

Effect of Oyster Mushroom on Biodegradation of Oil Palm Mesocarp Fibre

Mohammed Saidu, Afiz Busari, Ali Yuzir, Mohd Razman Salim

Abstract—The problem of degradation of agricultural residues from palm oil industry is increasing due to its expansion. Lignocellulosic waste from these industry represent large amount of unutilized resources, this is due to their high lignin content. Since white rot fungi are capable of degrading lignin, its potential for the degradation of lignocellulosic waste from palm oil industry was accessed. The lignocelluloses content was measured before and after biodegradation and the rate of reduction was determined. From the results of the biodegradation, it was observed that hemicellulose reduces by 22.62%, cellulose by 20.97% and lignin by 10.65% from the initials lignocelluloses contents. Thus, to improve the digestibility of palm oil mesocarp fibre, treatment by white rot-fungi is recommended.

Keywords—Biological, lignocelluloses, oil palm, white rot fungi.

I. INTRODUCTION

LIGNOCELLULOSIC waste is produced in large quantity by different industries. Anwar et al [1] reported that lignocellulosic material is one of the best-used feedstock used for natural and renewable resources. This potential valuable material is treated as waste in many part of the word and is still regarded as such in many developing countries, which raises many environmental concern [2]. In some countries, there have been significant effort of converting this waste to valuable products, but the major constraint of using them is due to its lignocellulose nature. This waste is generated in large quantity through several agricultural activities mainly from agro-based industries [3]. Lignocellulosic waste from this industry is not properly disposed thereby causing environmental problem. These agro wastes are classified as lignocellulosic materials containing three main components as cellulose, hemicellulose, and lignin [4]. Cellulose exists in form of D-glucose subunits, linked by β -1, 4 glycosidic bonds [5], which is bound together by intermolecular hydrogen bond. Hemicellulose has a complex carbohydrate structure which consists of different polymers like hexoses, pentoses, and sugar acids [6] while Hemicellulose is the second most available polymer (20-50% of lignocelluloses material) and is different from cellulose because it is not chemically homogenous [7]. Palm oil industry is one of the major types of agro industries that produce lignocellulosic material. A palm

oil mill industry produces waste after extraction of the crude palm oil. Over 6.0×10^2 million tons of harvestable palm oil biomass is produced annually worldwide [8], but only about 10% of these is used as finished product. The remaining, consisting of mesocarp fibres, empty fruit bunch, fronds, trucks, kernels, palm oil mill effluent, are discarded as waste. Although, these wastes can be converted to a valuable product, due to its lignocellulose content, it is usually avoided because of the expected low valuable product yield when converted. The primary challenge of converting it using biological method is due to lignocellulose contents, which consist of three major components: cellulose, hemicellulose, and lignin. The presences of these components bring about low yield due to low accessibility of micro crystalline cellulose fibres and presences of lignin and hemicellulose on the surface of the cellulose, which prevents cellulase from assessing the substrate efficiently [9].

Oil palm mesocarp fibre is readily available in Malaysia but due to its non-easily degradability; it is hardly used for generation of renewable energies. Since the present world economy is highly dependent on various sources of energy [10], oil palm mesocarp fibre can be valuable in this regard. It can be used as a substrate for the cultivation of edible mushroom. In [11], it was shown that white rot fungi can be cultured on oil palm mesocarp fibre to produced edible mushroom (*Pleurotus* spp). White rot fungi degradation is performed by complex mixtures of cellulases [12], hemicelluloses [13] and ligninase [14], [15]. White rot fungi degrade all wood fractions, including lignin and leave the wood with fibrous appearance of whitish material [16]. Since the fungi obtained its nutrient during decomposition of agricultural by-products, during this process the lignin are used as feed for the fungi, which leads to reduction of lignocellulose materials. White rot fungi is unique in its process biodegradation by mineralize lignin by ligninolytic enzymes [16].

