International Journal of Agricultural Research

ISSN 1816-4897

Academic Journals Inc.

www.academicjournals.com

International Journal of Agricultural Research 4 (8): 250-256, 2009
ISSN 1816-4897

Delignification of Oil Palm Empty Fruit Bunch using Chemical and Microbial Pretreatment Methods

[1]A.F. Hamisan, [1]S. Abd-Aziz, [2]K. Kamaruddin,
[1]U.K.Md. Shah, [2]N. Shahab and [1]M.A. Hassan
[1]Department of Bioprocess Technology,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia, 43400, UPM Serdang Selangor, Malaysia
[2]Environment and Bioprocess Technology Centre, SIRIM Berhad, No. 1,
Persiaran Dato' Menteri, Section 2, 40911 Shah Alam, Malaysia

Abstract: In this study, Oil Palm Empty Fruit Bunch (OPEFB) were subjected to chemical and microbial pretreatment for bioconversion of lignocellulosic biomass to fermentable sugars. For chemical pretreatment, 2% (w/v) sodium hydroxide (NaOH) was been used for delignification while for microbial pretreatment, *Phanerochaete chrysosporium* ATCC 32629 was used as model microorganism by liquid and solid state culture techniques. Microbial pretreatment showed significant lignin removal with longer delignification time as compared to chemical pretreatment. For the same value of Klason lignin, delignification by chemical pretreatment need only 3 h as compared to 7 days for microbial pretreatment. The optimum value of Klason lignin for microbial pretreatment and chemical pretreatment were 5.89 and 5.93, respectively. In conclusion, delignification of OPEFB can be achieved via chemical and microbial pretreatment.

Key words: Oil palm empty fruit bunch, delignification, chemical pretreatment, microbial pretreatment, Klason lignin

INTRODUCTION

Malaysia's oil palm industry has grown rapidly over the last four decades and it becomes very important agriculture-based industry. Since, then the country has maintained her position as the world leading palm oil producing country. Malaysia is blessed with favourable weather condition, which prevails throughout the year and advantageous for oil palm plantation. Nevertheless, the industry has also generated vast quantity of palm biomass, mainly from milling and crushing palm kernel. The main by-product and waste are Empty Fruit Bunches (EFB), Palm Oil Mill Effluent (POME), palm fibre and palm kernel shell.

Oil Palm Empty Fruit Bunches (OPEFB) as a lignocellulosic residue has potential as a cheap renewable feedstock for large scale production of bioproduct. Each year, more than 15 million tonnes of OPEFB were generated by palm oil industry in Malaysia (Rahman *et al*., 2007).

Corresponding Author: Dr. Suraini Abd-Aziz, Department of Bioprocess Technology,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
Tel: +6-03-89467479 Fax: +6-03-89467510

Bioconversion of lignocellulosic to bioethanol employs three major steps which include, pretreatment of OPEFB for breakdown lignin and open up crystalline structure of cellulosic materials, cellulosic hydrolysis using combination of enzymes for fermentable sugar (polyoses) production and bioconversion of fermentable sugar (polyoses) produced to bioethanol. Cellulose degradation by enzymatic hydrolysis is a reaction carried out by cellulase enzymes, which are very specific. Three major groups of cellulase are involved in the hydrolysis process of cellulose (Cao and Tan, 2002) and these are:

- β-1-4-endoglucanase, which attack low crystalline region in the cellulose fiber creating free chain ends
- β-1-4exoglucanase or cellobiohydrolase, which degrades the molecule further by removing cellobiose units from the free chain ends
- β-glucosidase or cellobiase, which hydrolyzes cellobiose to produce glucose

In the pretreatment of OPEFB, physicochemical technologies such as steam explosion, dilute acid, alkali and oxidant or their combination are several existing pretreatment method have mostly been developed by Moiser *et al*. (2005). However, physical and chemical pretreatment involve high energy (electricity and steam) and corrosion-resistant, high pressure reactors, which raise the cost of pretreatment operations and equipment usage (Shi *et al*., 2009a). In addition, chemical pretreatment can be unfavourable to successive of enzymatic hydrolysis and microbial fermentation apart from producing acidic or alkaline waste water which needs predisposal treatment for environmental safety (Keller *et al*., 2003).

