Journal of Tropical Biodiversity and Biotechnology
Volume 07, Issue 02 (2022): jtbb69743
DOI: 10.22146/jtbb.69743

Short Communications

# Microbial Degradation of Lignocellulose in Empty Fruit Bunch at Various Incubation Time

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**Keywords:**
cellulose
delignification
empty fruit bunch
incubation time
*P. ostreatus*
**Submitted:**
12 October 2021
**Accepted:**
03 February 2022
**Published:**
27 May 2022
**Editor:**
Miftahul Ilmi

**ABSTRACT**
Isolation of cellulose in empty fruit bunch (EFB) is hindered by lignin content. EFB was pretreated with *Pleurotus ostreatus* which has the ability to degrade lignin, in various incubation times (0th, 15th,30th, 45th, and 60th). The result showed lignin and cellulose decreased from 29.3% to 20.3% and from 40.2% to 26.2%, respectively. The optimum degradation time was on 30th day in which lignin and cellulose contents decreased from 29.3% to 20.3% and from 41% to 39.7% respectively. The cellulose to lignin (C/L) ratio increased from 1.40 to 1.99. These data revealed that *P. ostreatus* have a high potential for EFB delignification.



Indonesia is one of the largest palm oil-producing countries in the world with a total production of 40,5 million tons in 2018. Palm oil processing produces waste including empty fruit bunches (EFB). One ton of fresh fruit bunches processing produces 220-230 kg of EFB which is equal to 8.92-9.33 million tons/year (Asari et al. 2015). However, the utilization of EFB is still limited such as for fertilizer, piled up in the ground or just burned.

EFB contents cellulose, lignin, and hemicellulose by 37.3-46.5%, 27.632.5%, and 25.4-33.8%, respectively (Asari et al. 2015). The cellulose is able to be utilized in various applications such as bioethanol (Bukhari et al. 2014), adsorbents (Mahmood et al. 2018), etc. Unfortunately, isolation of the cellulose is difficult because of high lignin content in EFB. The presence of lignin around the cellulose makes chemical and enzymatic activities are restricted due to steric hindrance (Mahmood et al. 2018).

One of the biological methods which is generally known to pretreat EFB is by applying fungi. It uses low energy, is cheap, and environmentally friendly. White rot fungi are the most efficient for cellulose isolation because of their selectivity to degrade lignin, in contrast to brown and soft rot fungi where they are selectively degraded cellulose (Wang et al. 2013).

Oyster mushroom (*Pleteurotus ostereus*) is a white rot fungus that has been used previously to degrade lignocellulosic in various types of material, such as cotton husks (Li et al. 2001), and palm fronds (Metri et al. 2018).

According to literatures, it is known that the incubation time is an important factor to degrade lignocellulosic effectively. This study was conducted to determine the effect and optimum incubation time on *P. ostreatus* toward the lignocellulosic composition in EFB. Empty fruit bunch was subjected to fungal pretreatment using *P. ostreatus* at various incubation times 0th, 15th, 30th, 45th, and 60th days.

The empty fruit bunch was collected in Sungai Malaya Village, West Kalimantan. Mushroom spawn was obtained from the Mushroom Nursery in Parit Suka Maju, Pontianak, West Kalimantan. The chemicals are sulfuric acid ($H_2SO_4$), nitric acid ($HNO_3$), acetic acid ($CH_3COOH$) from Merck-Millipore, and distilled water. The study was run with five incubation times 0th, 15th, 30th, 45th, and 60th days. Each incubation time was made for 5 samples. Each sample was tested triple for lignin and cellulose contents.

EFB was washed with distilled water then dried for three days. Dried EFB was chopped and cut by 1-2 cm. The chopped EFB was put into a plastic bag and distilled water was added gradually until the moisture content reached 60% (Eq. 1). Each sample was autoclaved at 121 ˚C and 15 psi for 15 minutes. After the samples were cooled, each sample was inoculated and incubated with 0.4 grams of *P. ostreatus* for various incubation times (0th, 15th, 30th, 45th, and 60th days) with a temperature of 28±1 ˚C and relative humidity of 80%. Temperature and humidity are continuously monitored with the HTC humidity sensor. Temperature was controlled by a negative thermocouple (NTC) sensor and a soldering iron as a heater, while humidity was controlled by a mini fan and a manual sprayer.

$$\text{Moisture content} = \frac{Ww-Dw}{Ww} X100\% \quad \text{(Eq. 1)}$$

Ww: wet weight; Dw: dry weight.

