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Lignin decomposition of Oil Palm Frond by *Pleurotus ostreatus* with a variation of corn and rice-husk media

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Abstract
This study aimed to decompose lignin from oil palm midrib (OPF) bonds using the fungus Pleurotus ostreatus with various substrates (corn and rice husk). Lignin and cellulose levels before and after mushroom culture were tested by the Chesson-Datta method. Substrate variation with corn and husk rice showed that the addition of corn did not play a role in lignin decomposition. After being given treatment, the best degradation was using 0.6 grams of rice bran and 0.4 grams of $CaCO_3$, 22.01% for lignin and 32.74% for cellulose.

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INTRODUCTION

Lignocellulose is abundant organic component consists of three polymers, namely cellulose (35% - 50%), hemicellulose (20% - 35%) and lignin (10% - 25%) [1][2]. This component is a major source of sugar that can be converted through fermentation to form fuels, carbon, and various valuable chemicals [3-14]. Lignocellulose degradation by cellulase is mainly the initial step before fermentation [1][15]. The substrate must go through several stages in the degradation process, including delignification and Depolymerization. Lignocellulose is commonly found in various types of agricultural waste, from rice straw, corn weevil, and leather nuts - nuts to oil palm. Lignocellulosic biomass waste can then be used as a product obtained through processes.

The oil palm frond (OPF) is part of the biomass which has a composition 14,8% lignin; 62,3% α-Cellulose; 24,2% Hemicellulose; 1,8% Extractive; 11,672 Cellulose (dry ton) [16]. The palm frond lignin content is large enough, about 15%, making it difficult to use cellulose for further processing [17]. The lignin in the OPF can complicate the enzyme's action, bind to cellulose [1, 18, 19], and hinder the cellulose hydrolysis by chemical and biological means. So, to overcome these problems, a way to eliminate the bond formed between lignin and cellulose was needed. Several ways can be taken to eliminate such commitments, one of which is using the assistance of microorganisms through a process of enzymatic delignification. The use of white-rot fungi and brown rot fungi from the class Basidiomycetes

belongs to the class of rotting fungi derived from Ascomycetes and blue dye fungi [20]. The fungus *Pleurotus ostreatus* is a species of fungus belonging to the white-rot Basidiomycetes class [21]. The types of mushrooms used as yeast are easy to find in Indonesia. This fungus is easy to obtain and relatively does not require an expensive medium for its cultivation. In the weathering of oil palm fronds, the fungal mycelium takes up nutrients that support growth, namely carbon sources from carbohydrates, nitrogen in ammonium, and calcium (Ca) to neutralize oxalic acid released by the mycelium.

There are a variety of combinations of materials used as substrate mycelium. Therefore, in this study, differences in the composition and type of nutrients are added to determine the most optimum nutrition to enable the fungus to rotten the frond of oil palm in a short time and produce large amounts of cellulose.

METHOD

The method of this research is depicted in Figure 1.

Oil Palm Frond

Oil palm frond (OPF) was collected from Serang, province of Banten. OPF was grated to become separate fibres. The separated fibres were then blended to reduce the size and make it more homogeneous. The sieving was carried out with a mesh size of 0.250 mm. The OPF was placed in the oven for 1 hour at 105^{o} C until the weight did not change.

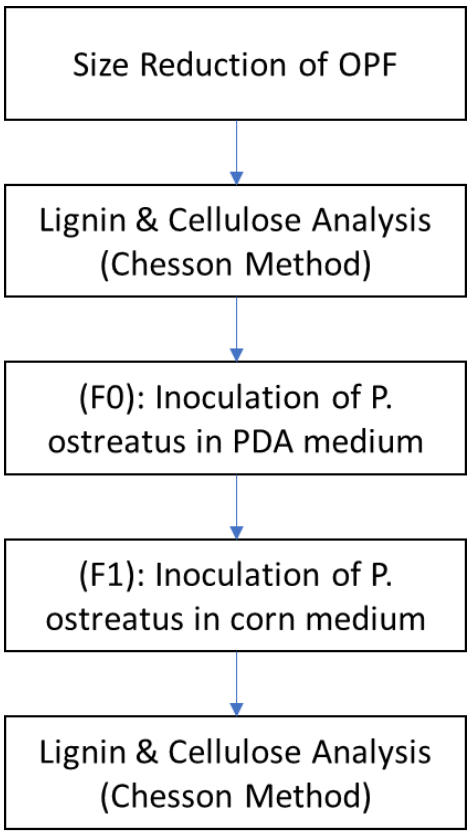


Figure 1. Research scheme of Oil Palm Frond decomposition by *P. ostreatus* with the husk and corn

