



The Utilization of Oil Palm Empty Fruit Bunches for Growth of Oyster Mushroom (*Pleurotus ostreatus*) and Biodelignification Process During Planting Cycle

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ABSTRACT

Indonesia produces a large amount of oil palm empty fruit bunch (EFB) that has potency used as media for mushroom cultivation. The research emphasized the use of EFB to grow oyster mushrooms as an alternative for delignification without chemicals. This research aimed to study the growth of oyster mushroom (*Pleurotus ostreatus*) at EFB based growing media treatments. The media consisted of a mixture of EFB and sawdust at the composition of 0%, 25%, 50%, 75%, and 100%. The media were also supplemented with rice bran, CaCO₃, and TSP fertilizer. The data were collected on the mycelium growth and the mushroom weight. During cultivation in baglog, there was no difference in mycelium growth rate. The EFB composition of 25% showed faster growth among the other compositions, followed by the EFB composition of 50%, 75%, and 0%. The highest Biological Efficiency Ratio (BER) at 56.25% was obtained at media composition of 50%. The EFB contained in baglog also showed biodelignification process. Biodelignification has the benefit which can reduce the use of chemicals in the delignification process to convert EFB into cellulose. The highest lignin reduction (40.12%) occurred in 75% of EFB media composition, while hemicellulose (49.56%) occurred in 100% EFB media composition.

INTRODUCTION

Empty fruit bunch (EFB) is one of the by-product materials from oil palm processing and may reach over 20% of total plant capacity (Hambali & Rivai, 2017). Indonesia is the largest producer of crude palm oil (CPO) in the world. CPO demand in International will reach 51 million tonnes at 2025 (Khatiwada et al., 2021). Research on the utilization of EFB has been widely carried out includes cellulose extraction (Nazir et al., 2013), composite production (Khalid et al., 2008), and bioplastic production (Isroi et al., 2017). EFB was also employed as a base catalyst in biodiesel production from *Jatropha curcas* oil (Yaakob et al., 2012) a raw material of compost fertilizer (Islam et al., 2021), and bioethanol production (Kim & Kim,

2013; Muryanto et al., 2012; Sudiyan & Hermiati, 2010). In oil palm plantations, many white rot fungi was found in EFB. Some of white rot fungi are edible mushroom. This phenomenon shows the potential of EFB as an edible mushroom growing medium. The utilization of EFB as mushroom growing media has not been widely explored as growing media for mushroom, EFB has some advantages. In Indonesia, the source is widely abundant. EFB can be used for production fertilizer (Arfiana et al., 2019). The materials also can be used as herbicide bioremediation in agricultural land (Corral-Bobadilla et al., 2019).

Some types of mushroom have been tested to grow in EFB media including *Volvariella volvacea* (Triyono et al., 2019), *Ganoderma boninense* (Sudirman et al., 2011) and *Pleurotus* sp (Ali et

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al., 2013; Sudirman et al., 2011). Those studies stated that EFB can be used as mushroom growing media; however, the productivity was low. It may be due to the complex lignocellulose structure of EFB so that mycelium becomes difficult to penetrate within media. One of the techniques to breakdown lignocellulose structure of EFB is by immersing EFB in a diluted acid solution to reduce sugar production on EFB hydrolysis (Tan et al., 2013). The novelty of this research is that the integration of the process of using EFB to grow oyster mushrooms is also one way of the delignification process without chemicals. So, after the oyster mushrooms were planted, EFB can be easily broken down into cellulose because of the low lignin content.

Classified as a white rot fungus, Oyster mushroom (*Pleurotus ostreatus* sp.) is an edible mushroom with meaty-like taste and contains high vegetable protein (10-30%) (Phat et al., 2016). Demand on oyster mushroom has been steadily increased for 5% since 2015 (Sucipto et al., 2015). Oyster mushroom contains essential amino acids that cannot be synthesized by human like isoleucine, lysine, metholin, cysteine, phenylalanine, tyrosine, glutamic acid, proline, and serine. A side from that unsaturated fatty acids in oyster mushroom is considered beneficial to be consumed by patients who suffers from hypertension, stroke, heart, and cholestrol, also for people with restrictive diets (Chirinang & Intarapichet, 2009). Furthermore, oyster mushroom can acts as immunomodulator (El Enshasy & Hatti-Kaul, 2013) that can improve human immune system.

In Indonesia, oyster mushroom is commonly cultivated in sawdust media. Oyster mushroom cultivation has been done in various agricultural waste such as naize stover (Chanakya et al., 2015),

coir pith (Bisaria et al., 1987), ground nut husk (Chang et al., 1981), sugarcane bagasse (Ragunathan et al., 1996), rice straw (Moonmoon et al., 2010), and mixes of these wastes as substrates (Ragunathan & Swaminathan, 2003). Oyster mushroom can potentially grow in lignocellulose waste such as oil palm empty fruit bunch (EFB) (Mahari, et al., 2020). However, the activity of delignification in the time of planting cycle have not report. Nutritions such as carbon (C) that is needed by oyster mushroom can be obtained in EFB containing cellulose, hemicellulose, and lignin. This research aimed to evaluate the potency of EFB waste as oyster mushroom growing media and the reduction of lignin and hemicellulose content in EFB (biodelignification) during incubation in mushroom planting period.

MATERIALS AND METHODS

This research was conducted from October 2016 to April 2017 at Indonesian Research Institute for Biotechnology and Bioindustry, Bogor, West Java.