Determination of cellulose was conducted by modifying Crampton and Maynard (1937) method (2). All fungal-treated EFB were separated from the *P. ostreatus* by removing mycelium. Next, treated EFBs were dried in an oven at 60 ˚C until a constant weight. A total of 0.1 grams of dried EFB from each incubation time was weighed separately in a test tube (a). Furthermore, dried EFB was added 2 mL of a solution consisting of 80% acetic acid ($CH_3COOH$) and 8.5% nitric acid ($HNO_3$). The test tube was closed and heated for 20 minutes at 100 ˚C, then 12 mL of distilled water was added. The solution was filtered through filter paper (b). The filter paper containing the cellulose residue was then washed with 95% ethanol ($C_2H_2O_5$) to clean from the remaining nitric acid. Finally, filter paper was dried in an oven at a temperature of 60 ˚C until constant weight (c). Calculation of cellulose content using Eq. 2.

$$\text{Cellulose content} = \frac{c-b}{a} x100\% \quad \text{(Eq. 2)}$$

The determination of lignin content was carried out by the Klason method (Pringle 1940). A total of 0.1 grams of dried EFB from each incubation time was weighed separately in a test tube (d). Then, the sample was added with 1 mL of 72% sulfuric acid ($H_2SO_4$) and left it for an hour at room temperature. Further, 28 mL of distilled water was added to the test tube and heated at 120 °C for 1 hour. The solution was filtered through filter paper (e). Subsequently, the filter paper containing the residue of lignin was washed with distilled water to remove the remaining sulfuric acid. The filter paper was heated in the oven at 100 °C until the constant weight (f). Calculation of lignin content using Eq. 3.

$$\text{Lignin content} = \frac{f-e}{d} x100\% \quad \text{(Eq. 3)}$$

The optimum incubation time was determined based on the ratio of the cellulose and lignin contents in EFB during the pretreatment process. The optimum incubation time is reached when C/L ratio is the highest.

Figure 1 showed growth of *P. ostreatus* from days of 15th to 60th. The first phase of fungal growth is shown from the appearance of mycelium on days 15th until 45th and the final phase was indicated by presence of basidia from 45th to 60th days.

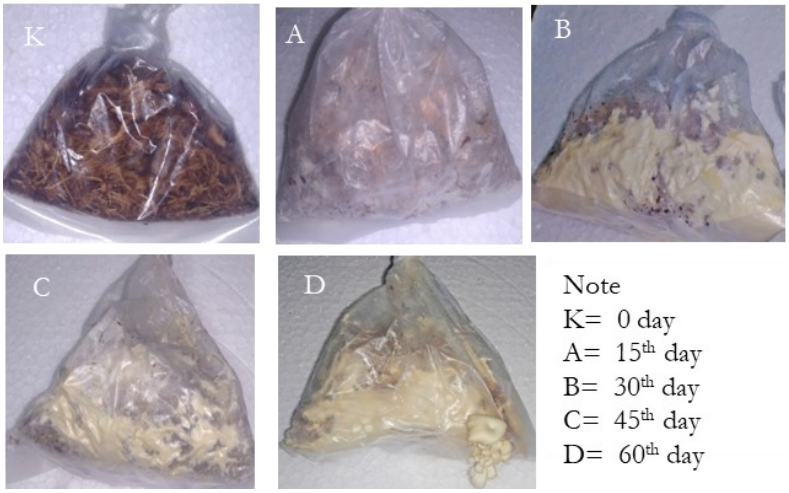


**Figure 1.** *P. ostreatus* growth in EFB.

Figure 2 showed lignin content in EFB started decreasing from 29.3% at the beginning of *P. ostreatus* growth (0th day) to 20.3% on 30th day. In contrast, the cellulose in EFB did not change significantly at early stage of incubation (days 15th until 30th). Subsequently, the cellulose began to degrade significantly from 40% on 30th day to 29.1% on the 45th day. The fungi use lignocelluloses in EFB for their daily nutrients. As a result, the lignin and cellulose composition in EFB is changeable. *Pleurotus ostreatus* degrades lignin in the beginning of their growth in order to access nutrients from carbon sources such as cellulose. Lignin binds cellulose strongly with ether and hydrogen bonds as a guard for cell resistance, a structural framework and protector (Harmsen et al. 2010).

