, the second generation technology. This paper investigates the delignification of sugarcane bagasse by the white rot fungus (Marasmius sp.). Sugarcane bagasse was delignified by fungus; then hydrolyzed by xylane enzyme and fermented by Saccharomyces cerevisiae through simultaneous processes of saccharification and fermentation (SSF). Pretreatment of the sugarcane bagasse was conducted by fungus with various time periods of incubations for 15, 30, and 45 days. SSF of pretreated sugarcane bagasse was conducted for 3, 6, 9, 12, and 15 days. Lignin, cellulose, and hemicellulose were determined by Chesson method. Bagasse structure before and after delignification were determined by Fourier Transform Infrared (FTIR) Spectroscopy. The results showed that the best result of bagasse delignification, containing 16.04% of cellulose, 26.61% of hemicellulose, and 51.89% of cellulose, was on 15 days of incubation. The bioethanol was obtained in 3 days of incubation with bioethanol concentration of 0.85% or 0.4 g/L.

Keywords*: sugarcane bagasse, bioethanol, Marasmius* sp, *simultaneous saccharification and fermentation*

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1. Introduction

Global warming, environmental pollution, and limitation of fossil fuels encourage researchers to find alternative environmental friendly energy resources. Bioethanol, second generation technology has high potential to be an alternative source of energy. Bioethanol is produced conventionally by fermentation of biomass containing sugar and starch from agriculture such as sugarcane, maize, corn, and cassava. This issue becomes a problem in bioethanol production because the sources of sugar and starch for bioethanol compete with the sources for food and feed [1, 2].

Furthermore, researchers found lignocellulose to be an alternative source for bioethanol production. The main components of lignocellulose are lignin, cellulose (45-50%), and hemicellulose [1, 3, 4, 5]. Lignin is a complex component with phenyl-propane bonds which are hard to be degraded. Lignin protects cellulose and hemicellulose from enzymatic hydrolysis [6]; thus, pretreatment of lignin is important for bioethanol production.

Previous studies of delignification were conducted by chemical, physical, and biological treatment. Chemical pretreatment was conducted with formic acid and sulfuric acid (H_2SO_4) which was known as acid hydrolysis [4, 7]. This pretreatment requires corrosive-resistant equipment, which generated higher cost and is not environmental friendly. Physical pretreatment was conducted by steam explosion which required high temperature [8]. Another delignification process was biological pretreatment. The advantages of this method are environmental friendly, requires low energy, and has higher yields than others treatments. White rot fungi were used for biological pretreatment because they produce delignification enzymes. There were many kinds of white rot fungi which had been used for bio-delignification, such as *Pleurotus eryngii, Pleurotus* sp., *L. edodes,* and *Marasmius* sp. [7, 8, 9, 10].

Yildirim et al reported that *P. eryngii* was used for delignification on swab. The process was conducted for 140 days with 5% and 10% w/w rice husk as addition. The results showed that there was a loss of 69.68% lignin in 5% rice husk addition [7]. Cellulose crystallinity on *Lantana camara* increased from 19.57% to 25.21% when it was delignified by *Pleurotus* sp. [9]. Therefore, the researchers showed that white rot fungi were effective for lignocellulose delignification.

Pretreatment of lignin increase the number of polysaccharides which are important for bioethanol production. Pretreated lignocellulose was hydrolyzed and fermented simultaneously which was known as simultaneous the process of saccharification and fermentation (SSF). The advantages of this method are direct conversion of monosaccharide into ethanol, decrease of cost, and reduction of contamination processes. The purpose of this research is to investigate the effect of *Marasmius* sp. on delignification of sugarcane bagasse for bioethanol production.

2. Experiment

a. Materials and pretreatment by *Marasmius* sp.

White rot fungus *Marasmius* sp. was purchased from the Biological Sciences Laboratory, Biological Department of Institute Technology of Bandung, Indonesia. Sugarcane bagasse was obtained from PT Madubaru, Bantul, Yogyakarta.

The fungus *Marasmius* sp. was grown on MEA (malt extract agar) for 7 days, then cultivated into sugarcane bagasse, and then incubated for various time periods (15, 30, and 45 days) at 25ºC with triplicate measurement. Pretreated bagasse was washed by fresh water and then dried under the sun.

Lignin, cellulose, and hemicellulose contents of bagasse before and after treatment were determined by using Chesson method [11].

b. Ethanol Production using Simultaneous and Fermentation (SSF) Process

Saccharomyces cerevisiae was obtained from Microbiology Laboratory, Inter University Center, Gadjah Mada University, Yogyakarta. It was cultivated on *Potato Dextrose Agar* (PDA) and then

incubated for 1-3 days at 28°C. Three needles dose of *S. cerevisiae* were transferred into flask containing 50 mL of yeast inoculum and then incubated for 24 hours at 30°C.