Baglog Media Production for *Pleurotus ostreatus*

Pleurotus ostreatus growth media consisted of EFB, sawdust from Sengon tree (*Albizia chinensis*), husk, TSP, and CaCO_3 . EFB content in baglog was varied at 25% (P1), 50% (P2), 75% (P3), 100% (P4), and 0% (P5). The composition variations for mushroom growing media are presented in Table 1. Each media composition consist of 5 baglog with 2 replication.

To prepare mixture media, first, EFBs were chopped and washed. Then, all raw materials were mixed homogenously and water was added they had 55-60% water content with pH 6-7. Media mixture was left overnight for decomposition process.

Table 1. Variation of media composition

Media substances	Weight of materials in media treatments (g)				
	P1	P2	P3	P4	P5
Empty Fruit Bunch	234.37	468.75	703.12	937.5	0
Sawdust	703.12	468.75	234.37	0	937.5
Rice bran	44.64	44.64	44.64	44.64	44.64
CaCO_3	13.39	13.39	13.39	13.39	13.39
Mineral Fertilizer	4.47	4.47	4.47	4.47	4.47
Isolate	0.1	0.1	0.1	0.1	0.1

***Pleurotus ostreatus* Inoculation**

Pleurotus ostreatus used in this study was obtained from CV. Asa Agro Corporation, Cianjur, West Java. The mushroom was grown in agar media (Potato Dextrose Agar) as the isolate stock and called as F2. Then, it was transferred to media contained 1% menir and bran and called as F1. After one week incubation on those media, about 1 cm isolates were taken out and inoculated into baglog media.

Inoculation were carried out in laminar and sterile room. All mixture media were sterilized using autoclave at 121°C and 1 atm pressure for 30 minutes. After the inoculation, baglog media was incubated for five months at temperature of 25°C. Incubation was carried out on an oyster mushroom rack with the humidity of 85%. The successful inoculation was indicated by appearance of mycelium fibers in baglog which grew as fruit body.

Mycelium Growth and Harvesting *Pleurotus ostreatus*

The pot experiment was laid out in a compartment. Mycelium growth was noticed after one day inoculation. Mycelium started to grow from upper part of baglog where isolates were inoculated. The length of mycelium in the baglog was measured every 7 days until 30 days. After mycelium vined covering the baglog surface, fruit body would appear. The harvesting of fruit body was done after fruit body has a quite big diameter, which was around 8-12 cm. The comparison between total weight of mushrooms harvested in one cultivation period per baglog and the weight of baglog resulted in Biological Efficiency Ratio (BER) value. High BER value represents a good composition of media used for mushroom cultivation. BER value was calculated using formula 1.

$$BER (\%) = \frac{\text{total weight of mushroom harvested per cultivation period}}{\text{the weight of baglog}} \times 100\% \dots (1)$$

Lignin Content Analysis

The lignin analysis was conducted according to Klason Method and in accordance with SNI 0492:2008. One gram sample was extracted with alcohol benzene with 1:2.15 ml of H₂SO₄ 72% was added to the mixture, then covered and stirred in every 2-3 minutes. The mixture was maserated at 20°C for two hours. Aquadest was added up to 575 ml and heated for 4 hours. After that, the mixture was stored for 24 hours until the lignin was completely precipitated. The lignin was filtered, washed gently

with water, and dried using an oven at 105°C. The dried lignin was weighted used formula 2.

$$\text{Lignin} = \frac{A}{B} \times 100\% \dots (2)$$

Where: A = weight of dried lignin (g); B = weight of sample (g).

EFB fibers from ex baglog was observed by using Scanning Electron Microscopy (SEM) to examine the changes occurred in lignin.

Cellulose Content Analysis

The cellulose content analysis was conducted according to SNI 0444: 2009. As many as 1.5 g cellulose was added into 75 ml of 17.5% NaOH solution. The mixture was continuously stirred until pulp became completely dispersed. NaOH solution 17.5% was added until the volume reached 100 ml. This suspension was stirred and stored in bath at 25°C ± 0.2°C for 30 minutes. The suspension was poured into a funnel. For about 10-20 ml of first released filtrate was discarded, then the rest filtrate was collected for 100 ml in a flask. Twenty five ml of this filtrate was separated in a 250-ml flask and 10 ml of K₂Cr₂O₇ 0.5 N was added. Then, 50 ml of concentrated sulfuric acid was slowly added. After 15 minutes, the solution was diluted with 50 ml of aquadest, heated up to 125-135°C, and then cooled down in room temperature. After 2-4 drops of ferroin indicator was added the solution was titrated using 0.1 N ferro ammonium sulphate solution until the colour changed to purple. Alpha-cellulose content (%) was calculated using following formula 3.

$$\text{Alpha cellulose} = 100 - \frac{6.85(V1 - V2) \times N \times 20}{A \times W} \dots (3)$$

Where: V1 = the volume of blank titration (ml); V2 = the volume of filtrate titration (ml); N = normality of the titrant; A = pulp volume (ml); W = dried pulp weight (%).

Hemicellulose Content Analysis

Hemicellulose content analysis was carried out by measuring pentosan level according to SNI method 14-1304-1989. Cellulose filtrate was diluted by distilled water. Then, 50 ml of this solution was mixed with 10 ml of K₂Cr₂O₇ 0.8 N and 80 ml of H₂SO₄ (p). The solution was stirred for 10 minutes, cooled down in room temperature, diluted by 500 ml of distilled water, and lastly, titrated by Na₂S₂O₄.

Data Analysis

The data were analyzed using IBM SPSS software 16.0 and subjected to a one-way analysis of variance (ANOVA) (p < 0.05).