Microbial pretreatment which employs microorganism especially fungi and their enzymes system for degradation of lignin present in the lignocellulosic biomass is a gentle substitute to harsh chemicals pretreatment. Fungal pretreatment has been formerly explored to improve cellulosic materials for feed and paper applications (Hadar *et al*., 1993). Recently, this environmental friendly approach has received renewed attention as a pretreatment technique for enhancing enzymatic saccharification and fermentation of lignocellulosic biomass to bioethanol (Camarero *et al*., 1994).

The white-rot basidiomycetes fungus, has attract an interest since it efficiently degrade lignin. Microbial pretreatment has potential advantages over the existing physicochemical pretreatment technologies due to reduced energy and material cost, relatively simple equipment and biological catalyst utilization (Keller *et al*., 2003). Though, the practicability of microbial pretreatment is still questioned essentially due to the really long duration of pretreatment as well as the complexity in selective degrading of lignin (Hatakka, 1983). *Phanerochaete chrysosporium* has high growth rate compared to many other basidiomycetes, exceptional oxidation potential and efficiency for lignin biodegradation (Singh and Chen, 2008). *Phanerochaete chrysosporium* delignification abilities could be enhanced by optimizing the media and cultivation methods to increase ligninolytic enzyme production (Reddy and D'Souza, 1994).

The objective of this study was to evaluate the lignin content of OPEFB by using kappa number after chemical pretreatment using dilute sodium hydroxide (NaOH) as compared with fungal pretreatment using *Phanerochaete chrysosporium* ATCC 32629 as model microorganism by liquid solid culture techniques. The production of lignin peroxidase in liquid and solid state fermentation was compared.

MATERIALS AND METHODS

Laboratory Scale Fermentation

This study was conducted in laboratory scale at Institute of Bioscience and Biomass Technology Center, Universiti Putra Malaysia and SIRIM Berhad, Malaysia, from October 2008 to April 2009.

Substrate

The biomass used in this study is shredded OPEFB. It was obtained from Sri Ulu Langat Palm Oil Mill at Dengkil, Selangor, Malaysia. The raw substrate was kept in the cold room at 4°C to avoid contamination. The OPEFB was soaked in detergent for overnight to removed any residual oil and then sun-dried. The OPEFB was ground by a hammer mill and kept in a dry place.

Organism and Cultivation Condition

Phanerochaete chrysosporium ATCC 32629 was purchased from American Type Culture Collection. Potato Dextrose Agar (Difco) was used for strain maintenance and sporulation.

Vegetative Inoculum

Spore production in slant required 5 days of growth at 39°C. Spores (conidia) are prepared by suspension in sterile water followed by passage through sterile glass wool. Spore concentration is determined by using spore count method (5×10^6 spores mL^{-1}). Two days old vegetative inoculums cultured in Potato Dextrose Broth was used for both liquid fermentation and solid state fermentation.

Cultivation Mode

Solid State Fermentation (SSF) was carried out in 250 mL Erlenmeyer flask. Two grams of untreated OPEFB was wetted with 8.45 mL media, setting the moisture content to approximate 80% (wet basis). The composition for lignin peroxidase and manganese peroxidase production was based on modified Basal III media (Tien and Kirk, 1988) supplemented with untreated OPEFB. Fermentation was done at temperature 39°C.

For submerged liquid fermentation, 100 mL media and 2 g of untreated OPEFB was used. Inocula size was fixed at 2% (v/v) using 2 days vegetative inoculum. Media composition and Erlenmeyer flask size were similar to SSF set up.

Chemical Pretreatment

The OPEFB was delignified using NaOH. Two grams of untreated OPEFB was mixed together with 200 mL of 2% (w/v) NaOH. The mixture was heated at 90°C for 1, 2 and 3 h, respectively.

Lignin Content Analysis

The kappa number analysis and Klason lignin determination was used to measure the extent of delignification (TAPPI, 1999). Kappa number based on potassium permanganate solution consumed by a moisture-free pulp under specific conditions. The percentage of Klason lignin approximately was determining based on Technical Association of the Pulp and Paper Industry (TAPPI, 1999).

