Glucose production from oil palm empty fruit bunch (OPEFB) using microwave and fungal treatment method

S R Juliastuti\(^*\), N Hendrianie\(^1\), K R Sabar\(^1\), S Anggita\(^1\)

\(^1\)Department of Chemical Engineering, Institut Teknologi Sepuluh Nopember, Jl Raya ITS, 60111 Surabaya, Indonesia

*Corresponding author: juliaz30@chem-eng.its.ac.id

**Abstract.** Palm oil is one of the plants that can be used to produce cooking oil, industrial oil, and fuel. Indonesia itself was one of the largest palm oil-producing countries in the world. OPEFB includes lignocellulose biomass, whose main content is 46.5% cellulose, 33.8% hemicellulose, and 32.5% lignin. This research method used 3 stages, namely microwave pretreatment, Fungal Treatment 1 (FT 1), and Fungal Treatment 2 (FT 2). The raw materials used are OPEFB, while the fungi used are *Phanaerochaete chrysosporium*, *Tricoderma harzianum*, *Aspergillus niger*, and *Tricoderma viride*. The first step to be prepared in this study was to prepare OPEFB powder measuring 20 mesh. OPEFB powder was then put in the microwave for 20, 40, and 60 minutes. OPEFB that had been heated in the microwave was then converted into a slurry with the addition of water with a ratio of 3:5 (w/w). The second and third phases were Fungal Treatment for ±10 days. The parameters analyzed were the introduction of OPEFB, product of microwave pretreatment, product of fungal treatment 1, and product of fungal treatment 2. The content to be analyzed were lignin, cellulose, hemicellulose, and glucose. The result of pretreatment revealed that microwave-based pretreatment with the addition of alkaline solution could eliminate lignin by 83.45% and increase holocellulose levels by up to 41.907% at 300 W power and 60 minutes. As for the result for fungal treatment, the best treatment for FT 1 was treatment ratio TH:PC 1:2, which could eliminate lignin for 47.55% and FT 2 with treatment ratio TV:AN 1:2 that could increase glucose up to 84.9%.

1. Introduction

Palm oil is an industrial plant/plantation valuable as a producer of cooking oil, industrial oil, and fuel [1]. Palm Oil is one of the leading commodities of plantation products that play a significant role in the progress of the economy in Indonesia due to its much-needed vegetable oil content in the industrial sector [2]. Indonesia is the world's largest palm oil-producing country, with total crude palm oil (CPO) production reaching 40.6 million tons in 2018 and contributing exports worth USD 18.5 trillion. The islands of Sumatra and Kalimantan are the right places to conduct oil palm processing activities. Riau is among the provinces with the largest area of oil palm plantations in Indonesia. Recorded in 2017, the area of oil palm plantations is around 2.703.199 hectares and continues to increase to approximately 2.806.349 hectares in 2019 [3]. This means that the area of oil palm plantations is more than a quarter the area of Riau Province itself which is more than 8.7 million hectares.

The production process of Crude Palm Oil (CPO) produces a lot of waste that is still not utilized, therefore it potentially pollute the environment. Oil Palm Empty Fruit Bunches (OPEFB) are the densest waste that is about 20% of the number of Fresh Fruit Bunches (FFB) of palm oil processed. Based on BPS data [2], Indonesia is the world's largest palm oil producer, producing above 40.56 million tons in 2018. Indonesia shifted Malaysia's position, which had previously occupied the first position for many
years. 23% of the total palm oil production is Oil Palm Empty Fruit Bunches (OPEFB) [2]. Only 10% of OPEFB has been used for boiler fuel and compost, even though many products can be processed from OPEFB waste.

OPEFB is lignocellulose waste from palm oil mills [4] and organic matter in large piles; there will be an anaerobic decomposition or large-scale decay process. The decay process causes the production of gases that pollute the atmosphere, such as CH$_4$, H$_2$S, NH$_3$, and Nox which can cause pests, home effects and global warming.

OPEFB includes lignocellulose biomass, whose main content is 41.3%-46.5% cellulose, 25.3%-33.8% hemicellulose, and 27.6%-32.5% lignin [5]. Cellulose and hemicellulose contained in OPEFB have the potential to be utilized into a variety of more valuable products such as glucose, organic acids, solvents ethanol, acetone, butanol, single-cell proteins, xanthan, antibiotic substances, and various other products. Lignin is a waste component of OPEFB that is relatively difficult to degrade. This compound is a structural polymer associated with cellulose and hemicellulose. To be transformed into a simpler element, OPEFB must be degraded first by pretreatment.

