Pertanika J. Sci. & Technol. 25 (S): 1 - 8 (2017)

SCIENCE & TECHNOLOGY

Journal homepage: http://www.pertanika.upm.edu.my/

Ganoderma boninense Efficacy in Delignifying Oil Palm Empty Fruit Bunches

Siti Sarah Jumali* and Shaleha Ismail

Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA (UiTM) Jasin, 77300 Merlimau, Melaka, Malaysia

ABSTRACT

Oil palm empty fruit bunches (EFB) were subjected to microbial pre-treatment of lignocellulosic biomass bioconversion to fermentable sugar. Microbial pre-treatment was carried out by inoculating *Ganoderma boninense* spores through solid state fermentation. The samples were initially treated with Sulphuric acid method prior to reading with UV-Visible Spectrometer. The readings were taken before and after inoculation of EFB with *G. boninense*. Bioconversion of 20 g EFB via solid state fermentation was done in five different amounts of *G. boninense* spore namely 0.0 g (control), 0.5 g (T2), 0.7 g (T3), 0.9 g (T4) and 1.1 g (T5) in 7 days. The result shows the highest delignification in sample inoculated with 1.1g of *G. boninense* spores, in which the spores are successfully reduced by 61.97% of lignin from total EFB biomass in 7 days compared to 60.08% (T4), 58.65% (T3) and 54.85% (T2). Meanwhile, for control the lignin content was reduced by 5.07% in 7 days. The study shows that *G. boninense* has the ability to remove lignin from EFB whereby longer incubation period and higher number of spores contribute to higher delignification percentage.

Keywords: Delignification, *G. boninense*, ligninolytic enzymes, oil palm empty fruit bunch (EFB)

INTRODUCTION

The palm oil industry has contributed significantly to the economic growth of Malaysia for more than four decades with 3.5 million in 2001 or 60% of the total agricultural land in the country (Ming & Chandramohan, 2002). Malaysia is also the largest country planting oil palm in Southeast Asia after Indonesia. Globally, the increase in crude oil palm production was 48.99 million metric tonnes per year in 2011 (Geng, 2013), where Indonesia together with Malaysia contribute 85% of the palm oil production in the world. This constitutes 23%

ARTICLE INFO

Article history:
Received: 28 September 2016
Accepted: 03 February 2017

E-mail addresses:
sarahjumali@melaka.uitm.edu.my (Siti Sarah Jumali),
gempax1992@yahoo.com (Shaleha Ismail)
*Corresponding Author

ISSN: 0128-7680

of world's oils and fats production (Abdullah & Wahid, 2010). In 2011, oil palm is the second most produced fats and oils after soya bean oil in the world in which oil palm produce is 23.6 million tonnes from 20% of the production (Ming & Chandramohan, 2002).

The processing of oil palm renders oil palm wastes demanding waste management strategies. Current practices include the use of oil palm empty fruit bunches (EFB) as mulching as well as biofertilizers after inoculation with certain types of fungi and bacteria. EFB is considered to be a special biomass of the palm oil industry (Ferrer, Vega, Ligero, & Rodriguez, 2011). The lignocellusic content of EFB indicates cellulose at 57.8%, Hemicellulose 21.2% and Lignin 22.8% (Nurhayati & Fauziah, 2013). EFB delignification allows the introduction of other valuable products such as biogas, bioethanol, butanol, biodiesel, hydrogen and compost as delignification allows the stripping off the cell wall and exposing the cellulosic material underneath. The cellulosic material may then be converted through thermo-chemical transformation, hydrolysis of cellulose and hemicellulose to sugars through fermentation.

The structural lignin is among the most abundant structures in the plant as it is responsible for plant organelle protection. It builds up the cell wall along with cellulose and hemicellulose resulting in lignin-carbohydrate network, a recalcitrant structure in the plant. The complexity of lignin causes its isolation and identification to be challenging making *G. boninense* element for a more efficient and environmentally sound deconstruction of plant cell wall (Martinez, Ruiz-Duen, Martinez, del Rio, & Gutierrez, 2009).