Peroxidase Activity

Lignin peroxidase (LiP) and manganese peroxidase (MnP) activities were measured according to the procedure of Castillo *et al*. (1994) using the substrate know as 0.167 mM 3-methyl-2-benzothiazolinone hydrazone (MBTH) upon mixing with 2.37 mM 3-(dimethylamino) benzoic acid (DMAB) to produced a purple colour solution. Substrates were prepared using succinic-lactic acid buffer at pH 4.5. 3 mM of $MnSO_4$ were used in order to determine the dependency of manganese and peroxidase in the culture. Reaction were carried out at 37°C and monitored by spectrophotometer at 590 nm for a min.

RESULTS

Alkaline Pretreatment

The lignin content of OPEFB after alkaline pretreatment using 2% NaOH is shown in Table 1. After soaking OPEFB in 2% NaOH solution at temperature 90°C, the kappa number measured was 61.32 and lignin content (Klason lignin) was 7.97%. As time increase to 3 h the kappa number decrease to 45.59 and the lignin content was 5.93%. For untreated OPEFB, kappa number could not be obtained due to high content of lignin.

Microbial Pretreatment

For microbial pretreatment, two type of cultivations mode was done, solid state and liquid fermentation. The lignin content of OPEFB after 3 days pretreatment using *Phanerochaete chrysosporium* ATCC 32629 in solid fermentation medium was 8.30% (Table 1). As the time of fermentation increased to 7 days the lignin content decreased to 5.89%. Comparing with liquid fermentation medium, the lignin content obtained after 7 days fermentation was 6.22%.

The higher delignification after 7 days fermentation was due to higher production of total peroxidase, lignin peroxidase and manganese peroxidase by the fungus (Fig. 1). The activities of total peroxidase, lignin peroxidase and manganese peroxidase at day 3 were 3.212, 2.847 and 0.365 U mL^{-1}, respectively, while the activities of total peroxidise, lignin peroxidase and manganese peroxidase at day 7 were 3.521, 2.616 and 0.905 U mL^{-1}, respectively. In liquid fermentation, the fungus produced a much lower activities of total peroxidase, lignin peroxidase and manganese peroxidase (Fig. 2). The activities of total peroxidase, lignin peroxidase and manganese peroxidase at day 3 were 0.812, 0.501 and 0.311 U mL^{-1}, respectively while the activities of total peroxidase, lignin peroxidase and manganese peroxidase at day 7 were 1.115, 0.495 and 0.620 U mL^{-1}, respectively. Result of this study showed that lignin peroxidase production in solid state fermentation (Fig. 1) is higher compared to liquid fermentation (Fig. 2).

Table 1: Kappa number, klason lignin and percentage of lignin removal for chemical and microbial pretreatments

Treatment	Duration	Kappa No.[a]	Klason lignin (%)[b]	Lignin content (%)[c]
2% (w/v) NaOH	1 h	61.32	7.97	1.63
	2 h	47.81	6.22	1.27
	3 h	45.59	5.93	1.21
P.chrysosporium ATCC 32629	3 day	47.77	6.21	1.27
(Solid state culture)	5 day	46.72	6.07	1.24
	7 day	45.32	5.89	1.20
P.chrysosporium ATCC 32629	3 day	63.87	8.30	1.70
(Liquid Culture)	5 day	54.49	7.08	1.44
	7 day	47.81	6.22	1.27

[a]Kappa number estimation was based on titration method (TAPPI, 1999)
[b]Klason lignin is a measurement of percentage residual lignin by equating the Kappa number with factor 0.13 (TAPPI, 1999). Hence, percentage of delignification can be quantified. [c]Assuming lignin content in OPEFB at 20.4% (Khalid *et al*., 1999)