Due to its abundance and renewability, there has been a lot of interest in utilizing lignocellulose oil palm mesocarp fibre inclusive for the production and recovery of many value-added products [17]-[19]. Just like any other lignocellulosic material including activated carbon, biogas, ethanol, animal feeds, fertilizer and other miscellaneous products, many products can be recover from oil palm mesocarp fibre [20], [21]. Apart of producing these products, it is also a source of environmental waste removal [22]. However, due to its longer period of hydrolysis, the usage, when compared to other raw materials, is not much. However, fungi and bacteria have been heavily employed for their abilities to produce a wide range of

Mohammed Saidu and Afiz Busari are with the Civil Engineering Department, Federal University of Technology, Minna, Niger State, Nigeria (corresponding author, phone:+2348034506678; e-mail: mohammed_saidu@futminna.edu.ng).

Ali Yuzir and Mohd Razman Salim are with the Department of Environmental Engineering, Faculty of Civil Engineering, Universiti Teknologi, Malaysia, 81310, Skudai, Johor Bahru, Johor, Malaysia.

cellulose and hemicellulases. Therefore, since fungi are capable of producing cellulases and hemicellulases which are secreted to the medium for easy extraction and purification [23], they can be used to hasten the hydrolysis process. Hence, cultivation of oil palm mushroom, using oil palm mesocarp fibre as a substrate has an impact on the biodegradation of the materials, and brings about improvement of digestibility [24]. The aim of this research was therefore to determine the potentials of using edible mushroom (*Pleurotus* spp.) for biological treatment of oil palm mesocarp fibre to enhance degradation.

II. MATERIALS AND METHODS

A. Fungi Strains and Culture Media

The fungi strains culturing was conducted at Mushroom Ambra Biotech Sdn Bhd, Kulai Jaya, Johor Bahru, Malaysia. The culture media of *Pleurotus* spp. was prepared as explained by [11]. Pure culture of *Pleurotus* spp. for the spawn preparation was prepared on wheat grain and the procedure by [25] for spawn preparation was adopted. Therefore, grains were covered by the mycelium, which rapidly colonizes the substrate [26].

B. Substrate Preparation for Cultivation

The oil palm mesocarp fibre used for this research was collected from Tai Tak Palm Oil Mill, Kota Tinggi, Johor State, Malaysia. The substrate was spread to dry and the impurities were removed manually. It was then milled by electric miller to reduce the particle size to less than 2 mm. A total of 10 kg was used and mixed to different ratios as shown in Table I. Sample A (100% mesocarp fibre), sample B (88% mesocarp fibre, 10% rice bran and 2% lime), Sample C (85% mesocarp fibre 10% rice bran, and 5 % lime), and Sample D (50% mesocarp fibre, 38% saw dust, 10% rice bran and 2% lime). The constituents of each sample were thoroughly mixed. Rice bran was added to balance carbon/nitrogen ratio and lime to maintain the pH of the mixture. The mixtures were then transferred to polypropylene bags of 15 by 30 cm long. The top of the bags were fitted with PVC necks, which served as the opening, and then covered with brown paper. The bags filled with the mixtures were taken to the autoclave and sterilized at 130°C for 4 hours. After sterilization, the bags were left to cool and later inoculated with prepared spawn: 20 g of spawn to 1.3 kg of the mixture. The bags were then marked and stored on the shelf with an indirect sun light at a temperature of 26°C under 80 to 85 % relative humidity.

C. Biodegradation Measurement

The lignocellulose contents determination was conducted at the Laboratory of Environmental Engineering Department, University Teknologi Malaysia. The analysis was carried according to [27] method and as modified by [28]. 1 g of lignocelluloses shredded material was suspended in 100 ml distilled water, and maintained at 100°C for 2 hours in a water bath and filtered on a tare crucible. The residue was dried at 90°C until constant weight. Weight loss was considered as water-soluble part. Dry residue was suspended in 100 ml of

0.5 M H₂SO₄ and maintained for 2 hours at 100°C in a water bath. The content was filtered, dried, and weighed as described above and the loss in weight was term as the hemicelluloses content. For cellulose and lignin estimation, 10 ml of 72% (v/v) H₂SO₄ was added to the above dried residue and maintained at 30°C for 1 hour on a rotary shaker at 200 rpm. After incubation, the mixture was diluted to up to 4 % of H₂SO₄ and autoclaved at 1.06 kg/cm² for 40 min. The content was filtered, dried, and weighed. The loss in weight was recorded as cellulose and the remainder residue considered as lignin. The same procedure was performed on the culture substrate to determine the loss in hemicelluloses, cellulose, and lignin and percentage loss was determined.