Five grams of bagasse were transferred to Erlenmeyer flask, containing 70 ml medium with composition of $(NH_4)_2PO_4$(1,0 g l^{-1}), $MgSO_4.7H_2O$ (0.05 g l^{-1}), and *yeast extract* (2 g l^{-1}). Furthermore, sodium citrate buffer (pH 5) and 0.5% HCl (10% (v/v)) were added to the mixture. It was sterilized for 20 minute at 121°C. Twenty fpu (20 fpu) of xylane enzyme and 15% (v/v) inoculum of *S. cerevisiae* were added on the Erlenmeyer flask. The flask was incubated for various time periods (3, 6, 9, 12, and 15 days) at 25°C. The conversion of sugarcane bagasse into bioethanol was performed at pH 5, which was controlled by sodium citrate buffer as the best condition for bioethanol production [10].

Bioethanol concentration was determined by GC HP 5890 FID col-CBP 525 m in Chemistry Department Laboratory, Gadjah Mada University,Yogyakarta.

c. Characterization of sugarcane bagasse

Characterization of bagasse structures before and after treatment was determined by using Fourier Transform Infrared (FTIR) in Chemistry Department Laboratory, Gadjah Mada University, Yogyakarta. FTIR spectra were recorded on Shimadzu spectrophotometer with detector of 16 cm^{-1} resolution and 10 scans per sample.

3. Results and Discussions

Delignification of lignin is important because it retains the hydrolysis of hemicellulose to monosaccharide by xylanase. Bio-delignification by using white rot fungi is one of the treatments for lignin breaking. These fungi will produce enzymes such as lignin peroxidase (LiP), manganese-depent peroxidase (MnP), and laccase to degrade phenolic subunits on lignin [9]. The effects of fungus pretreatment with variation of time periods on sugarcane bagasse were shown in Table 1.

The decrease of lignin and increase of cellulose contents are two main indicators of delignification process. This research showed that fifteen (15) days incubation time gave better result than 30 and 45 days incubation time. This research proposed that longer incubation time altered the pH and temperature of the system, which affected the enzymes production by *Marasmius* sp. There was a decrease of lignin content of sugarcane bagasse after pretreatment to 13% of the control, while hemicellulose and cellulose contents increased by 7.38% and 10.66%, respectively. The cellulose contents of sugarcane bagasse after 15, 30, and 45 days of incubation were significantly different to that of the control. On the other hand, the lowest lignin content was obtained at 15 days of incubation.

Tab. 1 Contents of sugarcane bagasse before and after pretreatment by *Marasmius* sp.

Incubation time (days)	Contents of sugarcane bagasse		
	Lignin (%)	Hemicellulose (%)	Cellulose (%)
0 (control)	18,47 [b]	24,78 [b]	46,89 [b]
15	16,04 [c]	26,61 [a]	51,89 [a]
30	19,43 [a]	26,61 [a]	50,48 [a]
45	19,48 [a]	24,05 [b]	50,67 [a]

Note: Numbers in columns followed by same letter indicates not significantly different according to Duncan's multiple range test at 5% level.

The IR spectrum showed differences in sugarcane bagasse between before and after pretreatment by white rot fungus *Marasmius* sp. as shown in Fig. 1. The IR spectra showed absorptions due to hydroxyl stretching at 3400-3500 cm^{-1} and methylene stretching at 2900 cm^{-1} [8]. The increase of peak intensity at 1250-1500 cm^{-1} was related with cellulose increment content after pretreatment of sugarcane bagasse by *Marasmius* sp. [4], and the increase of peak intensity at 1056 cm^{-1} showed C-O-C stretching of β-(1-4) glycoside chain. These differences showed that there are the structural change caused by the fungus treatment.