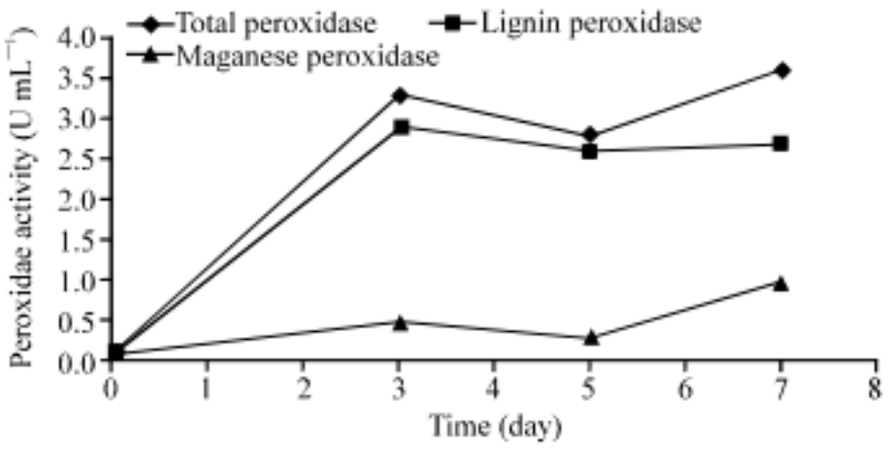

Fig. 1: Peroxidase activity in solid state fermentation

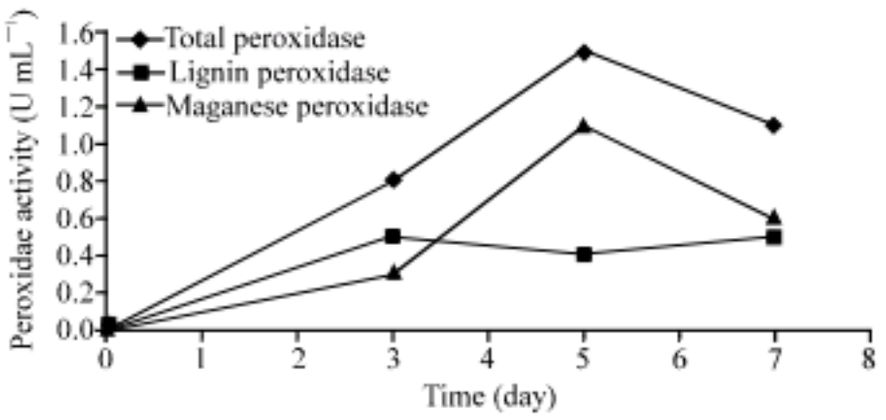

Fig. 2: Peroxidase activity in liquid fermentation

DISCUSSION

Two methods of pretreatment were attempted: Chemical pretreatment using simple heating in 2% (w/v) NaOH and microbial pretreatment using *Phanerochaete chrysosporium* ATCC 32629. For microbial pretreatment, two techniques were studied, solid state and liquid fermentation. Klason lignin and Kappa number (TAPPI, 1999) were used as an extent measurement of delignification processes. These quantification techniques were usually used in pulp and paper industries to evaluate the extent of delignification and residual lignin remaining in the cellulosic fractions prior to paper-making processes.

Compared with acid pretreatment, alkaline pretreatment with NaOH removed more lignin fraction from the biomass because of the solubilisation of lignin in alkaline solution (Chen *et al*., 2009). Delignification still can be achieved by using microbial method. Excreting of unique extra-cellular peroxidase (LiP and MnP) from *Phanerochaete chrysosporium* can decay lignin efficiently (Tien and Kirk, 1988). The growth and metabolism of lignin degrading microbe *Phanerochaete chrysosporium* shows a great effect on microbial pretreatment (Shi *et al*., 2009b). Nutrients supplementation and cultivation conditions affect the production of ligninolytic enzymes during cultivation of the microbes are depend on nutrients supplementation and cultivations conditions. So, the production of the enzymes still can be increase by optimization study. According to Brown *et al*. (1990) the balanced addition of trace metal, such as Mg^{2+}, Ca^{2+} and Mn^{2+} was important for ligninase production. From this study, it showed that higher delignification occurs in solid fermentation as compared in liquid fermentation. Solid state fermentation shown higher efficiency in delignification process due to environmental conditions being more similar to those in nature (Shi *et al*., 2009a).

Although, microbial pretreatment method using *Phanerochaete chrysosporium* ATCC 32629 could delignify OPEFB at the same level as the alkaline pretreatment method, alkaline pretreatment was preferred method, as only 3 h were required to achieve preferable delignification level. Reducing the lignin content is important to expose the crystalline structure of cellulose. However, less time consumed for chemical pretreatment means it is quite harsh for the substrate. According to Hendriks and Zeeman (2009) the result of the pretreatments is dependent on the biomass composition and operating conditions. As a result, microbial pretreatment showed significant of lignin removal, but timely as compared to chemical pretreatment.