RESULTS AND DISCUSSION

Mycelium Growth of Oyster Mushroom

Mycelium grew covering the baglog after the inoculation of oyster mushroom. The speed of the mycelium growth differed in different media compositions (Fig. 1). A mixture of media compositions between sawdust and EFB grew faster than the media only contained one carbon source such as at P4 and P5 treatments. Mycelium covered all baglog surface at the average of 28-30 days. This result shows that media contained EFB are suitable for oyster mushroom growth. In general, the use of EFB fibres and sawdust as growing media of oyster mushroom gave better results than the use of 100% sawdust (P5) and 100% EFB fibres (P4). According to Ali et al. (2013), the use only EFB as mushroom growth media cannot produce oyster mushroom fruit body. Fruit body can be developed if EFB media is mixed with sawdust or mesocarp. The addition of sawdust or mesocarp in the fermentation media can reduce the C/N ratio compared to pure EFB media. This is because lignocellulosic-based biomass contains little nitrogen, whereas nitrogen is needed for growth. The C/N ratio required for primordial growth of oyster fruiting bodies ranges from 21-30 while, for mycelium growth the C/N ratio

of more than 30 is required, and the C/N ratio below 20 is needed for the growth of fruiting body (Yang et al., 2013). Meanwhile, EFB only contains carbon as much as 46%, 0.85% total nitrogen, and the C/N ratio value of 54% (Mamimin et al., 2021). The addition of sawdust to the media has been shown to accelerate the growth of fruiting bodies. Where in previous studies that used EFB as a substrate with additional sawdust had slower mycelium growth. On day 28 the new mycelium could fill the baglogs (Marlina et al., 2015). While in this study, the primordia had grown on the 28th day of incubation (Fig. 2). EFB can substitute the conventional substrate for *P. ostreatus*. Another study showed that primordial growth in media containing EFB was faster than other media such as palm oil frond and trunks (Rizki & Tamai, 2011).

The mycelium growth of oyster mushroom on media variation of P1-P5 is presented in Fig. 3. The faster mycelium covers baglog, the faster primordia and fruit body will appear. The growth of oyster mushroom was classified into vegetative phase and reproductive phase. Vegetative phase starts from the inoculation until the first primordia appears (Fig. 2) while reproductive phase starts from primordia appears until the last fruit body is harvested.

