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Biodegradation of Oil Palm Empty Fruit Bunch Waste by *Pleurotus ostreatus* and *Volvariella volvacea* Using Solid-State Fermentation

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Abstract

Background: *Oil palm empty fruit bunches (OPEFB) represent the largest form of solid waste generated from palm oil production, comprising approximately 23 % of the weight of every ton of fresh fruit bunches (FFB) processed. Untreated OPEFB waste causing pollution problems and increase toxicity due to methane emission. This study aims to evaluate the potential of OPEFB as a growth substrate for the cultivation of Pleurotus ostreatus and Volvariella volvacea, as well as to assess the reduction in lignocellulosic content following fungal biodegradation.* **Methodology:** *The experiment was conducted using solid-state fermentation (SSF), and lignocellulose content was analyzed using the Chesson method and SNI 0429:2008 through descriptive quantitative analysis.* **Findings:** *Over a 21-day incubation period, mycelial growth of both fungi successfully colonized the OPEFB baglogs, resulting in a 1–2 % reduction in baglog weight. Both P. ostreatus and V. volvacea demonstrated the ability to degrade lignocellulose by secreting lignin peroxidase (LiP), manganese peroxidase (MnP), laccase, cellulase, and hemicellulase enzymes. Initial lignocellulose levels of OPEFB were 18 % lignin, 57 % cellulose, and 20 % hemicellulose. After 21 days of incubation, P. ostreatus reduced these levels to 10 % lignin, 47 % cellulose, and 19 % hemicellulose, while V. volvacea reduced them to 11 % lignin, 52 % cellulose, and 18 % hemicellulose.* **Contribution:** *These findings indicate that OPEFB is a viable substrate for mushroom cultivation and can be effectively biodegraded by these fungi, offering a sustainable approach to managing palm oil industry waste. The treated OPEFB can be used as organic fertilizer, animal feed, and briquettes.*

Keywords: *Biodegradation; linocellulose; empty oil palm fruit bunches (OPEFB); Pleurotus ostreatus; Volvariella volvacea*

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INTRODUCTION

According to the Central Statistics Agency of Lampung Province (Badan Pusat Statistik, 2022), Lampung Province produces the highest amount of palm oil, reaching 202,216 tons/year compared to rubber and sugar cane. Palm oil in Indonesia is processed into crude palm oil (CPO), in 1 ton of fresh fruit bunches (FFB) produces 210 kg of crude palm oil (CPO) and 50 kg of palm kernel oil (PKO) (Hartari et al., 2023). This palm oil production produces liquid and solid waste that has not been processed much further. Solid waste produced in large volumes includes empty oil palm bunches (OPEFB), oil palm shells (OPS), oil palm fibers (OPF), and oil palm trunks (OPT) (Santosa, 2008).

Empty oil palm fruit bunches (OPEFB) is the waste with the most significant amount from the palm oil production process; from 1 ton of fresh fruit bunches (FFB) produced, 23% or 230 kg of OPEFB waste is produced (Hartari et al., 2023). This OPEFB waste has not been widely utilized. It has no economic value, with its huge amount and less than optimal processing, so this waste is usually left alone and piles up in oil palm plantation areas (Ratnawati et al., 2018). OPEFB contains lignocellulose components that need to be degraded to break down the lignin structure and polysaccharides (Selman, 2021). If left untreated, this waste poses environmental hazards, contributing to soil and air pollution and methane emissions.

Biodegradation is decomposing organic matter by changing complex substrate components into simpler components using biological agents such as fungi, bacteria, and algae (Imsya et al., 2014). This biodegradation process can use P. *ostreatus* and V. *volvacea* fungi, because these fungi can be used as biological agents in solid state fermentation (SSF) to process and decompose lignocellulosic waste by producing extracellular enzymes (Herliyana et al., 2008; Wang et al., 2019).