This research is focused on discovering the efficiency of *G. boninense* in degrading EFB through solid state fermentation (SSF) using spores of differing amounts to investigate the efficiency of the basidiomycete *G. boninense*.

METHOD

Substrate

The shredded oil palm empty fruit bunch (OPEFB) was obtained from a local palm oil mill in Sime Darby Kempas Melaka, Malaysia. The EFB was soaked in detergent for 24 hours to remove dust and any oil residues. The EFB was washed with distilled water and oven-dried at 80°C for 24 hours. EFB was ground with a grinder and kept in a dry place.

Cultivation of fungus

The locally isolated fungus *G. boninense* was obtained from the Malaysian Palm Oil Board (MPOB) Bangi, Malaysia. The culture was maintained on potato dextrose agar (PDA) and incubated at room temperature (26°C – 28°C) for 7 days.

Experimental Design

Experimental design for the treatment is as shown in Table 1. Each of the flasks was filled with 20 g Oil Palm Empty Fruit Bunch (EFB), 5 ml sterilized distilled water and their respective amount of G. *boninense* spores.

Table 1
Experimental design to determine the effects of G. boninense *spore towards EFB*

Experimental designation	Number of spores (g)
T1 (Control)	0
T2	0.5
T3	0.7
T4	0.9
T5	1.1

Solid State Fermentation (SSF)

Solid State Fermentation (SSF) was conducted in sterile 250 mL Erlenmeyer flasks containing 20 g of oil palm empty fruit bunch (EFB). EFB was poured with 5 mL sterilized distilled water and spore was added according to designated treatment T1 (0.0 g of spore), T2 (0.5 g of spore), T3 (0.7 g of spore), T4 (0.9 g of spore) and T5 (1.1 g of spore). Number of spores were weighed using balance. Fermentation was done at 39°C.

Lignin Content Analysis

1 g of EFB sample was treated with 70% sulphuric acid in a water bath at 80oC for 2 hours. The sample was neutralized with 2% (w/v) NaOH. It was then filtered through Whatman 12 mm filter paper. The lignin content was read using UV-Visible spectrometer of 340 nm wavelength. Lignin content reading was repeated every day for 7 days to investigate on its decomposition.

RESULTS AND DISCUSSION

Basal stem rot in oil palm plantation is a very serious disease for the oil palm industry. It is spread by *G. boninense* and can cause severe economic loss of about 43% within 6 months (Assis, Chong, Idris, & Ho, 2016). This study showed after 7 days of inoculation the lignin content of the empty fruit bunches were reduced to up to 61% arising from the production of Lignin peroxidase (LiP), Manganese peroxidase (MnP) and laccase by *G. boninense* (Goh, Ganeson, & Supramaniam, 2014). Table 2 indicates that the ligninolytic enzymes produced by fungus were effective in reducing the lignin content in the EFB. The evaluation of lignin amount through UV-Visible spectrometer estimation revealed dramatic lignin decomposition in the sample inoculated with *G. boninense* compared to the the uninoculated sample. The significant decrease is related to the damage incurred to the lignin-carbohydrate network in the EFB whereby lignocelluloses are the substrates required for the growth of the white-rot fungus *G. boninense* (Sánchez, Sierra, Carlos, & Díaz, 2011). Comparatively, the lignin content of empty fruit bunches that are not inoculated with *G. boninense* shows a slow and steady decrease of 5.07% in 7 days.