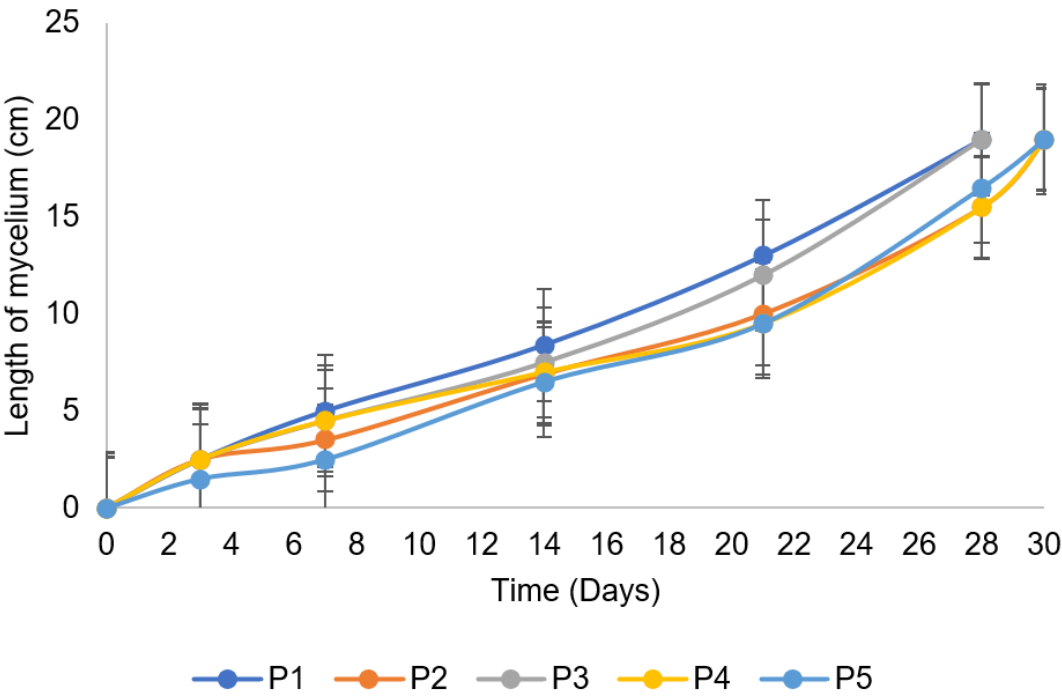


Fig. 1. The growth of mycelium length in different media composition



Fig. 2. Primordia phase of *Pleurotus ostreatus*

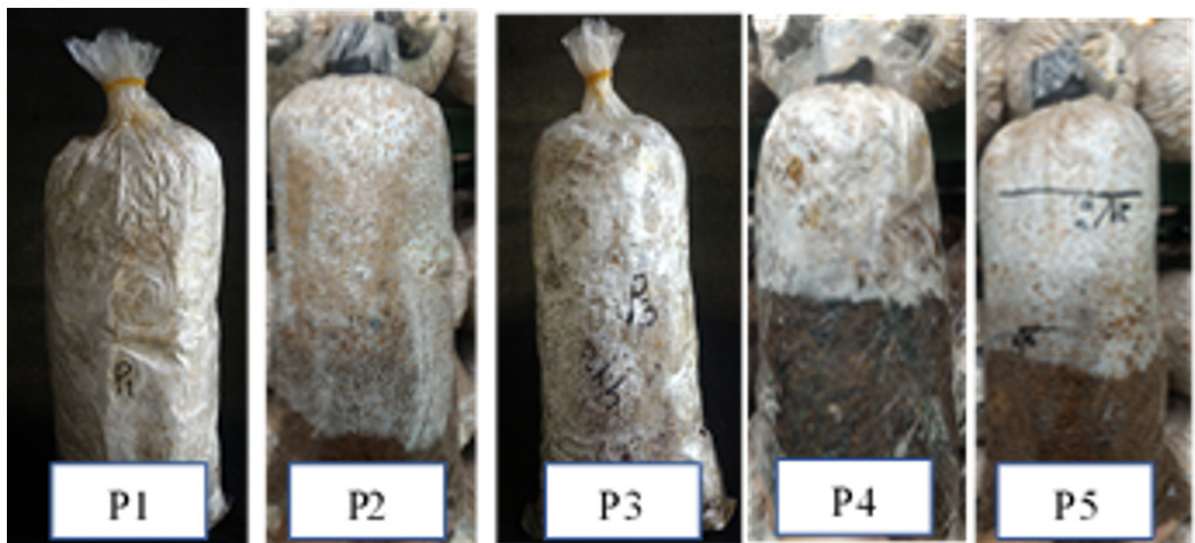


Fig. 3. Mycelium growth under different media composition

Mycellium is formed from hyphae threads originated from inoculated spores of oyster mushroom (Nam et al., 2018). The more complex a mycellium will fastened pinhead growth on surface media, resulted in the faster growth of fruit body.

The Growth and Yield of Oyster Mushroom Fruit Body

Primordia, as a pioneer of fruit body, grew after all mycellium covered the baglog surface. Different media composition gave different results.

Primordia firstly appeared in P1 and P3 on the 28th day, followed by P2, P4, and P5 on the 30th day. Primordial phase growth does not indicate a high Biological Efficiency Ratio (BER) value. Production-harvesting period shows the period the baglog could produce the fruit body.

The highest harvest of fruit body was obtained from P2, which produced 3300 g per 5 baglog in the first month and 3550 g in the second month (Fig. 4). The harvest results drastically declined in the third and fourth month, with the value of 1725 g and 350

g, respectively. The fruit body of oyster mushroom were presented in Fig. 5. Media compositions containing EFB (P1-P4) produced a high yield of mushroom in the first month, compared to P5, which did not contain EFB. Mushroom need carbon source which was obtained from EFB (lignin and cellulose), calcium source from CaCO_3 , and a small amount of nutrients, such as N, P and S from brans (Rambe et al., 2020). EFB contains 26.49% lignin, 32.57% cellulose, and 27.7% hemicellulose (Mamimin et al., 2021). Sawdust and EFB contain a limited amount of nutrients, such as nitrogen, phosphor, sulfur, and others. Therefore, needed additional nutrients could be obtained from brans.

Media compositions have a great influence on mushroom growth. EFB was mixed with sawdust to get the optimal media composition in producing a high BER value of mushroom. Sawdust used in this research was Sengon wood. Sengon wood is classified as a hard wood and does not contain a lot of sap, which fulfill the criteria for good sawdust (Bhattacharjya et al., 2014). Sawdust used as cultivation media should be from wood that does not contain preservatives as it can inhibit the oyster mushroom growth (Ogidi et al., 2020). The selection of sawdust should be based on some criteria including dryness level, cleanliness, not overgrown with mushrooms or mold, and not rotten.

Biological Efficiency Ratio (BER) and Oyster Mushroom Weight

Biological Efficiency Ratio (BER) is the efficiency of mushroom production in one planting cycle to form a fruit body. A high BER value implies a good mushroom ability in using the substrate media. In this research, the highest BER value was 56.25% which obtained from P2. P2 consisted of 50% EFB and 50% sawdust. P5, which acted as control, also shows a high BER value, which was slightly lower than P2 (52.38%) (Fig. 6). This mixture media could result in high mushroom weight, up to 193 g/baglog, but the biological efficiency remained low (Max. 11.3%). A study conducted by Sudirman et al., (2011) showed that EFB mixture with sawdust from *Paraserianthes falcataria* resulted in high mushroom weight up to 209 g/baglog and biological efficiency up to 167%. This result showed that sawdust is one of the important composition as the mixture with EFB for growth media of oyster mushroom to reach the optimum BER.

Media P4 with 100% EFB had the smallest BER values (Fig. 6). In contrast, media with 50% and 100% sawdust composition (P2 and P5) had the highest BER values. The media added by sawdust (P1, P2, P3) gave better result in a BER value than the media which only consisted of EFB (P4). This is probably due to the difficulty of mushrooms in breaking down carbon sources from EFB that inhibits the growth of mushroom.