D. Statistical Analysis

The results obtained were statistically analysed using analysis of variance (ANOVA), and tests of significance carried out by Duncan's multiple range tests [29] at P≤0.05.

III. RESULTS AND DISCUSSION

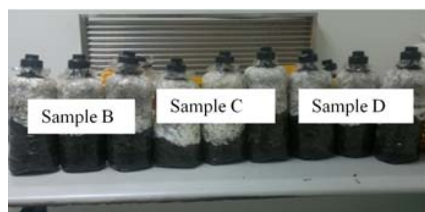
Lignocellulose complex in oil palm mesocarp fibre (OPMF) and other plants residue is degraded very slowly by ruminants because of the physical barrier imposed by lignin polymers, which prevents free access of hydrolytic enzymes such as cellulases and hemicellulases to their substrates [30]. Hence, hemicellulose degradation is required before efficient lignin removal can commence. The mean initial pH of the mixed substrate was 10.6, but after disinfection and during the mycelium growth, the pH drop to 7.7 which was due to microbial activities which makes the carbon-nitrogen ratio to balance. Usually pH during culturing is an index of fungal activity and vice versa [24]. Fig. 1 (a) shows Sterilized substrates inoculated with different mixture. Sample A contained 100% mesocarp fibre showed a slow rate of mycelium running rate of 4.0 cm for the first week as compared to other spawn running time of samples B, C, and D which were 5.5, 6.4, and 6.2 cm, respectively,. They showed a faster rate of mycelium growth during the spawn running. Sample A was attributed to its high water holding capacity of 75%, high oil content of 6.7%, lack of nitrogen to balance the C/N ratio and the lime to maintain the pH. While samples B, C, and D were mixed with a supplement and added with lime of 2%, and 5%. The added supplements balanced the C/N ratio to 30.4, 30.1, and 29.8 for samples B, C and D respectively as shown in Fig. 1 (b) and final mycelium cover is shown in Fig. 1 (c). In a related study, it was established that low yield and slow spawn running during cultivation of oyster mushroom was due to the non-additional supplement to balance the production process [31]. Hence, sample C gave a faster rate at the beginning and with higher growth rate between the second week and the third week in mother culture, and it completely covered the substrate at fifth week of the inoculation and reduced the rate lignocellulosic material in the substrate.

The mycelium growths of the different mix ratios are shown in Table I. The results showed that samples C and D moved faster than sample A and B during the mycelium running time. However, sample C running time was faster during the third

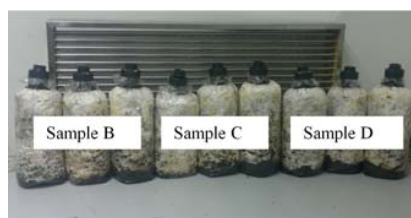
week, which indicates that the mixtures in these samples were better.



(a)



(b)



(c)

Fig. 1 (a) Sterilized substrates inoculated with different mixture, (b) Downwardly growing mycelium, (c) Downwardly growing mycelium at final week

However, sample A showed a slow spawn running time compared to other samples. This is due to non-addition of other substrate and lime to balance C/N ratio and pH [31]. It is known that the lignin C/N ratio and N contents of residue normally affect the rate of decomposition [32], [33]. From the colonization rates, the mycelial growth rates in samples B and C were found to be best for the substrate mixture for Oyster mushroom (*Pleurotus* spp.) culturing on palm oil mesocarp fiber. The effect of using a substrate that has non-mixture shows a slow mycelium running time.

Biodegradation pattern of palm oil mesocarp fibre during biological pretreatment with *Pleurotus* spp. were evaluated. The spectrum of mesocarp fibre pretreatment with *Pleurotus* spp. revealed a proportional decrease in the lignocellulose contents. From Fig. 2, the raw lignocellulose mesocarp fibre shows 35.1% of hemicellulose, 29.95% of cellulose, and 21.62% of lignin. Although, hemicellulose, cellulose, and lignin are the main constituents of lignocellulose materials, it still has some primary polymers. Hemicellulose is heteropolysaccharide composed of different hexoses, pentoses, and glucuronic acid, which makes it more soluble than cellulose, while lignin is highly irregular and soluble polymer consisting of phenylpropanoid subunits namely *p*-

hydroxyphenyl (H-type), guaiacyl (G-type), and syringyl (s-type) units.