Table 2
Percentage of lignin after day(s) of inoculation

Day(s) after inoculation	Lignin percentage (%)				
	T1	T2	T3	T4	T5
1	1.740	1.707	1.519	1.308	1.173
2	1.701	1.604	1.427	0.958	0.971
3	1.647	1.478	1.219	0.910	0.876
4	1.620	1.307	1.041	0.829	0.769
5	1.633	0.960	0.839	0.754	0.630
6	1.645	0.880	0.760	0.665	0.515
7	1.652	0.771	0.628	0.552	0.446

Table 3
Total reduction percentage of lignin from initial inoculation

Designation	Amount of spores (g)	Percentage of lignin reduction (%)	Rate of reduction (%/day)	Rate of reduction (%/hour)
T1 (Control)	0.0	5.07	0.724	0.030
T2	0.5	54.85	7.835	0.326
T3	0.7	58.65	8.379	0.349
T4	0.9	60.08	8.582	0.357
T5	1.1	61.97	8.853	0.369

Following Solid State Fermentation (SSF), sugar and carbohydrate concentration will rise due to the action of ligninolytic enzymes which catalyses the removal of lignin. SSF technique pose better delignification rate as considerable amount of ligninolytic enzymes are produced quickly with minimum downstream processing due to the smaller amount of impurities (Robinson, McMullan, Marchant, & Nigam, 2001). Research conducted in treating lignin through enzymatic delignification via indirect usage of fungi such as delignification of *Bambusa bambos* resulted in 84% delignification after 8 hours using laccase and cellulose (Kuila, Mukhopadhyay, Tuli, & Banerjee, 2011). Meanwhile in *Saccharum spontaneum*, maximum delignification was 84.67% at 6.21 hour of incubation with laccase (Rajak & Banerjee, 2015) which implies a total reduction of 13.63% per hour. A research conducted on oil palm EFB through an attempt of ionic liquid utilization to delignify EFB showed an overall reduction of 15.5% from the total biomass recorded (Financie, Moniruzzaman, & Uemura, 2016). The study employs the use of ionic liquid 1-ethyl-3-methylimidazolium-diethyl phosphate and commercial laccase attained from *Trametes* sp. as delignification agent. Compared to which the production of ligninolytic enzymes produced by *G. boninense* is found to be far more efficient than *Trametes* sp..

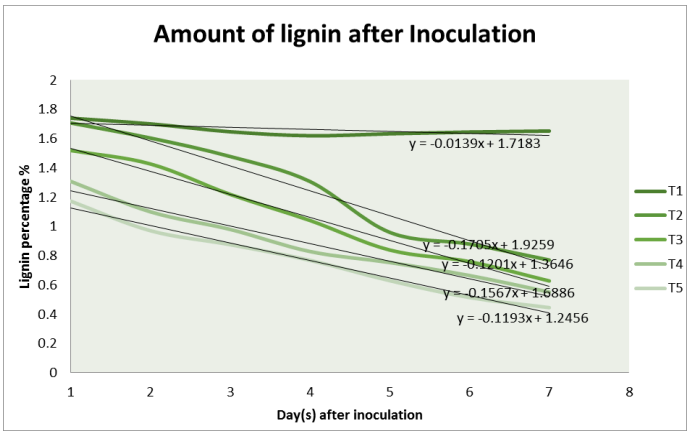

Figure 1. Changes in residual lignin level after inoculation with of *G. boninense* strains

From Figure 1, the highest rate for lignin degradation is shown by the slope T2 where the slope is the steepest at m=-0.1705 followed by T4, T3, T5 and T1. Meanwhile, Table 3 exhibits the total reduction percentage of each lignin from initial inoculation where T5 which is 1.1 g of spores contributed to the highest delignification percentage of 61.97% followed by T4 (60.08%), T3 (58.65%), T2 (54.85%) and T1 5.07%. As expected, the higher the contact with surface areas, the higher the delignified EFB. It strongly suggests the production of ligninolytic enzymes during the course of decomposition. The higher number of spores contribute to