In this study, enzymatic saccharification occurs, where the cellulase enzyme helps to convert cellulose into simple sugar. The main challenge of saccharification of the OPEFB is the hemicellulose and lignin content. Pretreatment of lignocellulose is a crucial step for the efficient utilization of biomass for ethanol production. Effective pretreatment of these lignocellulosic materials is needed to liberate the cellulose from the lignin seal and at the same time to reduce the lignin content, reduce cellulose crystallinity, and increase cellulose porosity.

Microwave irradiation has been widely used in many areas because of its high heating efficiency and easy operation. Some studies have shown that microwave irradiation could change cellulose's ultrastructure, degrade lignin and hemicelluloses in lignocellulosic materials and increase the enzymatic susceptibility of lignocellulosic materials. When using microwave-assisted alkali pretreatment of wheat straw, lower sugars losses and higher hydrolysis rates than conventional alkali pretreatment methods were observed. Combination microwave treatment with either acid or alkali or combined acid/alkali might be an alternative for pretreatment of lignocellulosic materials has been recently explored.

Therefore, this study aims to reduce the value of lignin present in waste tendan empty coconut (OPEFB) with pretreatment using a microwave and know the yield of glucose produced from the Fungal Treatment process.

2. Materials and method

2.1. Materials
OPEFB was obtained from PT. Wilmar and NaF was obtained from SAP chemicals. While the fungi used were four kinds of fungi, Tricodherma harzianum and Tricodherma viride were obtained from the Lumajang Agricultural Laboratory. Phanerochaete chrysosporium was obtained from Airlangga University, and the last fungi, Aspergillus niger, was obtained from the Laboratory of Microbiology in ITS Chemical Engineering. Microwave treatments were carried out using a domestic microwave oven (Kris Microwave 20L). The microwave oven provided microwave radiation at variable power levels of 300 W and 350 W.

2.2. Method
2.2.1. Pretreatment. Alkaline pretreatment was carried out with 50 grams of OPEFB and was added by 0.05 grams of NaF and 500 mL aquadest on a beaker glass of 1 L, then put in the microwave according to variable 300 W for 20, 40, and 60 minutes and 350 W for 20, 40, and 60 minutes. The decrease in lignin, cellulose, and hemicellulose after microwave-alkaline pretreatment was calculated.

2.2.2. Fungal treatment. OPEFB porridge was made by mixing OPEFB and water with a weight ratio of 3:10. Tricodherma harzianum, Aspergillus niger, Tricodherma viride, and Phanerochaete chrysosporium were bred on Potato Dextrose Agar (PDA). Then fungi were calculated using a microscope (counting chamber). Furthermore, the delignification and hydrolysis process in OPEFB was done by adding fungi Tricodherma harzianum, Phanerochaete chrysosporium, and bred for four days.
Chesson and DNS methods were used to analyze cellulose, hemicellulose, lignin, and glucose levels in OPEFB. Then Aspergillus niger, and Tricoderma viride with a particular concentration (according to the variable), were added on the 5th day, and fungal treatment was continued until the 9th day. All of it was put into 30 grams of OPEFB porridge for nine days at a temperature of 37°C. Furthermore, analysis of cellulose, hemicellulose, lignin, and glucose levels in OPEFB with Chesson and DNS methods

2.2.3. Chesson method. One gram of dry sample (weight a) was added by 150 mL H2O and reproduced at 100°C with a waterbath for 1 hour. The result was filtered, the residue was washed with 300 mL of hot water. Then, the residue was dried with the oven until constant weight and weighed (weight b). Residue plus 150 mL H2SO4 1 N, then was refluxed with water bath for 1 hour at a temperature of 100oC. The result was filtered and washed to neutral (300 mL), and the residue was dried to a constant weight, then weighed (weight c). The dry residue was added by 100 mL H2SO4 72% and soaked at room temperature for 4 hours, then was added by 150 mL H2SO4 1 N and was refluxed at 100°C with a water bath for 1 hour. Residues were filtered and were washed by H2O until neutral (400 mL). The residue was then preheated in an oven at 105°C until constant weight and weighed (weight d). Furthermore, the residue was furnaced and weighed (weight e).