TABLE I
WEEKLY SPAWN RUNNING TIME WITH DIFFERENT MIX RATIO

Sample	Mix ratio (%)	Mycelium running per week (cm)				
		1 st week	2 nd week	3 rd week	4 th week	5 th week
A	100 (MF ONLY)	4.00	5.50	5.60	5.78	5.86
B	88:10:2 (MF:RB:L)	5.5	14.00	21.20	25.30	27.00
C	85:10:5 (MF:RB:L)	6.4	14.3	21.30	25.50	27.10
D	50:10:38:2(MF:RB:SD:L)	6.20	13.80	21.0	25.40	26.90

Note: MF, Mesocarp Fibre; RB, Rice Bran; SD, Saw Dust; L, lime.

The results (Fig. 2) of the biological degradation of the OPMF show that there was reduction in the content of hemicellulose, cellulose, and lignin. According to [34], lignocellulose is not dependent on environmental conditions alone, but also the degradation capacity of microbial population. Hemicellulose shows 10.91% remaining on the substrate, which indicates 24.17% reduction, the pattern of hemicellulose degradation of cellulose, since biodegradation reduced the cellulose content by 29.97%.

Lignin degradation by white rot-fungi (*Pleurotus* spp.) is an oxidative process and phenol oxidases are the enzymes [35], [36]. The lignin reduced after the biodegradation by 10.65%, indicating that the hemicellulose and cellulose are now exposed to any form of degradation. The enzymes act and remove hemicellulose-lignin association without mineralization of the lignin [37], but lignocellulose is essentially a race between cellulose and lignin degradation. It shows that the treated OPMF can be better hydrolyzed and converted to other usage than the untreated one. The removal of lignin gives a better accessibility for cellulose degradation. These results confirm the finding of [38]. It shows a better degradation within the period of fungi cultivation, since lignin is responsible for integrity, structural rigidity, and prevention of swelling lignocelluloses [39].

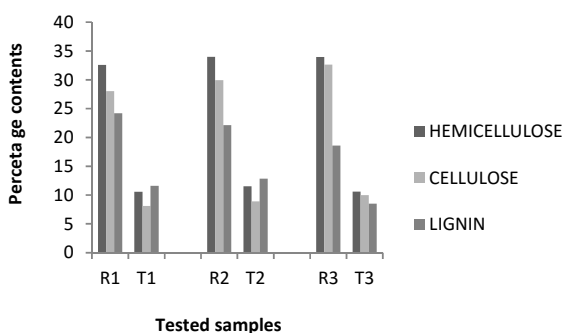


Fig. 2 Level of biodegradation of lignocellulosic materials

The degradation of POMF by *Pleurotus* spp., which resulted to average of about 10% reduction of lignin after 30 days of incubation, is a selective degradation system by the fungi. The results presented in Fig. 2, together with spawn

running time, suggested that the edible mushroom grown on the POMF play an important role in lignin degradation.

Statistical analyses of the variation on the rate of biodegradation on samples A, B, C and D with that WSC were performed by one-way ANOVA test and showed a significance difference of $P < 0.05$.

The difference may be due to the non-supplement added to sample A which showed no mycelium run in the column bag of the sample.

IV. CONCLUSION

Due to the increase in palm oil production in Malaysia, more waste is produced which contribute to environmental problems. Their bioconversion potential is problem because of lignocellulose content. Treating of these agricultural wastes before any bioconversion will help in reducing the lignocellulose of the materials. The research serves as a system of upgrading lignocellulosic waste. One potential of the study is also the production of edible mushroom (*Pleurotus* spp.) which also improved the digestibility of the substrate.