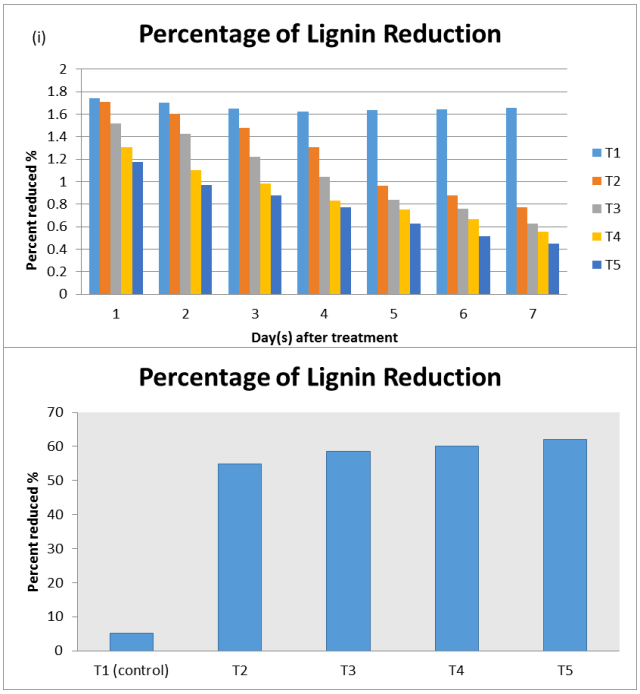

Figure 2. Percentage of lignin reduction

more enzyme production thus more substrates are hydrolysed. Table 3 shows that 1.1 g of *G. boninense* spores is able to reduce as much as 8.853% of EFB per day which is equal to 0.369% per hour. Although the production is slower than that of *S. spontaneum* which degrades 13.63% lignin per hour (Rajak & Banerjee, 2015); the figure represented is still significant due to the locally abundant *G. boninense* and the high disparity of lignin content before and after application. Other research has found out that laccase can be produced to up to 43.07 Ul-1 while Manganese Peroxide 16.21 Ul-1 (Goh et al., 2014).

Microbial biocatalysis for decomposition is relatively inexpensive therefore enzymatic action of microbes is now the predominant feature for biological substitutes to speed up reaction (Grommen & Verstraete, 2002). The fast reaction of its containing enzymes (Robinson et al., 2001) may be further exploited through enzyme extraction for waste management and transforming biomasses into biofuel and energy generation. Researches have demonstrated the outstanding performance of ligninolytic enzymes of basidiomycetes in assisting oxygen delignification of craft pulp, and acting as a substitute to conventional chemical bleaching (Bourbonnais, Paice, Freiermuth, Bodie, & Borneman, 1997). Similar to the other white-rot fungi, *G. boninense* is also a potent bioremediation agent in degrading various xenobiotic compounds, dyes and polymeric products, thus protecting the environment from chemical pollution (Maciel, Silva, & Ribeiro, 2010). The lignonolytic enzymes that is produced may also contribute in pulp and paper industry (Kaur & Nigam, 2014; Górska et al., 2014) as well as benefit agriculture, forestry, sawmill, woodworking, furniture, milling and papermaking industry when produced in a large enough scale (Górska et al., 2014).

CONCLUSION

The white-rot fungi *G. boninense*, although a potent destroyer of oil palm, is also a potential basidiomycete that can promote numerous enzymatic degradation and decomposition of wastes and by-products. The lignocellulases that is produced reduces the need for chemicals and thereby improve environmental hygiene and sustainability.

ACKNOWLEDGEMENTS

The author would like to acknowledge Malaysian Palm Oil Berhad (MPOB), Bangi, Malaysia for the supply of *G. boninense* culture and also Sime Darby Kempas Malacca, Malaysia, and Universiti Teknologi MARA (UiTM).

REFERENCES

Abdullah, R. & Wahid, M. B. (2010). *World Palm Oil Supply, Demand, Price and Prospects: Focus on Malaysian and Indonesian Palm Oil Industry*. Malaysian Palm Oil Board Press: Malaysia.

Assis, K., Chong, K. P., Idris, A.S., & Ho, C. M. (2016). Economic Loss due to Ganoderma Disease in Oil Palm. *International Journal of Social, Behavioral, Educational, Economic, Business and Industrial Engineering, 10*(2), 631-635.

Bourbonnais, R., Paice, M. G., Freiermuth, B., Bodie E., & Borneman, S. (1997). Reactivities of Various Mediators and Laccases with Kraft Pulp and Lignin Model Compounds. *Applied Environmental Microbiology, 63*(12), 4627–4632.