Medium

Medium Potato Dextrose Agar (PDA) as much as 500 ml was sterilized at 121° C for 15-20 minutes in an autoclave. Fungal cultures of *P. ostreatus* were inoculated onto the PDA. The part of the mushroom used is the flesh, located between the stem and cap. Samples were incubated at 25°–26° C for 1-2 weeks and named as F0 medium. F1 medium was prepared by washing a mixture of corn and rice husk, 56.7 grams, then drained for 1 hour. Steaming is then carried out for 1.5 hours and cooled. Then, Calcium Carbonate (CaCO3) was added and stirred evenly. The variation of corn, husk rice and $CaCO_3$ composition is depicted in Table 1.

Table 1. Sample composition of corn, husk and $CaCO_3$

Sample Code	Corn [g]	Husk [g]	$CaCO_3$ [g]	OPF [g]
A1	--	0.4	0.4	20
A2	10	0.4	0.4	20
A3	15	0.4	0.4	20
A4	20	0.4	0.4	20
A5	25	0.4	0.4	20
B1	-	0.2	0.4	20
B2	-	0.3	0.4	20
B3	-	0.4	0.4	20
B4	-	0.5	0.4	20
B5	-	0.6	0.4	20

The mixture was put into a bottle and then sterilized in an autoclave at 121° C, 1.1 atm for 1 hour. Next, the white-rot fungal mycelium (from F0) was put in a bottle, tightly closed, and allowed to stand until the bottle was filled with fungal mycelium for two weeks.

Analysis

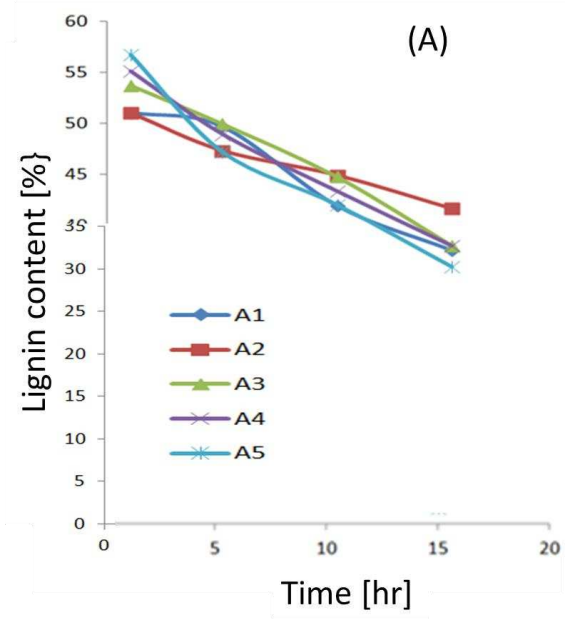
Cellulose and Lignin (acid-insoluble lignin, AISL) were analyzed using a Chesson-Datta method [22]. One-gram dry sample was added into 150 mL water (a), then was refluxed at 100°C on a water bath for an hour. The result was filtered, and then the residue was washed with 200 mL of hot water. The residue was dried in an oven until it had a constant weight, and then it was weighed (b). The residue was added 150 mL H_2SO_4 1 N, then refluxed in a water bath for an hour at 100°C. The result was filtered until neutral (300 mL) and dried (c). The dry residue was added with 72% of 10 mL H_2SO_4 and immersed at 28°C for 4 hours. It was added with 150 mL H_2SO_4 1 N and refluxed on a water bath for an hour on cooling back. The residue was filtered and washed with H_2O to neutral (400 mL), then heated in the oven at 105°C and weigh the result (d). Knowing the content of lignin and cellulose, counted with (1) and (2).

$$\% \, cellulose = \frac{c-d}{a} \, x \, 100\% \quad (1)$$

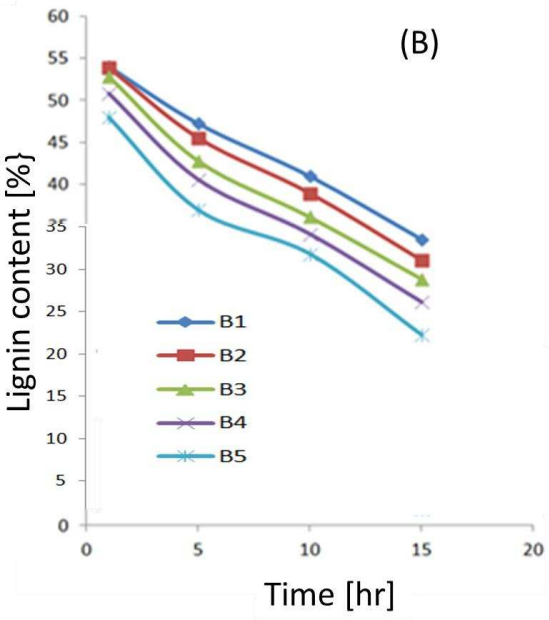
$$\% \, lignin = \frac{d}{a} x \, 100\% \quad (2)$$

