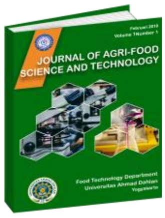
Journal of Agri-Food Science and Technology (JAFoST)
Vol 5(2) 2024: 134 – 143

P-ISSN 2686-0716
E-ISSN 2746-5519

**Journal of Agri-Food Science and Technology (JAFoST)**

Journal homepage http://journal2.uad.ac.id/index.php/jafost
Journal email jafost@tp.uad.ac.id




# Physical, Chemical, and Biological Pretreatment of Lignocellulose in Oil Palm Empty Fruit Bunches (OPEFB)

Devi[1*], Ika Fitriana Dyah Ratnasari[1], Dwi Astutik[2], Hery Widodo[3], Roni Ismoyojati[1], Nimra Sajjad[4]

[1]Plantation Crop Production Technology, Lamandau Polytechnic, Lamandau, Indonesia
[2]Industrial Plant and Refreshment Instrument Standard Testing Center, Sukabumi, Indonesia
[3]Agribusiness Study Program, Universitas Pembangunan Tunas, Surakarta, Indonesia
[4]Faculty of Health, Deakin University, Melbourne, Australia

**Corresponding Email:* devidede@gmail.com

**ARTICLE INFO**

***Article history***

Received 15/10/24
Revised 20/11/24
Accepted 29/11/24

***Keywords***

Cellulose;
Empty palm oil fruit bunches;
Hemicellulose;
Lignin;
Pretreatment

**ABSTRACT**

*Oil Palm Empty Fruit Bunch (OPEFB) constitutes a solid waste generated by the palm oil industry. Empty palm oil bunches contain cellulose or fiber. This component is the primary source for generating valuable products, including fermented sugar, chemicals, liquid fuel, carbon sources, and energy. This research contributed to determine the lignin, hemicellulose, and cellulose content of empty palm oil fruit bunches during biological, physical and chemical Pretreatment, as well as to determine the degradation of lignin, hemicellulose and cellulose. Physical treatment uses steam explosion, chemical treatment uses NaOH and biological treatment uses Trichoderma reesei FNCC 6012. Pretreatment using steam explosion has temperature levels ranging from 120 ℃, 140 ℃, and 160 ℃. Treatments using NaOH consisted of concentrations of 2%, 4%, and 6%, while treatments using Trichoderma reesei were based on fermentation times of 5 days, 10 days, and 15 days. This preliminary treatment functions to reduce the lignin levels in empty palm oil fruit bunches. The parameters observed in this research were lignin, hemicellulose and cellulose content. The research results showed that treatment using Steam explosion at temperatures of 140 ℃ and 160 ℃ was able to reduce lignin levels by 16.03% and 15.90%. Treatment using steam explosion at a temperature of 160 ℃ and Trichoderma reesei for 15 days was able to increase Hemicellulose levels by 35.84% and 36.21%. Treatment using Steam explosion at a temperature of 160 ℃ gave the best effect on cellulose of 51.09%.*

10.12928/jafost.v5i2.12008



## 1. INTRODUCTION

Indonesia's various plants were good sources of cellulose, antioxidants, vitamins, polyphenols, and many more nutrients (Indriani & Khairi, 2023). Oil Palm Empty Fruit Bunch (OPEFB) is one of the solid wastes produced by the palm oil industry. Palm oil plays a significant role in supporting Indonesia's economy (Hidayah & Wusko, 2020). One palm tree can produce 22 fronds, and one hectare will produce around 6.3 tons of fronds each year. Empty palm oil bunches contain cellulose or fiber, whereas paper is made from cellulose or fiber. The composition of palm fronds consists of cellulose at 34.89%, hemicellulose at 27.14%, and lignin at 19.87%. This component is the main source for producing valuable products such as fermented sugar, chemicals, liquid fuel, carbon sources, and energy. Lignocellulose is a polysaccharide component consisting of three types of polymers, namely cellulose, hemicellulose, and lignin. The usage of cellulose, which is a polymer, is quite restricted. It is extremely rare to find cellulose in its purest form in the natural world; rather, it is usually always found in combination with other substances, such as lignin and hemicellulose. The most significant barrier for the hydrolysis of cellulose is the presence of lignin and hemicellulose in the surrounding environment. For the purpose of degrading lignocellulose, the high cellulose content of palm fronds can be subjected to additional processing through pretreatment. (Hu et al., 2020).