Based on previous studies, P. *ostreatus* and V. *volvacea* can degrade lignin in corn cob waste by 5.43 % (Safitri, 2023), 22% lignin in empty oil palm bunches (Yenie & Utami, 2017), 25.26 ± 1.57 % lignin in bagasse or sugarcane pulp (Anita et al., 2016), and 3.9 % cotton lignin (Sienita, 2021). The results of biodegradation can be used as organic fertilizer (Hunaepi et al., 2018), briquettes (Kurniawan & Syukron, 2019). Although *P. ostreatus* and *V. volvacea* have been individually studied for lignocellulose biodegradation, there is a limited body of research directly comparing the effectiveness of these two fungal species under identical conditions, particularly on OPEFB substrates. The volume and limited utilization of OPEFB waste, coupled with its high lignocellulosic content lead a need of research into sustainable waste management strategies. One promising approach involves the use of OPEFB as a cultivation substrate and biodegradation medium, employing efficient biological agents such as *Pleurotus ostreatus* and *Volvariella volvacea*.

METHOD

This study is an experimental research employing a completely randomized design (CRD) with three treatments: (1) control (OPEFB substrate without fungal inoculation), (2) *P. ostreatus* (OPEFB substrate inoculated with *Pleurotus ostreatus*), and (3) *V. volvacea* (OPEFB substrate inoculated with *Volvariella volvacea*), each with three

replicates. The OPEFB (Empty Fruit Bunch) biodegradation process involved several stages: OPEFB sampling, preparation of F1 media and fungal inoculation, preparation of OPEFB baglog media followed by inoculation from the F1 culture, observation of mycelial growth and baglog weight, and measurement of lignocellulose content. In this study, dried empty oil palm fruit bunches (OPEFB) sourced from Lampung Selatan were used as the primary substrate

Preparation of *P. ostreatus* and *V. volvacea* Seeds Media

The seed media is made using 5 kg of corn seeds boiled until the seeds crack. 185 g corn seed, add 5 % (v/w) water, and put into a 250 ml glass bottle. Corn seed used as seed media due to their high carbohydrates and proteins content which provide essential nutrients for fungal growth. Additionally, corn seed has good moisture retention capacity hence maintaining humid environment that support the fungal metabolism and hyphal extension (Hamzah et al., 2022). The seed media is sterilized by autoclave at a temperature of 121 ℃ and a pressure of 15-20 psi for 15 minutes and cooled for 12 hours (Alhidayatullah et al., 2014).

Propagation of P. *ostreatus* and V.*volvacea* Seed (F1)

P. *ostreatus* and V.*volvacea* parent seeds from laboratory collection aseptically inoculated into the seed medium and incubated at a temperature of around 26-28 ℃ until the entire substrate was covered with mycelium or for 26 days (Alhidayatullah et al., 2014).

Baglog Media Preparation

OPEFB is chopped into small pieces or in the form of fibers, the degradation media is made with a weight of 300 g with a composition of 82 % OPEFB, 15 % bran, 1.5 % lime, and 1.5 % gypsum mixed by adding water with a content of 70 % or until the media can be clenched and crushed slowly and a little water drips. Furthermore, the media is composted for 2 days, then the media is put into a polypropylene folding plastic and compacted, the top of the plastic is given a plastic ring, then plugged with cotton and closed using a ring and plastic cover. Then, the media is sterilized using an autoclave at a temperature of 121 ℃ and a pressure of 15-20 psi for 20 minutes, then the media is cooled for ± 24 hours (Alhidayatullah et al., 2014).