RESULTS AND DISCUSSION

This study utilized P. ostreatus to decompose lignin in the oil palm frond bonds. The fungi used are P. ostreatus previously carried out in nursery F1 for three weeks. After that, it was moved to media, mixed with the front of oil palm, husk and corn or husk only. The OPF input for all experiments was fixed at 20 grams. The results of observations on lignin levels in experiments with variations in corn content and husk content are depicted in Figure 2.



(a)



(b)

Figure 2. Lignin content after decomposition by *P. ostreatus* with the variation of (A) husk and corn composition; and (B) Husk composition.

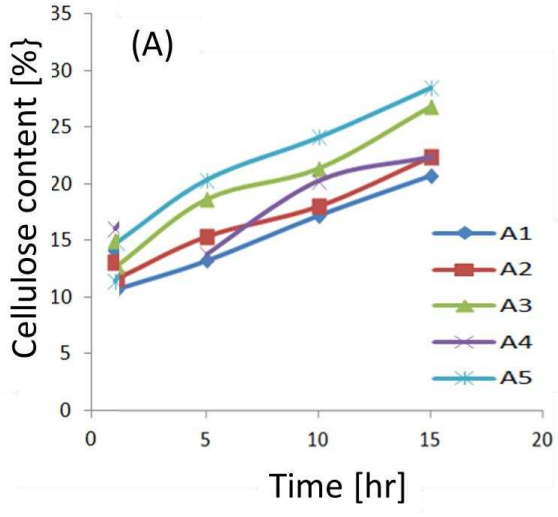
Figure 2 shows that the lignin composition continued to decrease in all samples of corn variety (A1 to A5) and husk variety samples (B1- to B5). The initial lignin content of all samples was relatively the same. However, a sharper decrease in lignin content was shown in the experiment with the husk variety (Figure 2b) compared to the experiment using only the corn variety (Figure 2a).

An oxidation process causes the decreasing lignin content from the mycelium of *P. ostreatus* that comes into the wood's pores. Therefore, enzymes are produced for degrading lignin such as LiP, MnP, laccase and cellulase, xylanase, hemicellulose [23]. These enzymes

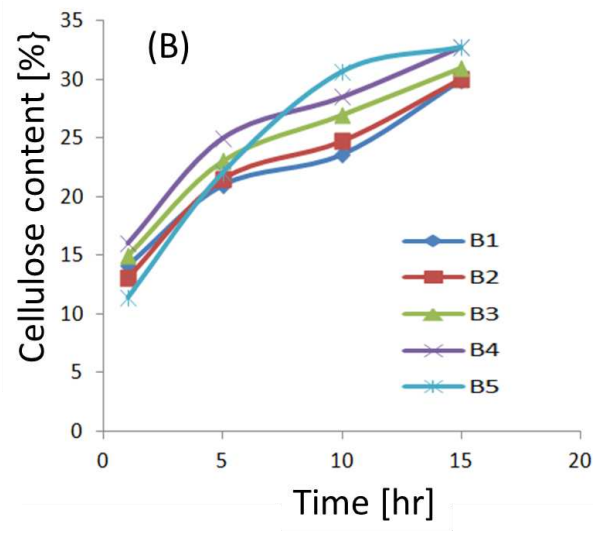
can reduce the content of methoxy, phenolic, and aliphatic lignin, break the aromatic ring, make the new carbonyl group [24] and produce carbon dioxide. LiP and MnP work continuously for degrading lignin.

First, LiP will oxidize non-phenolic lignin, whereas MnP will produce Mn3+, making the oxidation process happen in phenolic or non-phenolic lignin [25]. Then, the laccase, the blue copper, will accelerate electron oxidation in the phenolic and the substrate. The third enzyme would then lead to a radical increase in aromatic compounds. The husk's optimum composition for lignin degradation is 0.6 grams and 0.4 grams of CaCO3. The remaining lignin was 22.01%.

The results of the analysis of the cellulose content in the experiment with variations in the content of corn and husk using the same amount of OPF input are shown in Figure 3.