2.2.4. DNA analysis. Before DNS analysis was carried out, the important thing was to make a standard glucose solution first. The first step was to make a solution by mixing 0.9 g of glucose in 100 mL aquadest. Then, a standard glucose solution is created by diluting the supply solution with dilution 1:1 (without dilution), 1:2, 1:3, 1:4, and 1:5.

Next, create a DNS solution, first by dissolving 16 g NaOH in 200 mL aquadest, then added 10 g of DNS solution and stirred using stirrer until completely dissolved. Then, 30 g of NaK-tartrate and 8 g of Na-metabisulfite were dissolved into 500 ml of aquadest. The solution (1) was mixed with solution (2) and was added by water up to 1000 mL.

After making a standard glucose solution and a DNS solution, a standard glucose curve is created, the curve will be used to analyze the fermentation-extractive process sample. 2 mL of glucose-containing inventory solution with 3 mL of DNS solution were mixed. Then, the mixture is heated in boiling water for 15 minutes. Then, the mixture was cooled using ice or running water. The same process was done for different concentrations of glucose solution. Absorbance is measured and recorded on spectrophotometers with wavelengths of 540 nm. Create a graph by plotting between glucose levels as a reduction sugar with absorbance.

The production of the blank solution is done by mixing 2 mL aquadest with 3 mL of DNS solution. Heat the mixture in boiling water for 5 minutes. Then, the mixture is cooled using ice or running water.

To analyze fermented-extractive samples, 2 mL of diluted sample solution (50 times using aquadest) were mixed with 3 mL of DNS solution. Then, the mixture is heated in boiling water for 15 minutes. The mixture is cooled using ice or running water. Repeat these steps for different samples. Absorbance is measured and recorded on spectrophotometers with a wavelength of 540 nm. The absorbance is obtained by the equation of the standard glucose curve.

3. Result and discussion

3.1. Characterization of OPEFB
Characterization was carried out to determine the main component of OPEFB, and the composition is shown in table 1. As can be seen, the main content of OPEFB is holocellulose (cellulose + hemicellulose), which can be interpreted that OPEFB has the potential to be utilized as a glucose producer. One of the obstacles in the process of glucose production is lignin. Lignin contained in OPEFB was high, which is about half of the holocellulose contained. Therefore, pretreatment is used to perform the delignification process by breaking hydrogen bonds in lignin, thus increasing the porosity of biomass [6,7]. Reducing the content of lignin in biomass can increase the conversion of cellulose into glucose to increase glucose yield.
Table 1. Composition of Oil Palm Empty Fruit Bunch (OPEFB).

| Component          | Content (%) |
|--------------------|-------------|
| Lignin             | 31.53       |
| Cellulose          | 43.57       |
| Hemicellulose      | 19.53       |
| Moisture           | 4.98        |
| Other components   | 0.38        |

3.2. Pretreatment
The size of biomass material could affect the process of lignin removal, wherein smaller biomass particles obtained better results [8]. Particle size reduction increases surface area to volume ratio and improves enzyme accessibility to active binding sites for the subsequent enzymatic hydrolysis and fermentation steps. In this study, we choose the biomass size in 20 mesh.

This study used NaF, reported by [6] NaF could break the hydrogen bonding of lignin in much lower temperature and milder catalysts (in small amounts). Moreover, the application of alkaline solutions leads to the removal of the lignin barrier, disruption of structural linkages, reduction of cellulose crystallinity, and decrease in the polymerization degree of carbohydrates [9].