Baglog Inoculation with P.*ostreatus* and V.*volvaceae*

200 g of P.*ostreatus* and V.*volvaceae* seed inoculated to OPEFB baglog aseptically. Baglog incubated at 28-32 ℃ with a relative humidity of 70–80 % for 21 days. Observations were conducted at 7-day intervals—on the 7th, 14th, and 21st days of incubation. Parameters observed are mycelium length and baglog weight (Iksan, 2015).

$$Mycelium\ length\ percentage = \frac{\text{mycelium length}}{\text{baglog length}}\ x\ 100\% \quad \text{..... (1)}$$

$$Baglog\ weight\ percentage = \frac{\text{final weight}}{\text{initial weight}}\ x\ 100\% \quad \text{..... (2)}$$

Lignocellulose Content Measurement

Lignocellulose content was measured by Chesson method and SNI 0492:2008. Chesson method is based on gravimetry analysis using hydrolized or dissolved component. Lignocellulose content (lignin, cellulose, and hemicellulose) measured on day 0 and day 21 by weighing 1 g of OPEFB baglog sample (*weight a*), refluxed by dissolved into 150 ml aquadest for 1.5 hours on 150 °C. The solution was filtered and separated using masir cup. Residue were washed using aquadest and dried using oven for 24 hours on temperature 105 °C. Dried residue were weighed and calculated as *weight b*. Dried residue were added 150 ml H_2SO_4 1 N and refluxed for 1.5 hour on 150 °C temperature. After that, residue separated using masir cup and washed with aquadest and dried using oven for 24 hours on temperature 105 °C. Weight of this residue calculated as *weight c*. Next, the residue was transferred into an Erlenmeyer flask and soaked with 50 mL of 72 % H_2SO_4 at room temperature (28 °C) for 4 hours, then 150 ml of 1N H_2SO_4 was added and refluxed at 150 °C for 1.5 hours. Next, the residue was separated using a masir dish and rinsed using distilled water, the residue was dried using an oven at 80 °C for 20 hours (*weight d*). Then, the residue was washed using a furnace at 575±25 °C for 1 hour (*weight e*). The weights obtained can be used to calculate the levels of lignin, cellulose, and hemicellulose using the following calculation formula (Chesson, 1981):

- Lignin content = $\left(\frac{d-e}{a}\right) x\ 100\%$ ..... (3)
- Cellulose content = $\left(\frac{c-d}{a}\right) x\ 100\%$ ..... (4)
- Hemicellulose content = $\left(\frac{b-c}{a}\right) x\ 100\%$ ..... (5)

Description:
a = dry weight of sample (grams)
b = weight of first residue (grams)
c = weight of second residue (grams)
d = weight of third residue (grams)
e = weight of fourth residue (grams)

RESULT AND DISCUSSION

Growth of *P. ostreatus* and *V. volvacea* in OPEFB Waste

P. *ostreatus* and V. *volvacea* grown on OPEFB media through the SSF fermentation stage for 21 days showed the growth of fungal mycelium starting from the top to the bottom of the OPEFB baglog (Figure 1). Based on Figure 1, the growth rate of the P. *ostreatus* and V. *volvacea* mycelium is different. In P. *ostreatus*, mycelium growth was 100 % on the 14th day while V. *volvacea* mycelium growth was 100 % on the 21st day. This proves that OPEFB fermented using the SSF technique has the potential as a growth medium for P. *ostreatus* and V. *volvacea* fungi because the distribution and growth of mycelium fills the entire OPEFB baglog.

Figure 1. Growth of P. *ostreatus* and V. *volvacea* on OPEFB Media with an incubation period of 21 days. (a) Control; (b) P. *ostreatus*; (c) V. *volvaceae*.

Extracellular enzymes produced by fungi influence the speed of mycelium growth on this OPEFB substrate. The enzymes produced by fungi to degrade lignocellulose are cellulase, xylanase, hemicellulase, laccase, lignin peroxidase (LiP), and manganese peroxidase (MnP) (Andlar et al., 2018; Suryadi et al., 2022). OPEFB grown by this mycelium indicates the success of P. *ostreatus* and V. *volvacea* in degrading lignocellulose.

Internal and external factors can influence the success of lignocellulose degradation. Internal factors that affect mycelium growth are fungal species, substrates containing lignin, cellulose, hemicellulose, and nitrogen (Neville et al., 2018). Meanwhile, external factors for mycelium growth are temperature, humidity, water content, light intensity, pH, and air circulation (Bellettini et al., 2019; Kusumaningrum et al., 2017).