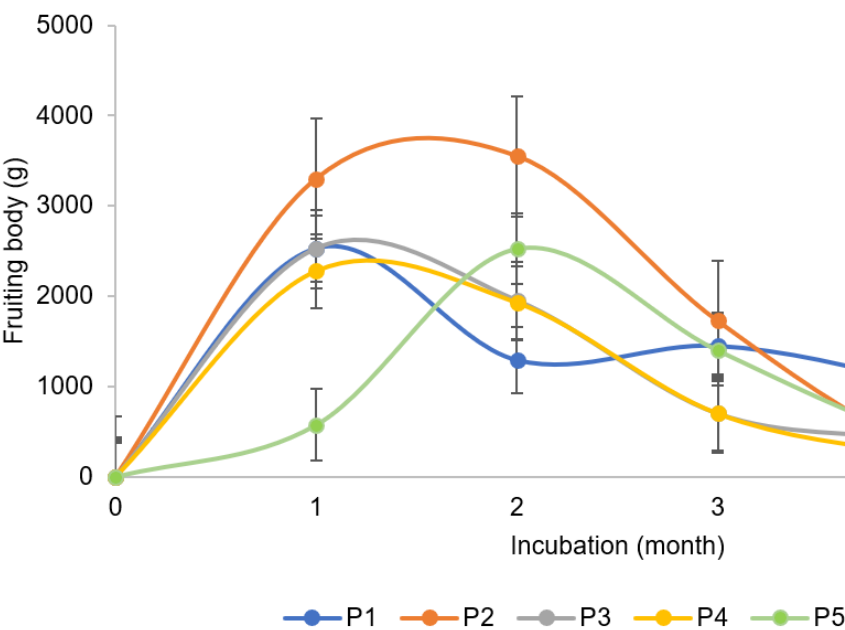


Fig. 4. Fruiting body production in one planting cycle

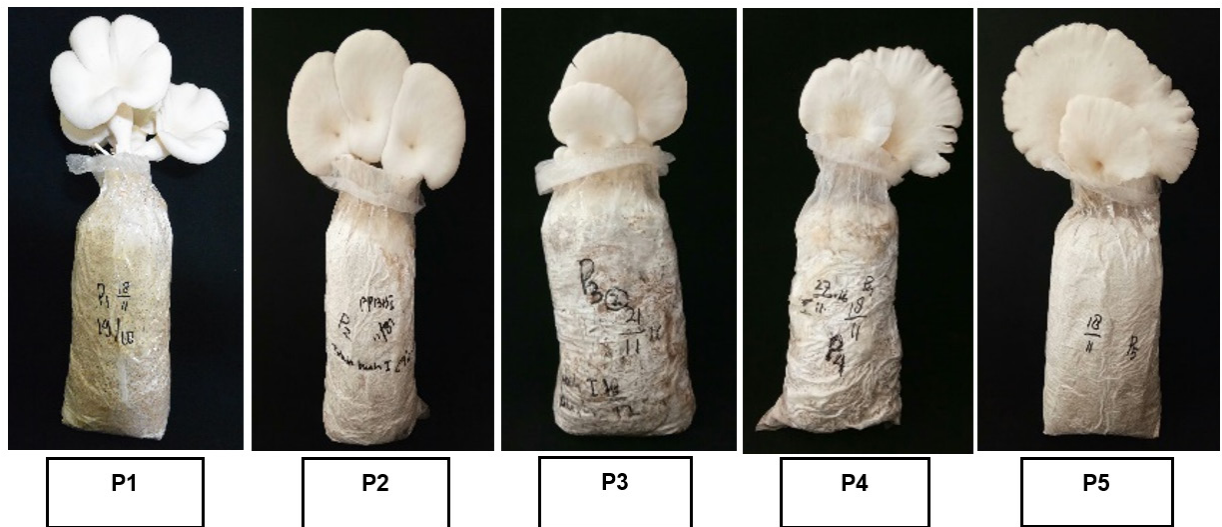


Fig. 5. The growth of fruit body of oyster mushroom

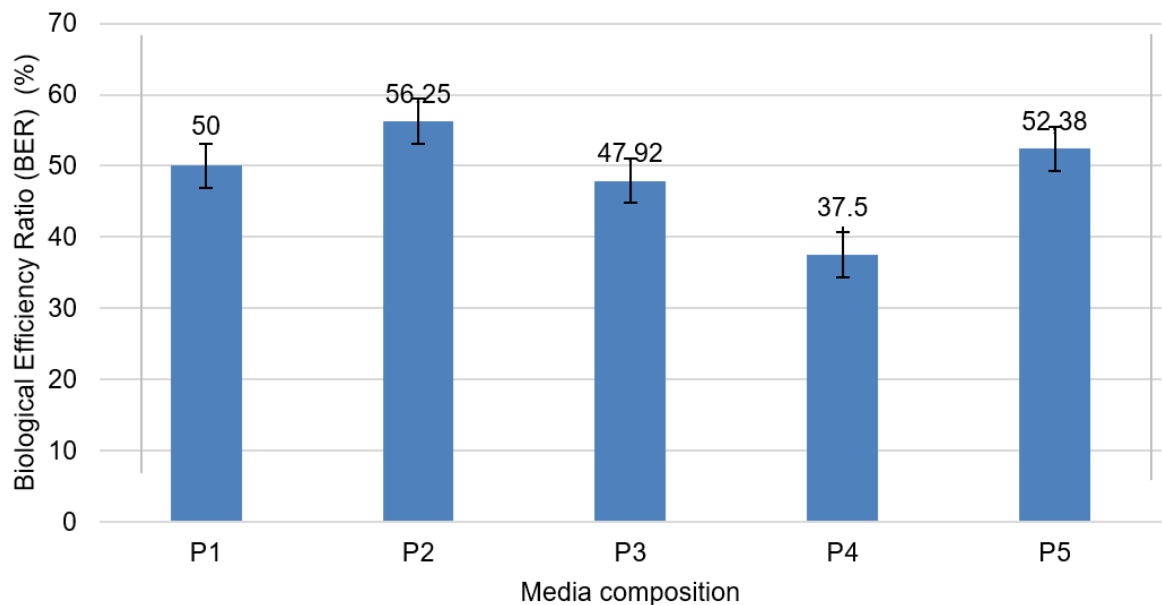


Fig. 6. Biological Efficiency Ratio of oyster mushroom in different media composition