Ferrer, A., Vega, A., Ligero, P., & Rodriguez, A. (2011). Biorefinery of empty fruit. *BioResoures, 6*(4), 4282-4301.

Financie, R., Moniruzzaman, M., & Uemura, Y. (2016) Enhanced enzymatic delignification of oil palm biomass with ionic liquid pretreatment. *Biochemical Engineering Journal. 110*, 1–7.

Geng, A. (2013). Conversion of Oil Palm Empty Fruit Bunch to Biofuels, Liquid, Gaseous and Solid Biofuels - Conversion Techniques, *InTech, 16*, 479-490.

Gellerstedt, G. & Henriksson, G. (2008) Lignins: major sources, structure and properties. M*onomers, Polymers and Composites from Renewable Resources*. (pp. 201-224) Amsterdam: Elsevier.

Goh, K. M., Ganeson, M. & Supramaniam, C. V. (2014). Infection potential of vegetative incompatible Ganoderma boninense isolates with known ligninolytic enzyme production. *African Journal of Biotechnology, 13*(9), 1056-1066.

Górska, E. B., Jankiewicz, U., Dobrzynsyki, J., Gałązka, A., Sitarek, M., Gozdowski, D., Russel, S., & Kowalczyk, P. (2014). Production of Ligninolytic Enzymes by Cultures of White Rot Fungi. *Polish Journal of Microbiology, 63*(4), 461–465.

Grommen, R. & Verstraete, W. (2002). Environmental biotechnology: the ongoing quest. *Journal of Biotechnology, 98*(1), 113–123.

Kaur, S. & Nigam, V. (2014). Production and Application of Laccase Enzyme in Pulp and Paper Industry. *International Journal of Research in Applied, Natural and Social Sciences, 2*(4), 153-158.

Kuila, A., Mukhopadhyay, M., Tuli, D. K., & Banerjee, R. (2011). Accessibility of Enzymatically Delignified *Bambusa bambos* for Efficient Hydrolysis at Minimum Cellulase Loading: An Optimization Study. *SAGE-Hindawi Access to Research Enzyme Research*.

Maciel, M. J. M., Silva, A. C., & Ribeiro, H. C. T. (2010). Industrial and biotechnological applications of ligninolytic enzymes of the basidiomycota: A review. *Electronic Journal of Biotechnology. 13*(6).

Martinez, A. T., Ruiz-Duen, F. J., Martinez, M. J., del Rio, J. C., & Gutie´rrez, A. (2009). Enzymatic delignification of plant cell wall: from nature to mill. *Current Opinion in Biotechnology, 20*, 348–357.

Ming, K. K. & Chandramohan, D. (2002). Malaysian Palm Oil Industry at Crossroads and its Future Direction. *Oil Palm Industry Economic Journal, 2*(2), 10-15.

Nurhayati, A. & Fauziah, S. (2013). The Properties of the Washed Empty Fruit Bunches of Oil Palm. *Journal of Physical Science, 24*(2), 117–137.

Sánchez, O., Sierra, R., Carlos, J., & Díaz, A. (2011). Delignification Process of Agro-Industrial Wastes an Alternative to Obtain Fermentable Carbohydrates for Producing Fuel, Alternative Fuel, *InTech*.

Pilotti, C. A. (2005). Stem rots of oil palm caused by Ganoderma boninense: pathogen biology and epidemiology. *Mycopathologia, 159*(1), 129-37.

Rajak, R. C. & Banerjee, R. (2015). Enzymatic delignification: an attempt for lignin degradation from lignocellulosic feedstock. *Royal Society of Chemistry Advances, 5*, 75281-75291.

Robinson, T., McMullan, G., Marchant, R., & Nigam, P. (2001). Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. *Bioresource Technology, 77*, 247-255.

Yusof, B. (2007). Palm oil Production through Sustainable Plantations. *European Journal of Lipid Science and Technology, 109*, 289–295.