Pretreatment was done with the variable of power 300 W and 350 W and time 20, 40, and 60 minutes. In each variable, the content of cellulose, hemicellulose, and lignin was analyzed to find out the influence of the pretreatment process on raw materials OPEFB. Figures 1 and 2 could be seen by comparing the content of lignin and holocellulose from raw OPEFB with OPEFB after pretreatment. Results showed an influence of time and power on the pretreatment process on the loss of lignin, where the maximum lignin removal could reach 83.45%. From figure 1, it could be seen the influence of time on the removal of lignin, where increasing pretreatment time would increase the loss of lignin. In addition, power also had an effect where the lower power, 300 W, is more efficient in eliminating lignin because with lower power, the lost lignin content is higher. From figure 2, it could be seen as a comparison between holocellulose (cellulose + hemicellulose) contained from raw OPEFB and OPEFB after pretreatment. It could be seen that there was a significant increase in holocellulose levels to reach 43.17% at 350 W, 20 minutes. This can occur due to the use of alkaline solvents. According to Kanani et al., alkaline solvents can increase the content of hemicellulose in biomass materials [10]. In addition, the decrease in lignin levels also affected the increase in cellulose levels in biomass materials. The use of microwaves was considered a promising pretreatment process, whereby microwave irradiation helps by utilizing the thermal and non-thermal effects produced by microwaves. Electromagnetic waves from the microwave could occur due to contact between microwave energy directly to the polysaccharides. These contacts created ion movements and vibrations from polar molecules, leading to heat generation and wider molecular collisions. This triggers acceleration in chemical, biological, and physical reactions. From this study, the most efficient pretreatment process occurred at 300 W power and 60 minutes with the addition of NaF (0.1%).

3.3. Fungal treatment
Fungal treatment was done in two stages, namely Fungal treatment 1 (FT 1) and Fungal Treatment 2 (FT 2). Fungal Treatment 1 was done to degrade lignin contained in OPEFB. This method was performed on bioreactors for 5 days at a temperature of 37°C with stirring and the addition of fungi Trichoderma harzianum (TH) and Phanaerocaete chrysosporium (PC) by comparison, 1:1, 1:2, and 2:1. Fungal Treatment 2 was done by continuing the process of fungal treatment one for 4 days at a temperature of 37 °C with stirring and the addition of fungi Trichoderma viride (TV) and Aspergillus
In this experiment, Chesson-datta analysis method was conducted to analyze the content of cellulose, hemicellulose, and lignin contained after the fungal treatment process 1 and the DNS analysis method to analyze the glucose content in OPEFB after the fungal treatment process.

![Figure 1](image1.png)

**Figure 1.** Comparison of lignin content of raw OPEFB, microwave pretreatment 300 W and microwave pretreatment 350 W.

![Figure 2](image2.png)

**Figure 2.** Comparison of holocelullose content of raw OPEFB, microwave pretreatment 300 W and microwave pretreatment 350 W.

From table 2, it could be seen that the largest decrease in lignin was in the treatment of TH : PC 1:2 by 47.55%, from the initial OPEFB of 7.97% to 4.18%. The smallest decrease in lignin levels was in the treatment of TH : PC 2:1 which was 12.3% and did not much different from the treatment of TH:PC
1:1, which was 12.55%. There was an increase in cellulose levels from the initial OPEFB by 34.34% to 46.55% after treatment (for best treatment results). In addition, there was also a decrease in hemicellulose levels in the initial OPEFB by 54.74% to 18.66% after treatment (for best treatment results). The decrease in lignin levels was due to the fungi P. chrysosporium and T. harzianum having extracellular enzymes, lignin peroxide (LiP), and Manganese peroxide (MnP), which were lignonlytic enzymes. LiP oxidized aromatic compounds (phenolic and non-phenolic) through the release of one electron, which then forms radical cations and phenoxy radicals. It then reacted with nucleophile and oxygen molecules to produce enzymatic combustion that would break the bonds of C-C and C-O, polarized the polymer compounds, and opened the aromatic ring [11]. From fungal treatment 1, it could see that the best results obtained occurred in the treatment of TH: PC 1:2. This was in accordance with Kristianto et al., stating that the comparison of PC:TH 2:1 resulted in the highest decrease in lignin levels [11].