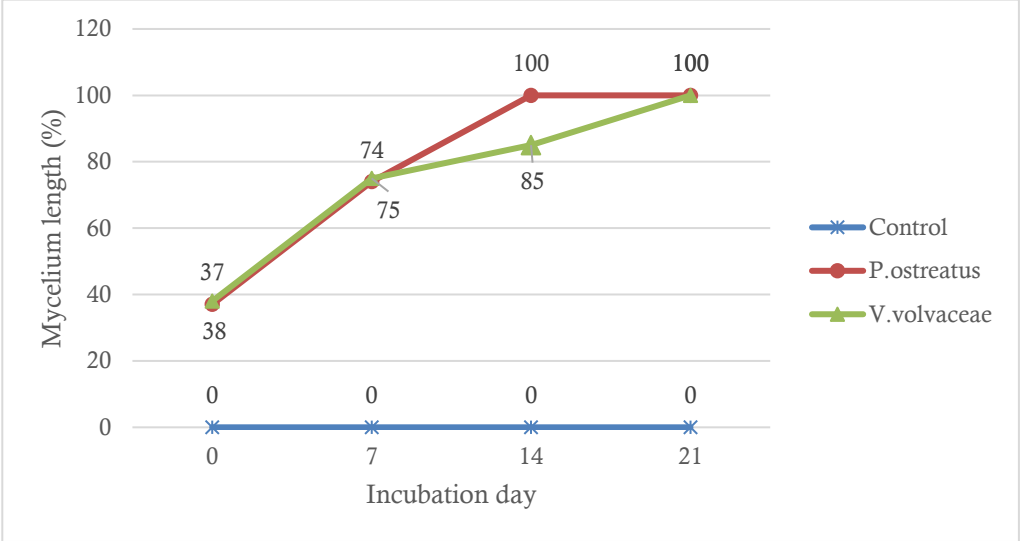

Figure 2. Growth of P. *ostreatus* and V. *volvaceae* on OPEFB Media with an incubation period of 21 days. (a) Control; (b) P. *ostreatus*; (c) V. *volvaceae*.

The environmental temperature of the mushroom house ranges from 28-31 °C with a humidity of 76-83 %. The humidity of the mushroom house is optimal for the growth of P. *ostreatus* and V. *volvacea*. Meanwhile, the temperature of the mushroom

house is quite optimal for the growth of P. *ostreatus*, where the optimal growth temperature ranges from 16-30 °C (Hoa & Wang, 2015), but less optimal for the growth of V. *volvacea*, where the optimal growth temperature is at 33-36 °C (Hamzah et al., 2022). The difference in growth rate between P. *ostreatus* and V. *volvacea* is thought to be due to the difference in the optimal growth temperature and mycelial density of P. *ostreatus* and V. *volvacea* mushroom seeds.

Based on Figure 2, the decrease in baglog weight over the 21-day fermentation period was not significant. On day 0, all OPEFB baglogs had an initial weight set at 100 %. After 21 days, the weight percentages were 99 % for the control, 98 % for *P. ostreatus*, and 98 % for *V. volvacea*. This minimal reduction indicates that the 21-day incubation period was insufficient for effective decomposition of the OPEFB. As a result, the lignocellulose content remained largely undegraded and unutilized by the fungi, leading to the insignificant change in baglog weight.

The biodegradation process can lead to a reduction in baglog weight (Hanif et al., 2019). Fungal mycelium continues to decompose the lignocellulose content in the OPEFB substrate throughout the incubation period, utilizing available nutrients to support its growth. As the fungi consume these nutrients, the substrate mass gradually decreases, resulting in a reduction in baglog weight. This weight loss is primarily attributed to the degradation of the lignocellulosic structure by the fungal mycelium, which renders the substrate more brittle. The extent of degradation correlates with the degree of weight loss—the more extensive the fungal activity, the greater the reduction in mass. In a study by (Hanif et al., 2019), oyster mushroom baglogs incubated for five months showed a significant weight reduction, decreasing from 1000 g to 500 g.