REFERENCES

- [1] Anwar, Z., Muhammad, G., Muhammad I. "Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: A brief review". 2014. *J. Rad. Appl. Sci.* 7 163-173.
- [2] Palacios-Orueta, A., Chuvieco, E., Parra, A. and Carmona-Moreno, C. Biomass Burning Emissions: "A Review of Models Using Remote-Sensing Data". *Environ. Model. Assess.* 2005. 104(1), 189-209.
- [3] Pe'rez J. Mun "oz-Dorado de la Rubia T, Marti'nez J. "Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview". 2002. *Int. Microbiol.* 5: 53-63.
- [4] Adebayo E. A., Martinez-Carrera D. "Oyster mushroom (*Pleurotus*) are useful for utilizing lignocellulosic biomass". *Afr. J. Biotechnol.* 2015. 14 (1), 52-65.
- [5] Fengel, D., Wegener, G. Wood: Chemistry, Ultrastructure, Reactions. De Gruyter, Berlin. 1984.
- [6] Pandey, R. K. "A study of chemical structure of soft and hardwood and wood polymers by FTIR spectroscopy". *J Appl Polym Sci.* 1999 71, 1969-1975.
- [7] Agbor, V. B., Cicek, N., Sparling, R., Berlin, A. and Levin, D. B. (2011). "Biomass pretreatment: Fundamentals toward application". *Biotech Adv.* 29(6), 675-685.
- [8] Kumar, R., Singh, S. and Singh, O. "Bioconversion of lignocellulosic biomass: biochemical and molecular perspectives". *J. Ind. Microbiol. Biotechnol.* 2008. 35(5), 377-391.
- [9] Zhang, Y.-H. P., Ding, S.-Y., Mielenz, J. R., Cui, J.-B., Elander, R. T., Laser, M., Himmel, M. E., McMillan, J. R. and Lynd, L. R. "Fractionating recalcitrant lignocellulose at modest reaction conditions". *Biotechnol. Bioeng.* 2007. 97(2), 214-223.
- [10] Sarkar, N., Ghosh, S. K., Bannerjee, S. and Aikat, K. "Bioethanol production from agricultural wastes: An overview". *Renew. Energy.* 2012.37(1), 19-27.
- [11] Saidu, M., Salim, M. R. and Yuzir, M. A. M. "Cultivation of oyster mushroom (*Pleurotus* spp.) on palm oil mesocarp fibre". *Afr. J. Biotechnol.* 2011. 10(71), 15973-15976.
- [12] Bayer, E. A., Chanzy, H., Lamed, R. and Shoham, Y. "Cellulose, cellulases and cellosomes". *Curr. Opin. Struct. Biol.* 1998. 8(5), 548-557.
- [13] Ljungdahl, L. G. "The Cellulase/Hemicellulase System of the Anaerobic Fungus *Orpinomyces* PC-2 and Aspects of Its Applied Use". *Ann. Ny. Acad. Sci.* 2008. 1125(1), 308-321.
- [14] Sánchez, C. "Lignocellulosic residues: Biodegradation and bioconversion by fungi". *Biotechnol Adv.* 2009. 27(2), 185-194.
- [15] Weng, J.-K., Li, X., Bonawitz, N. D. and Chapple, C. "Emerging strategies of lignin engineering and degradation for cellulosic biofuel production". *Curr. Opin. Biotechnol.* 2008. 19(2), 166-172.
- [16] Isroi, Millati R, Syamsiah S, Niklasson C, Cahyanto M N, Lundquist K and Taherzadeh M J. Biological Pretreatment of Lignocelluloses with white-rot fungi and its application: A review. *BioResources.* 2011. 6(4), 5224-5259
- [17] Das, H. and Singh, S. K. "Useful Byproducts from Cellulosic Wastes of Agriculture and Food Industry—A Critical Appraisal". *Crit. Rev. Food. Sci.* 2004. 44(2), 77-89.
- [18] Foyle, T., Jennings, L. and Mulcahy, P. "Compositional analysis of lignocellulosic materials: Evaluation of methods used for sugar analysis of waste paper and straw". *Bioresor. Technol.* 2007. 98(16), 3026-3036.
- [19] Pandey, A., Soccol, C. R., Nigam, P. and Soccol, V. T. "Biotechnological potential of agro-industrial residues. I: sugarcane bagasse". *Bioresor. Technol.* 2000. 74(1), 69-80.
- [20] Galbe, M. and Zacchi, G. "Pretreatment of Lignocellulosic Materials for Efficient Bioethanol Production Biofuels". In *Olsson, L. (Ed.)* 2007 (Vol. 108, pp. 41-65) Springer Berlin / Heidelberg.