(a)



(b)

Figure 3. Cellulose content after decomposition by *P. ostreatus* with the variation of (A) husk and corn composition; and (B) Husk composition.

Cellulose content increased consistently across all experimental variations, as shown in Figure 3. However, in line with Figure 2, a sharper increase in cellulose content was shown in the experiment with the husk variation as depicted in Figure 3b compared to the experiment using only the corn variety as shown in Figure 3a.

The increase in cellulose content did not occur because there was no addition of cellulose to the system and no reaction to produce cellulose. However, the cellulose content increased due to the loss of lignin decomposed by P. ostreatus. Initially, the bond between lignin and cellulose (lignocelluloses bond) had been broken, and lignin had been degraded. Thus, the cellulose content artificially increased. The growth of *P. ostreatus* in the medium containing corn appeared to be more fertile than without corn, as shown in Figure 4.

The addition of corn, in general, showed more growth (Figure 4b) but less reduction in lignin than the growth media without corn (Figure 4c). The activity of *P. ostreatus* likely uses more corn as a substrate in experiment A so that only a small amount of lignin was decomposed. Whereas in experiment B, more lignin decomposed, causing the apparent cellulose content to increase (Figure 4b). However, the growth of *P. ostretaus* is less (4c) fertile. Some studies have shown that corn was in favour of the growth of *P. ostreatus* [26, 27, 28]. However, the increase in the growth of *P. ostreatus* was not accompanied by a decrease in lignin. Lignin degradation is expected in the processing of lignocellulosic medium for bioethanol utilization.

The addition of corn medium will increase the costs not only for the cost of medium but also the process of adding medium. Therefore, the recommended medium for further results from this research is the main composition of OPF along with husk and $CaCO_3$.

Although there is no significant difference between the ten experiments, the promising composition for lignin weathering process, the largest producer of cellulose is using husk 0.6 gram and CaCO3 0.4 gram which is the last content of lignin is 22.01% and for the content of cellulose is 32.74%.

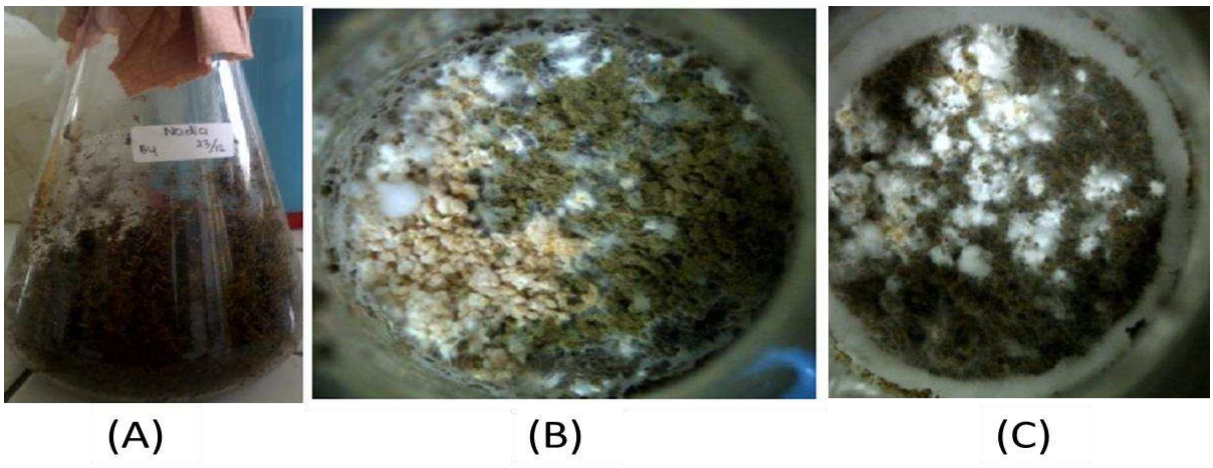


Figure 4. The appearance of the early growth of *Pleurotus ostreatus* on a mixed medium consisting of 20 grams of oil palm frond and variations of corn, husk, CaCO3 and the rest of Potato dextrose agar. (A) before growth, day 0; (B) Medium with corn, growth day 7th; and (C) Medium without corn, growth day 7th

CONCLUSION

This study aims to observe the effect of differences in the addition of corn and rice husks on lignin reduction. The addition of corn will increase the growth of *Pleurotus ostreatus* in the presence of corn substrate, as corn is favorable for the growth of this fungi. However, the increase in the growth of *P. ostreatus* was not accompanied by a decrease in lignin. The addition of corn medium will also increase costs, so the recommended medium for further results from this research is the main composition of OPF along with husk and $CaCO_3$.

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