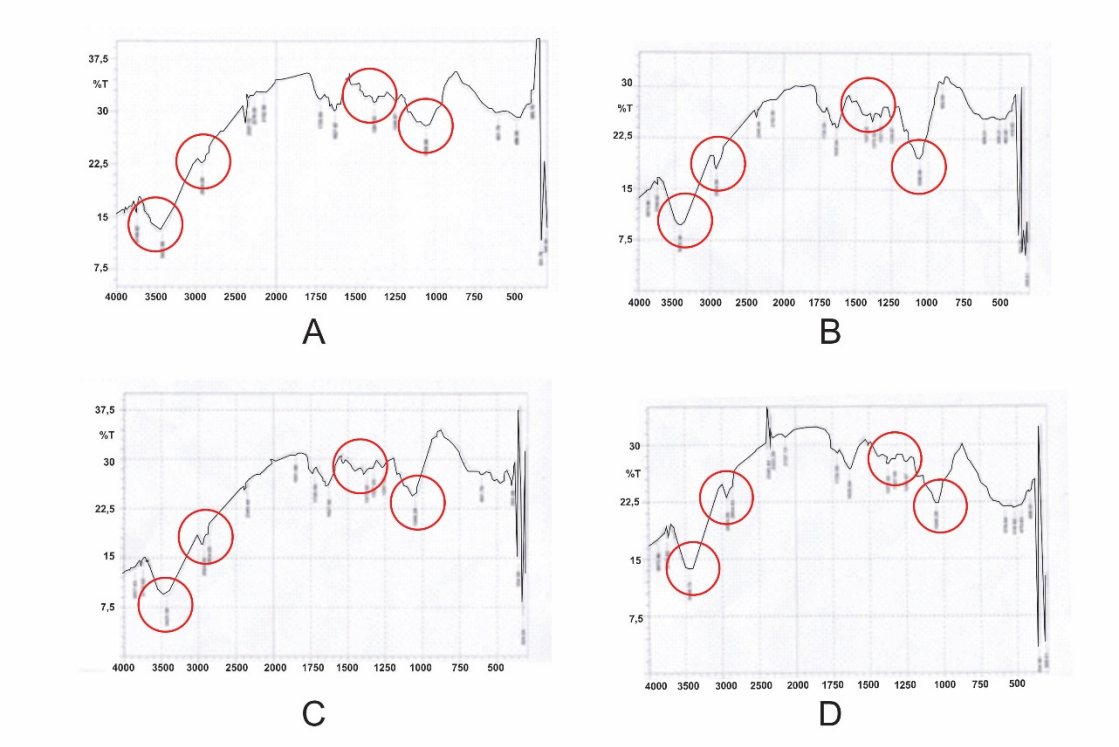

Fig. 1 Bagasse FTIR Spectra: A. Raw bagasse (control); B. *Marasmius* sp.15 days pretreatment; C. *Marasmius* sp. 30 days pretreatment; D. *Marasmius* sp. 45 days pretreatment

The bioethanol was obtained as 0.85% (0.4 g/L) in 3 days by SSF process, and there were no additional ethanol productions for the next incubation time. The SSF process after 6, 9, 12, and 15 days of incubation could not produce bioethanol because the activity of *S. cerevisiae* decreased due to the formation of acetic acid and furfurals, which inhibited the activity of yeast [12]. During the process of saccharification and fermentation, polysaccharide were converted to monosaccharaides by using xylanase enzyme and 0.5% HCl, and further those monosaccharaides are fermented to produce bioethanol by *S. cereviceae* [13].

4. Conclusion

The best condition for bagasse delignification by *Marasmius* sp was 15 days of incubation with the lowest lignin content of 16.04%. The bioethanol was obtained in 3 days with bioethanol concentration of 0.85% (0.4 g/L).

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REFERENCES

[1] T. Anindyawati. Prospek Enzim dan Limbah Lignoselulosa untuk Produksi Bioetanol, *Berita Selulosa*, 44(1): 49-56, 2009.

[2] B. Buaban, *et. al*. Bioethanol Production from Ball Milled Bagaase using an On-site Produced Fungal Enzyme Coctail and Xylose-Fermenting. *Pichiastripitis*, *J. Biosch. Bioeng*, 110(1): 18-25, 2010.

[3] L. L. G. Fuentes, S.C. Rabelo, R. M. Filho, and A. C. Costa. Kinetics of Lime Pretreatment of Sugarcane Bagasse to Enhance Enzymatic Hydrolysis. *Appl. Biochem. Biotechnol*, 163: 612-625, 2011.

[4] R. Sindhu, *et. al*. Formic Acid as Potential Agent for the Convertion of Sugarcane Bagasse to Bioethanol. *Appl Biochem Biotechnol*, 162: 2313–2323, 2010.

[5] E. Hermiati, *et. al*. Pemanfaatan Biomassa Lignoselulosa Ampas Tebu untuk Produksi Bioetanol. *Jurnal Litbang Pertanian*, 29 (4): 121-130, 2010.

[6] M. J. Taherzadeh, and K. Karimi. Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A Review. *Int. J. Mol. Sci*, 9: 1621-1651, 2008.

[7] N. Yildirim, and A. Yildiz. The Effectiveness of *Pleurotuseryngii* Strains in Biodelignification of Cotton Stalk, Causing Environmental Hazard and Disposable Problem. *Int. J. Agic. Biol*,13: 257-260, 2011.

[8] C. N. Hamelinck, G.V. Hooijdonk, and A. P. C. Faaij. Ethanol from LignocellulosicBiomass: Techno-economic Performance in Short, Middle, and Long Term. *Biomass& Bioenergy*, 28: 384-410, 2005.

[9] A. Kuila, M. Mukhopadhayay, D. K. Tuli, and R. Banerje. Production of Ethanol from Lignocellulosics : A Enzymatic Venture. *EXCLI Journal*, 10: 85-96, 2011.

[10] S.H. H. Risdianto, P. W. Suhardji, Nilo, and T. Setiadi. *Produksi Lakase dan Potensi Aplikasinya dalam Proses Pemutihan Pulp. Berita Selulosa*. 43(1):1-10, 2006.

[11] R. Datta. Acidogenic Fermentation of Lignocellulose-acid Yield and Convertion of Component. *Biotechnology and Bio engineering*, 23(9): 2167-2170, 1981.

[12] C. Bellido, *et. al*. Effect of Inhibitors Formed During Wheat Straw Pretreatment on Ethanol Fermentation by *Pichiastipitis*. *Bioresource Technology*. 102(23): 10868-10874, 2011.

[13] M. Samsuri, *et.al*. Pemanfaataan Selulosa Bagas untuk Produksi Ethanol Melalui Sakarifikasi dan Fermentasi Serentak dengan Enzim Xylanase. *MakaraTeknologi*, 11(1): 17-24, 2007.