- [21] Mtui, G. Y. S. "Trends in industrial and environmental biotechnology research in Tanzania". *Afr. J. Biotechnol.* 2007. 6 (25), 2860-2867.
- [22] Mtui, G. Y. S. "Recent advances in pretreatment of lignocellulosic wastes and production of value added products". *Afr. J. Biotechnol.* 2009. 8(8), 1398-1415.
- [23] Maki, M., Leung, K. T. and Qin, W. "The prospects of cellulase-producing bacteria for the bioconversion of lignocellulosic biomass". *Int. J. Biol. Sci.* 2009. 5(5), 500-516.
- [24] Sinegani, A. A. S., Emtiazi, G., Hajrasulih, S. and Shariatmadari, H. "Biodegradation of some agricultural residues by fungi in agitated submerged culture". *Afr. J. Biotechnol.* 2005. 4(10), 1058-1061.
- [25] Patil, S. S., Ahmed, S. A., Telang, S. M. and Baig, M. M. V. "The nutritional value of *Pleurotus ostreatus* (JACQ.:FR.) kumm cultivate on different lignocellulosic agrowaste". *Innov. Rom. Food. Biotechnol.* 2010. 7, 66-76.
- [26] Sánchez, C. "Cultivation of *Pleurotus ostreatus* and other edible mushrooms". *Appl. Microbiol. Biotechnol.* 2010.85(5), 1321-1337.
- [27] Datta, R. "Acidogenic Fermentation of Lignocellulose-Acid Yield and Conversion of Components". *Biorechnol. Bioeng.* 1981. 23(9), 2167-2170.
- [28] Arora, D. S. and Sharma, R. K. "Comparative ligninolytic potential of *Phlebia* species and their role in improvement of *in vitro* digestibility of wheat straw". *J. Anim. Feed Sci.* 2009. 18, 151-161.
- [29] Steel R. G. D., Torrie J. H. "Principles and procedures of statistics". McGraw Hill Co, Inc. 1080. New York, USA.
- [30] Hadar, Y., Kerem, Z., Gorodecki, B. and Ardon, O. "Utilization of lignocellulosic waste by the edible mushroom *Pleurotus*". *Biodegradation.* 1992. 3(2), 189-205.
- [31] Pani, B. K. "Evaluation of Some Substrates for Cultivation of White Summer Mushroom (*Calocybe indica*)". *J. Agric. Sci.* 2010. 1 (4), 357-359.
- [32] Müller, M. M., Sundman, V., Soininvaara, O. and Meriläinen, A. "Effect of chemical composition on the release of nitrogen from agricultural plant materials decomposing in soil under field conditions". *Biol. Fert. Soil.* 1988. 6(1), 78-83.
- [33] Summerell, B. A. and Burgess, L. W. "Decomposition and chemical composition of cereal straw". *Soil Biol. Biochem.* 1989. 21(4), 551-559.
- [34] Waldrop, M. P., Balser, T. C. and Firestone, M. K. (2000). "Linking microbial community composition to function in a tropical soil". *Soil Biol. Biochem.* 2000. 32, 1837-1846.
- [35] Kumar, R., Singh, S. and Singh, O. "Bioconversion of lignocellulosic biomass: biochemical and molecular perspectives". *J. Ind. Microbiol. Biotechnol.* 2008. 35(5), 377-391.
- [36] Leonowicz, A., Matuszewska, A., Luterek, J., Ziegenhagen, D., Wojtaś-Wasilewska, M., Cho, N.-S., Hofrichter, M. and Rogalski, J. "Biodegradation of Lignin by White Rot Fungi". *Fungal Genet. Biol.* 1999. 27(2-3), 175-185.
- [37] Borneman, W. S., Hartley, R. D., Morrison, W. H., Akin, D. E. and Ljungdahl, L. G. "Feruloyl and p-coumaroyl esterase from anaerobic fungi in relation to plant cell wall degradation". *Appl. Microbiol. Biotechnol.* 1990. 33, 345-351.
- [38] Bisaria, R., Madan, M. and Mukhopadhyay, S. N. "Production of biogas from residues from mushroom cultivation". *Biotechnol. Lett.* 1993. 5(12), 811-812.

- [39] Hossain, S., Khalil, M. I., Alam, M. K., Khan, M. A. and Alam, N.
“Upgrading of Animal Feed by Solid State Fermentation by *Pleurotus*
sajor-caju”. *Euro. J App. Sci.* 2009.1(4), 53-58.