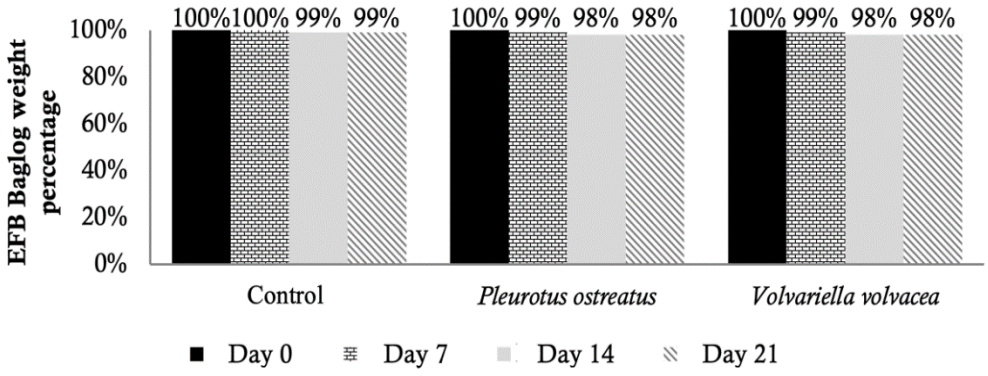

Figure 3. OPEFB baglog weight during incubation

Lignocellulose content

The degradation process of solid organic waste such as OPEFB can be characterized by a decrease in the lignocellulose content contained in OPEFB. Based on Figure 4, it shows that the fungi P. *ostreatus* and V. *volvacea* have the ability to degrade OPEFB waste which is characterized by a decrease in lignocellulose content. The decrease in lignocellulose content is caused by the growth of fungi and the degradation process carried out by utilizing the components and nutrients contained in the OPEFB substrate for its growth.

Based on the analysis results, OPEFB lignin content decreased during incubation (Figure 4). On day 0, the lignin content of all OPEFB baglog was 18%. After incubation for 21 days, there was a change in lignin content; in the control, it became 15%, *P. ostreatus* 10%, and *V. volvacea* 11 %. This shows that both fungi can secrete ligninolytic enzymes to reduce the lignin content in OPEFB bags. Following the research of Villas-Bôas et al., (2002), lignin can be effectively degraded by fungi. P. *ostreatus* can reduce lignin levels by 25.26 ± 1.57 % (Anita et al., 2016) and V. *volvacea* by 22 % (Yenie & Utami, 2017) by producing extracellular ligninolytic enzymes such as lignin peroxidase, manganese peroxidase, and laccase.