The harvestable mushrooms had the optimal fruit body size with a thinner edge and usually it takes 2-3 days after the appearance of primordia. The average harvest of mushrooms was done twice a day, in morning and afternoon. Late in harvesting mushrooms lowered the quality of mushrooms. The fruit body became dry, specially the edge parts were perming and browning, so that reduced the weight of fresh mushroom. Therefore, the harvesting time should be right in order to get fresh mushrooms with optimal weight. Harvesting was

done by removing the whole part of the mushroom until there was nothing left in the growing media. Leaving some parts of mushrooms in baglog could make the baglog decay and therefore will lower the productivity of mushrooms.

The highest harvest of oyster mushroom was produced in P2 media, yielded 8.94 kg oyster mushroom in one planting cycle (Fig. 7). Mushroom produced from P2 also showed the highest BER value. These results confirmed that P2 is the most optimal media composition for oyster mushroom

growth media. It can be concluded that BER value is directly proportional with total weight of mushroom produced in one planting cycle.

Biodelignification of EFB

Biodelignification is the biological process of removing lignin compounds. In this case, cultivating oyster mushrooms could reduce lignin content of the growth media which is called biodelignification. Oyster mushrooms that have been inoculated on baglog used lignin as a source of nutrition for growth. A long incubation process during the growth of oyster mushroom (approximately 5 months) caused the degradation of the compounds contained in the media, one of them is lignin. Lignin content removal is beneficial because in some processes such as cellulose extraction lignin is undesirable material.

Lignin content declined continuously from from 0 to 5 months (Fig. 8). Lignin content of P1 was initially 26.5%, and slightly decreased to 26.11% in the 1st month and 24.49% in the 5th month, with a total decrease was 20%. Next, a 35.59% reduction in lignin occurred in P2 media composition. The greatest reduction in lignin occurred in the P3 media composition where lignin content initially was 26.49% and in the 5th month declined to 15.86%, the reduction reached 40.12%. In P4 there was no decrease in lignin levels because the media composition used was 100% EFB. It may due to the

fact that it is very difficult for mushroom to degrade the lignin content in the EFB. This is because lignin is a complex carbohydrate with a 3D structure and is difficult to use as a substrate (Niu *et al.*, 2021; Sukri *et al.*, 2021).

Overall, Fig. 9 shows the change in the cellulose content depending on the composition of the medium. In media containing more sawdust, there was a decrease in cellulose content. On the other hand, in the media containing more EFB, there was an increase in the cellulose content. This is because in terms of size. Sawdust in the form of powder has extensive surface contact with high fungal mycelium, thus cellulose is more easily decomposed. On the other hand, EFB is still in the form of fibers measuring 3-5 cm, so that the fungal mycelium is more difficult to decompose cellulose.

The highest decrease of hemicellulose is found in P4 at 49.56% then followed by P5 at 48.95%. P4 and P5 consist of 100% EFB and 100% sawdust respectively (Fig. 10). It seems that hemicellulose was well degraded in homogeneous medium. Fig. 11 present the micrographs of the EFB fibers before and after after being cultivated with mushroom. Before treatment, EFB fibers had rough surface and bonded with lignin while after mushroom cultivation, EFB fibers are no longer intact because EFB had been decomposed.