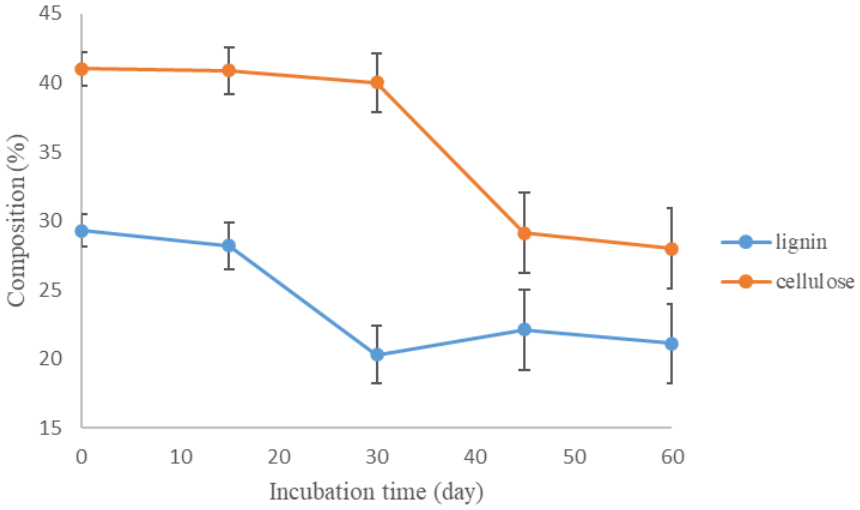


**Figure 2**. Lignocellulose composition in EFB at various incubation time.

Mustafa et al. (2016), mentioned that higher of C/L ratio indicates higher isolation yield of cellulose. Figure 3 showed that the optimum incubation time for the highest C/L ratio was 30th day. The cellulose to lignin (C/L) ratio of empty fruit bunch increased from 1.40 on 0th day to 1.99 on 30th day. Some previous studies on degradation of lignocelluloses revealed that *P. ostreatus* have also performed effectively to reduce lignin content in cottonseed husks (Li et al. 2001) and oil palm midribs (Metri et al. 2018) by giving (C/L) ratio 2.06 and 1.92, respectively.

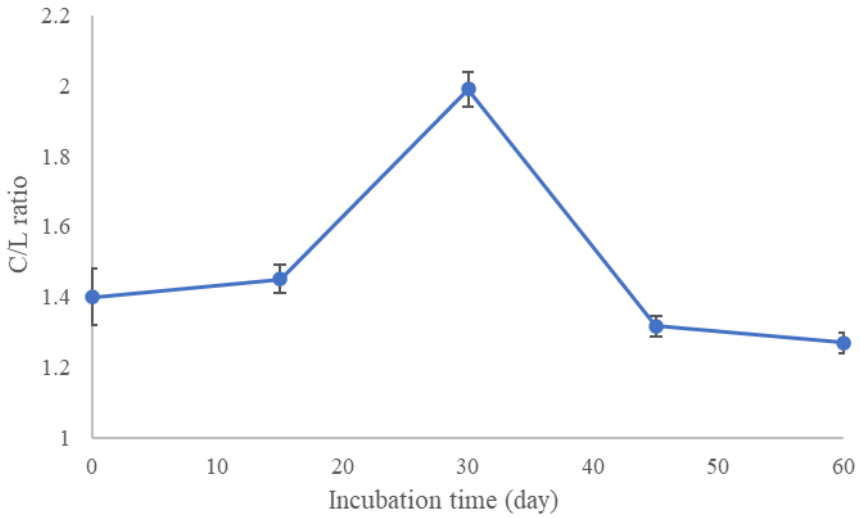


**Figure 3.** C/L changes in EFB at various incubation time.

In summary, *P. ostreatus* shows a good capability to pretreat EFB substrate by the formation of mycelium, pinning, and mushroom fruiting bodies. The incubation time of the fungus gave a significant effect on the composition of lignin and cellulose. The ability of *P.ostreatus* to degrade lignin was occurred in the early growth phase of the fungus, in contrast, highest degradation of cellulose arise in the final phase of fungal growth. The optimum incubation time was reached on 30th day.

## AUTHORS CONTRIBUTION

NRA designed, collected, and analyzed the research data, R and G.G helped, supervised all the process, and re-wrote the manuscript.

## ACKNOWLEDGMENTS

This research was helped and financed by UNTAN as well as some family member.

## CONFLICT OF INTEREST

There is no conflict of interest regarding the research or the research funding.

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