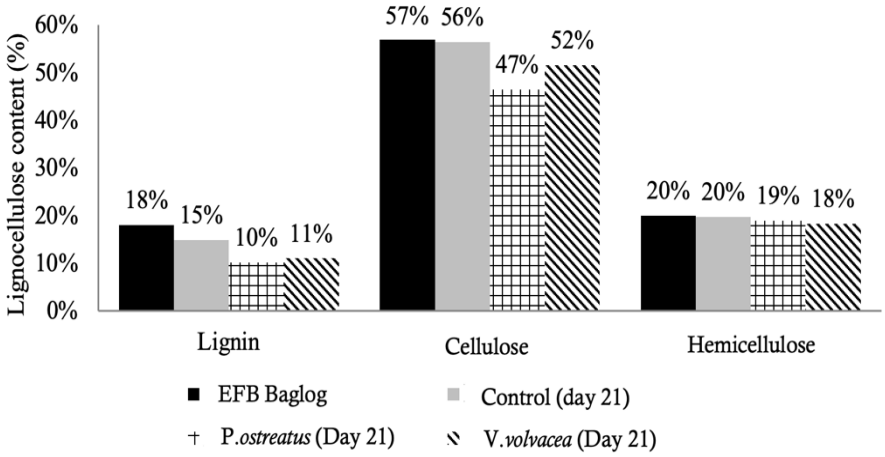

Figure 4. Percentage of lignocellulose content in OPEFB baglog

Fungal mycelium degrades lignin, leading to the thinning of plant cell walls. As the lignin structure breaks down, it releases lignin components and creates a porous, honeycomb-like pattern that is more susceptible to further degradation. This structural change facilitates access to cellulose and hemicellulose, allowing these polysaccharides to be broken down into simpler components. The resulting sugars and other degradation products are then utilized by the fungi for growth and metabolic processes (Bilal & Asgher, 2016; Krishaditersanto, 2018; Suryadi et al., 2022).

Based on Figure 4, the initial cellulose content of all baglogs on day 0 was 57 %. After 21 days of incubation, the cellulose content decreased to 56 % in the control, 47% in the *P. ostreatus* treatment, and 52 % in the *V. volvacea* treatment. This reduction indicates that cellulose degradation occurred during the incubation period, driven by the activity of cellulase enzymes. These enzymes break down cellulose into linear chains and disaccharide units (cellobiose), which are further hydrolyzed into glucose and utilized for fungal growth. According to Herliyana et al., (2008), the decrease in cellulose content in sengon wood due to *Pleurotus* isolates ranged from 18.9% to 87.4%, highlighting the potential effectiveness of these fungi in lignocellulose degradation.

In Figure 4, the hemicellulose content on day 0 of incubation in all OPEFB baglogs was 20 %. After 21 days of incubation, the hemicellulose content decreased to 19 % in the P. *ostreatus* treatment and 18% in the V. *volvacea* treatment, while the control baglog showed no reduction. This decrease indicates that P. *ostreatus* and V. *volvacea*

are capable of producing hemicellulase enzymes, which degrade hemicellulose into simpler sugars such as araban, galactan, and xylan (Krishaditersanto, 2018). However, the relatively small reduction in hemicellulose content may be due to its amorphous structure, which includes complex chemical bonds—such as ethyl, acetyl, and glucosidic linkages—that are more difficult to break down (Khadafi & Fordini, 2023; Lisin & Hutomo, 2015).

The difference in lignocellulose degradation between P. *ostreatus* and V. *volvacea* was not substantial, suggesting that both fungi are effective in reducing lignocellulose content in OPEFB. However, the 21-day incubation period may not have been sufficient for optimal lignocellulose degradation. Fadilah et al., (2008) reported that longer incubation times result in greater reductions in lignocellulose levels.

A slight decrease in lignocellulose content observed in the control baglog may be attributed to contamination. Such contamination could result from incomplete sterilization or the accidental introduction of airborne spores during handling. Common fungal contaminants in mushroom baglogs include *Aspergillus* sp., *Trichoderma* sp., *Paecilomyces* sp., *Rhizopus* sp., *Fusarium* sp., *Syncephalastrum* sp., *Cyllodes* sp., and *Mucor* sp., many of which possess cellulolytic capabilities that contribute to lignocellulose degradation (Handayani & Purwantisari, 2015).

CONCLUSION

Empty oil palm bunches (OPEFB) show potential as a substrate or baglog medium for the cultivation of P. ostreatus and V. volvacea. Mycelial growth and distribution were observed to fully colonize the baglog by day 14 for P. ostreatus and by day 21 for V. volvacea. A 21-day incubation period with these fungi resulted in a reduction of lignocellulose content in the OPOPEFB. Initially, the lignocellulose composition of OPOPEFB was 18% lignin, 57% cellulose, and 20% hemicellulose. After incubation, the P. *ostreatus*-treated baglogs showed a reduction to 10% lignin, 47% cellulose, and 19% hemicellulose, while the V. *volvacea*-treated baglogs contained 11% lignin, 52% cellulose, and 18% hemicellulose. P.*ostreatus* more effective than V.*volvacea* in OPEFB degradation. The treated OPEFB can be used as organic fertilizer, animal feed, and briquettes.

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