CONCLUSION

Delignification can be achieved either by chemical pretreatment using 2% NaOH or via microbial pretreatment using *Phanerochaete chrysosporium* ATCC 32629. However, in microbial pretreatment, lignin removal is dependent on the microbial ability either to consume lignin or to produce biological products such as enzymes, to remove lignin. Microbial pretreatment can be used as a gentle process yet, longer time is required (7 days). Chemical pretreatment using 2% (w/v) NaOH can be used as an alternative process of delignification. Furthermore, similar percentage of lignin removal was achieved within less than 3 h.

ACKNOWLEDGMENT

The authors would like to thank the Ministry of Science Technology and Innovation of Malaysia for financially support throughout this research project.

REFERENCES

Brown, J.A., J.K. Glen and M.H. Gold, 1990. Manganese regulates expression of manganese peroxidase by *Phanerochaete crysosporium*. J. Bacteriol., 172: 3125-3130.

Camarero, S., G.C. Galletti and A.T. Martinez, 1994. Preferential degradation of phenolic lignin units by two white rot fungi. Applied Environ. Microbiol., 60: 4509-4516.

Cao, Y. and H. Tan, 2002. Effects of cellulase on the modification of cellulose. Carbohydrate Res., 337: 1291-1296.

Castillo, M.P., J. Stenstrom and P. Ander, 1994. Determination of manganese peroxidase activity with 3-methyl-2-benzothiazolinone hydrazone and 3-(dimethylamino) benzoic acid. Anal. Biochem., 218: 399-404.

Chen, M., J. Zhou and L. Ming, 2009. Comparison of four different chemical pretreatments of corn stover for enhancing enzymatic digestibility. Biomass Bioenerg, 33: 1381-1385.

Hadar, Y., Z. Kerem and B. Gorodecki, 1993. Biodegradation of lignocellulosic agricultural wastes by *Pleurotus ostreatus*. J. Biotechnol., 30: 133-139.

Hatakka, A.I., 1983. Pretreatment of wheat straw by white rot fungi for enzymatic saccharification of cellulose. Eur. J. Applied Microbiol. Biotechnol., 18: 350-357.

Hendriks, A.T.W.M. and G. Zeeman, 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour. Technol., 100: 10-18.

Keller, F.A., J.E. Hamilton and Q.A. Nguyen, 2003. Microbial pretreatment of biomass: Potential for reducing severity of thermo-chemical biomass pretreatment. Applied Biochem. Biotechnol., 105: 27-41.

Khalid, M., C.T. Ratnam, T.G. Chuah, S. Ali and T.S.Y. Choong, 1999. Comparative study of polypropylene composites reinforced with oil palm empty fruit bunch fiber and oil palm derived cellulose. Mate. Design., 29: 173-178.

Moiser, N., C. Wyman, B. Dale, R. Elander, Y.Y. Lee, M. Holtzapple and L. Michael, 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour. Technol., 96: 673-686.

Rahman, S.H.A., J.P. Choudhury, A.L. Ahmad and A.H. Kamruddin, 2007. Optimization studies on acid hydrolysis of oil palm empty fruit bunch fiber for production of xylose. Bioresour. Technol., 98: 554-559.

Reddy, C.A. and T.M. D'Souza, 1994. Physiology and molecular biology of the lignin peroxidase of *Phanerochaete chrysosporiom*. FEMS Microbiol. Rev., 13: 137-152.

Shi, J., R.R. Sharma-Shivappa and M.S. Chinn, 2009a. Microbial pretreatment of cotton stalks by submerge cultivation of *Phanerochaete chrysosporium*. Bioresour. Biotechnol., 100: 4388-4395.

Shi, J., R.R. Sharma-Shivappa, M. Chinn and N. Howell, 2009b. Effect of microbial pretreatment on enzymatic hydrolysis and fermentation of cotton stalks for ethanol production. Biomass Bioenerg., 33: 88-96.

Singh, D. and S. Chen, 2008. The white-rot fungus *Phanerochaete chrysosporium*: Conditions for the production of lignin-degrading enzymes. Applied Microbiol. Biotechnol., 81: 399-417.

TAPPI, 1999. Kappa Number of Pulp Method-T236 om-99. Technical Association of the Pulp and Paper Industry, Atlanta, GA.

Tien, M. and T.K. Kirk, 1988. Lignin peroxidase of phanerochaete chrysosporium. Methods Enzymol., 161: 238-249.