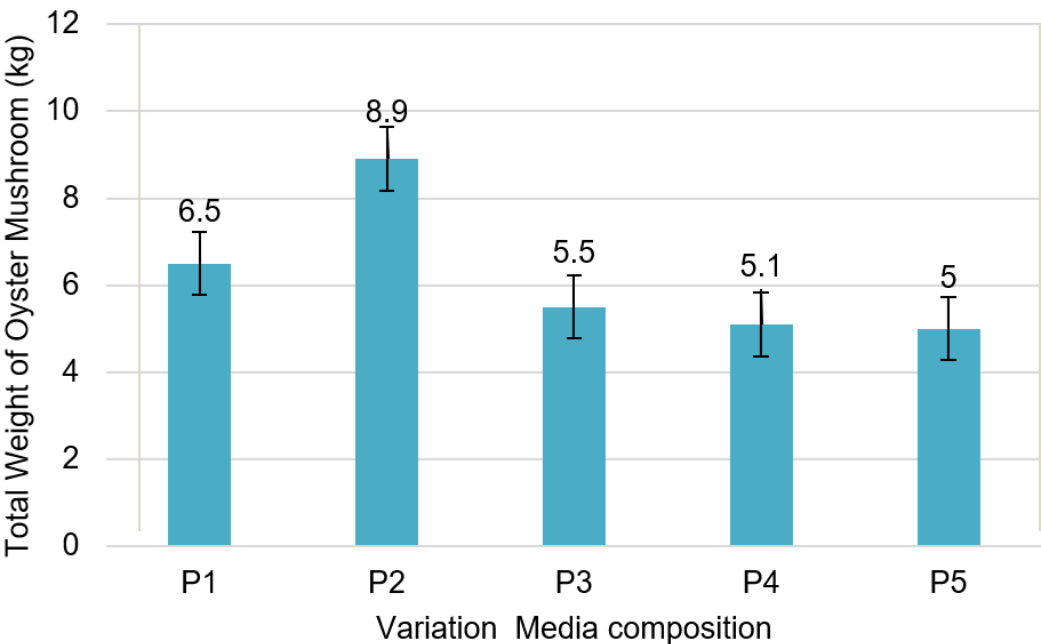


Fig. 7. Total weight of oyster mushroom

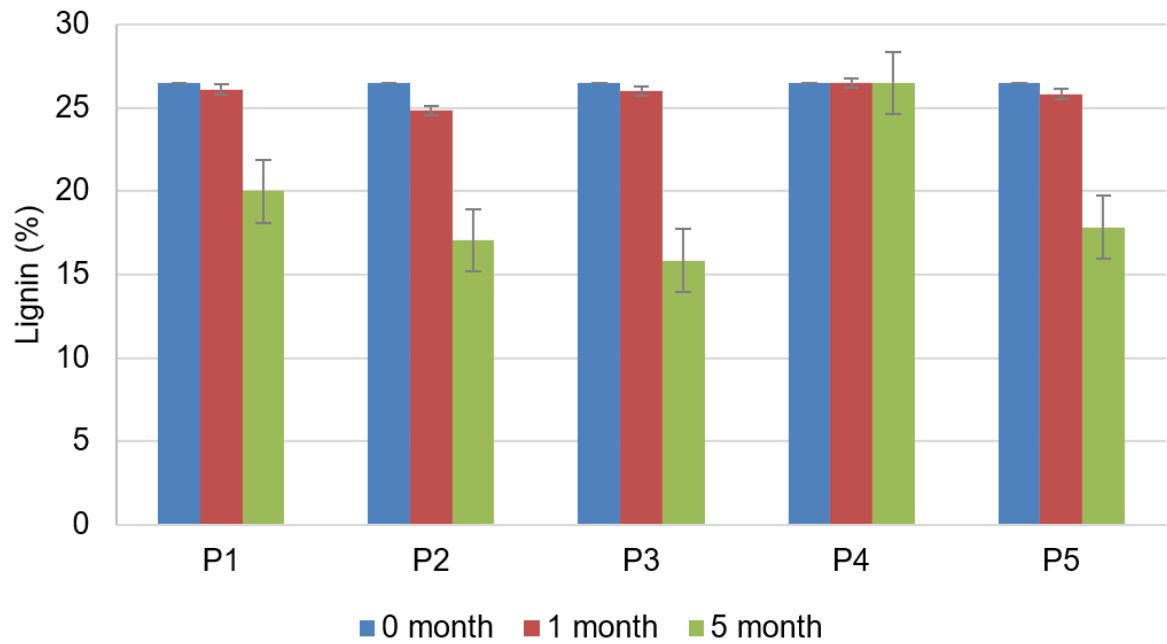


Fig. 8. Decrease in lignin during 5-month period of mushroom cultivation

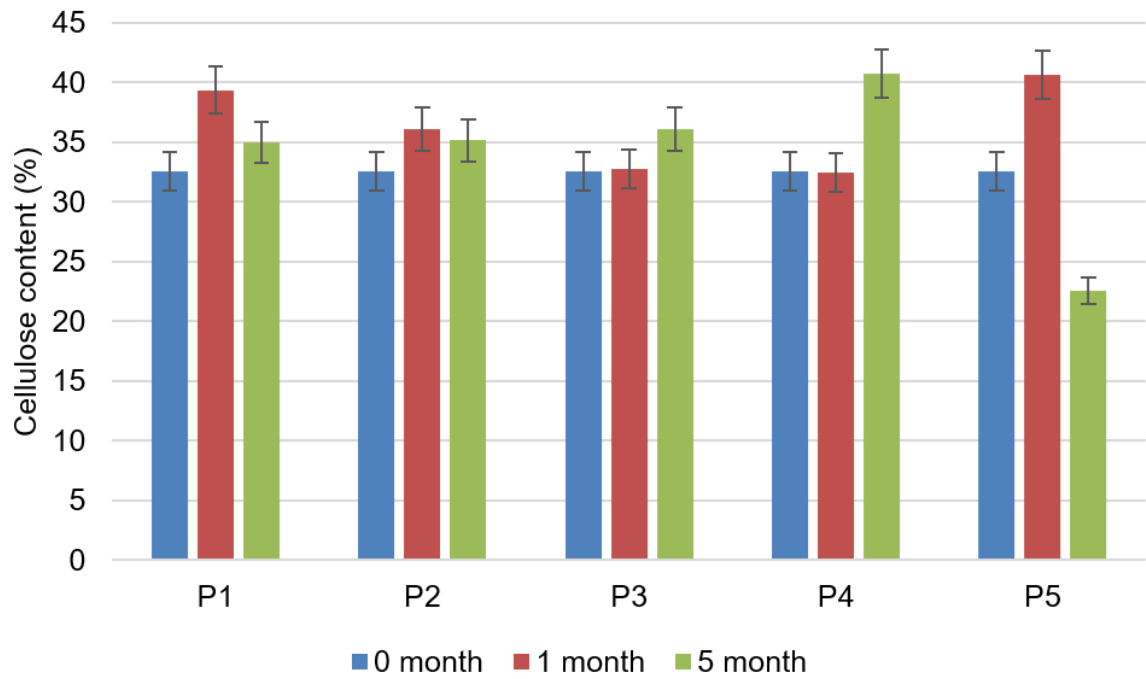


Fig. 9. Change in cellulose content during 5-month period of mushroom cultivation