Table 2. Cellulose, hemicellulose and lignin content of OPEFB after fungal treatment 1 (FT 1).

| Variable     | Cellulose (%) | Hemicellulose (%) | Lignin (%) |
|--------------|---------------|-------------------|------------|
| OPEFB        | 34,34         | 54,74             | 7.97       |
| TH : PC 1:1  | 42,87         | 31,26             | 6.97       |
| TH : PC 1:2  | 46,55         | 18,66             | 4.18       |
| TH : PC 2:1  | 35,22         | 34,73             | 6.99       |

From table 3, the highest increase occurred in treatment TV:AN 1:2, where there was an increase in glucose levels up to 84.9% from the initial glucose level (FT 1) of 0.0912% to 0.1687%. This was followed by treatment TV:AN 1:1, which increased by 46.6%, and treatment TV:AN 2:1, which increased by 38.2% from the initial glucose level (FT 1). It could occur because the fungi T. viride and A. niger were able to produce cellulosic enzymes that had the ability to hydrolyze the bonds of β-1-4 in the cellulase and convert them into monomers. This was in accordance with [11] study that in FT 2 glucose content in each treatment would experience an increase caused by the ability of A. niger, T. viride, P. chrysosporium, and T. harzianum that had been added previously in degrading cellulose in the fungal treatment process 2 to produce cellulosic enzymes such as cellulase.

Table 3. Glucose content of OPEFB after fungal treatment 1 and fungal treatment 2.

| Variable | FT 1 | FT 2 |
|----------|------|------|
|          | Absorbance | Glucose (%) | Absorbance | Glucose (%) |
| 1:1      | 0.0380 | 0.0797 | 0.1317 | 0.1169 |
| 1:2      | 0.0670 | 0.0912 | 0.2623 | 0.1687 |
| 2:1      | 0.1440 | 0.1218 | 0.2613 | 0.1683 |

4. Conclusion
This study obtained the potential use of microwave-based pretreatment with the addition of an alkaline solution, NaF. It can also be concluded that the process is more efficient at lower power with the addition of NaF. The largest decrease in lignin occurred in microwave pretreatment at 300 W for 60 minutes with an increase of 0.1% NaF of 83.45%. As for the fungal treatment, the use of P. chrysosporium and T. harzianum with treatment TH : PC 1:2 are effective for use as a lignin-degrading microbe, where lignin can be reduced by up to 47.55%. Also, the use of T. viride and A. niger with treatment TV:AN 1:2 in fungal treatment 2 are effective to degrade cellulose into glucose as well as to increase the glucose content in OPEFB by 84.9%.
Lignin surrounds cellulose is a major problem in the process of cellulose degradation. Therefore, by maximizing the process of removal of lignin, higher levels of cellulose will be obtained, and the yield of glucose will be increased.

5. Reference

[1] Kadim L A N 2014 Informasi dan Teknologi Ilmiah III(2) 49-52
[2] Badan Pusat Statistik Indonesia 2019 Statistik Kelapa Sawit Indonesia accessed from https://www.bps.go.id/indicator/54/768/1/produksi-perkebunan-rakyat-menurut-jenis-tanaman.html
[3] Kementerian Pertanian Republik Indonesia 2020 Palm Oil Area by Province in Indonesia 2017-2021 accessed from https://www.pertanian.go.id/home/index.php?show=repo&fileNum=229
[4] Rivani M., Herawan T 2013 Prosiding Pertemuan Teknis Kelapa Sawit 2013 7-9
[5] Syafwina, S, Honda Y, Watanabe T, Kuwahara M 2002 Wood research: bulletin of the Wood Research Institute Kyoto University 89 19-20
[6] Shaveta K H, Singh P 2014 Pharm Anal Acta 5 308
[7] Silverstein R A, Chen Y, Sharma-Shivappa R R, Boyette M D, Osborne J, 2007 Bioresource Technology 98(16) 3000-3011
[8] Chen C, Boldor D, Aita G, Walker M 2012 Bioresource Technology 110 190-197
[9] Jiele x, Cheng J, Sharma-Shivappa R R, Burns J C 2010 Energy & Fuels 24(3) 2113-2119
[10] Kanani N, Wardono E Y, Hafidz A M, Octavani H R 2018 Teknika Jurnal Sains dan Teknologi 14(1) 87-96
[11] Kristianto F, Gumilang A J 2013 Penurunan Kadar Lignin Dari Tandan Kosong Kelapa Sawit (TKKS) Dan Pemecahan Material Selulosa Untuk Pembentukan Glukosa Dengan Proses Fungal Treatment (Thesis, Institute Sepuluh Nopember Surabaya)

Acknowledgments

Authors wishing to acknowledge assistance or encouragement from colleagues, special work by technical staff or financial support from organizations should do so in an unnumbered Acknowledgments section immediately following the last numbered section of the paper.