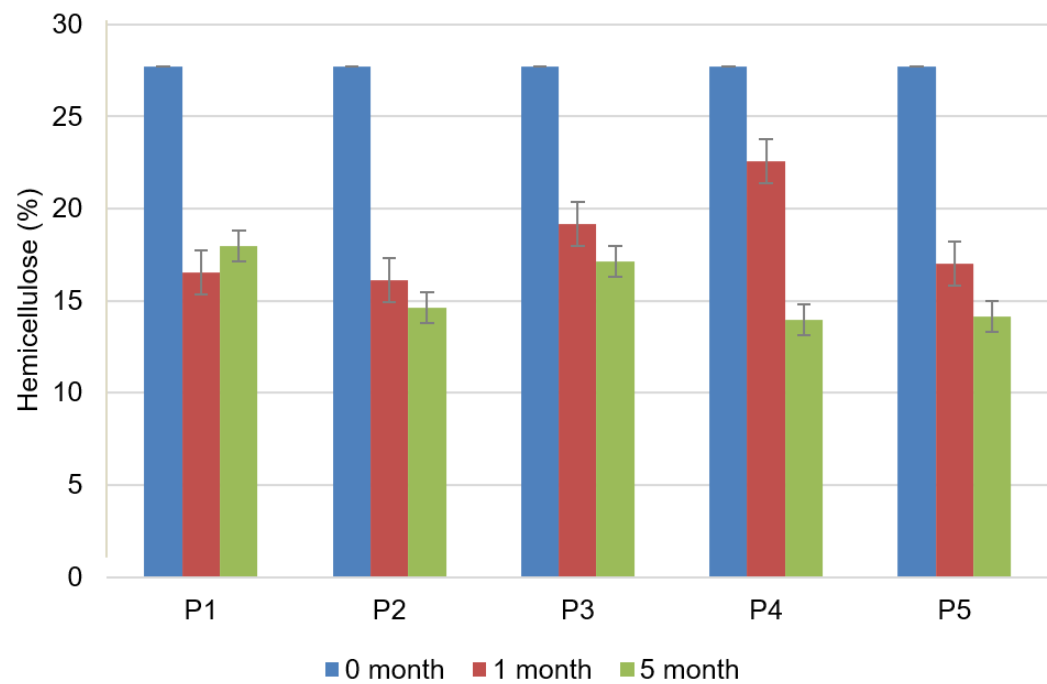


Fig. 10. Decrease in hemicellulose during 5-month period of mushroom cultivation

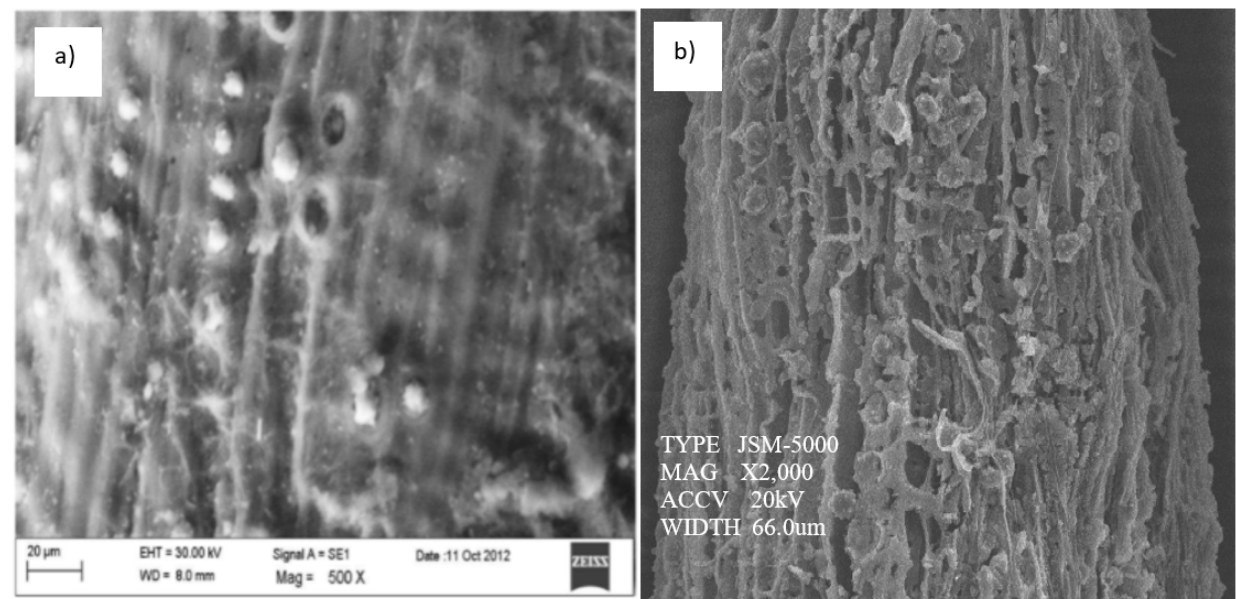


Fig. 11. The micrograph of EFB before (a) (Fatah et al., 2014) and after (b) mushroom cultivation period.

CONCLUSION AND SUGGESTION

Based on this research, BER value was directly proportional with total weight of mushroom. The highest BER value was 56.25% from P2 that contained of 50% EFB and 50% of sawdust. Bidelignification occurred during the incubation of oyster mushroom as lignin content was continuously decrease, started from 0 to 5 months. The highest decrease of lignin was found in P3 at 40.12%, from 26.49% to 15.86%. The highest decrease of hemicellulose was found in P4 (49.56%) and P5 (48.95%).

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