Research on starch-based treatment stated that the result will enhance mechanical properties and high-water resistance (Arifani & Tamalea, 2024). Pretreatment is an important stage in the biochemical conversion of lignocellulosic biomass into biofuel. This stage requires changing the structure of cellulose biomass so that access to cellulose is higher for enzymes so that it can be converted into fermentable sugar. The pretreatment process is a preliminary treatment process for lignocellulosic materials by breaking down and reducing the lignin and hemicellulose content, destroying the crystal structure of cellulose, and increasing the material's porosity (Sun & Cheng, 2002). The pretreatment purpose is to open the lignocellulose structure to make it easier for enzymes to break down polysaccharides into monosaccharides (Goshadrou, 2019). Chemical pretreatment aims to increase cellulose biodegradation by removing lignin and/or hemicellulose (Muryanto et al., 2016). This method also aims to reduce the level of polymerization and crystallinity of the cellulose component. This chemical pretreatment was originally developed in the paper industry to delignify cellulosic materials to produce quality paper products (Menon & Rao, 2012). The initial chemical treatment is use NaOH. NaOH treatment requires a long time at low temperatures. Research by Muryanto et al., (2016), using NaOH at a temperature of 160 ℃ for 40 minutes had the highest delignification process. Pretreatment of various lignocellulosic biomass such as wheat straw, grass, hardwood, and softwood using NaOH is also able to reduce lignin content to less than 26% (Zhao et al., 2008). The use of NaOH accompanied by 50 KHz ultrasonic waves for 30 minutes increases the cellulose and hemicellulose content and reduces the lignin content (Rilek et al., 2017). Good quality cellulose can be used as a stabilizer and prevent precipitation (Giovani et al., 2024).

Biological pretreatment can be carried out using microorganisms that have cellulase enzymes that work to degrade cellulase. Biological pretreatment uses the fungus *Trichoderma reesei* (Naher et al., 2021) and *Streptomyces griseus* (Saritha et al., 2012). *Trichoderma reesei* is widely used in industry because of its ability to produce extracellular hydrolase enzymes for degrading lignocellulose in large quantities. *Trichoderma reesei* has up to 80% cellulase enzymes (Lynd et al., 2002). *Trichoderma reesei* had enzyme activity reaching 1.0313 IU/ml at a temperature of 35 ℃, pH 6 for 8 days of incubation. Pretreatment using *Trichoderma reesei* produces up to 80% endoglucanase and exoglucanase but the β-glucosidase is lower so that the main product of hydrolysis is not glucose but cellobiose (Ahamed & Vermette, 2008; Naher et al., 2021). Physical pretreatment can be done using a tool called a steam explosion, which is made of stainless steel. Pretreatment of empty fruit bunch (EFB) at 100 ℃ for 1 hour increased the cellulose content by 51% and reduced lignin by 81% (Rocha et al., 2012). The contribution of this research is to determine the cellulose, hemicellulose, and lignin content in empty oil palm bunches with physical, biological, and chemical pretreatment (Koesoemadinata et al., 2021).

## 2. MATERIALS AND METHODS

### 2.1. Materials

The study examined Oil Palm Empty Fruit Bunch (OPEFB), obtained from Bagan Kusik Village, Ketapang Regency, West Kalimantan. *Trichoderma reesei* FNCC 6012 and *Saccharomyces cerevisiae* FNCC 3012 fungi were obtained at the PAU microbiology laboratory, Universitas Gadjah Mada. Additional raw materials used were wheat flour, distilled water, mandel mineral solution, (($(NH_4)SO_4$, $KH_2PO_4$, $CaCl_2$, $MgSO_4.7H_2O$, $MnSO_4.7H_2O$, $CoCl_2.6H_2O$), 1% NaOH, 0.1% tween 80 solution, DNS acid, Potato Dextrose Agar (PDA) media, pH paper, Whatman filter paper no. 1, phenol solution, concentrated $H_2SO_4$ solution, 0.05 M citrate buffer solution pH 4.8. The tools used in the study were steam explosion, autoclave, vacuum pump, laminar, incubator, centrifuge, homogenizer, water bath, vortex.

### 2.2. Research Methods

This study used a Completely Randomized Design (CRD) consisting of one treatment factor, physical, chemical, and biological treatments. Physical treatment using Steam explosion (SE) consisted of A1 (120 ℃), A2 (140 ℃), and A3 (160 ℃). Chemical treatment using NaOH consisted of A4 (2%), A5 (4%), and A6 (6%). Biological treatment using *Trichoderma reesei* (T.r) fungus, including A7 (5-day fermentation), A8 (10-day fermentation) and A9 (15-day fermentation). Each of these treatments was repeated 3 times. The observation results were analyzed statistically with ANOVA, and if there was a significant difference between treatments, Duncan's multiple range test (DMRT) was carried out at a significant level of 5% (Fitriani et al., 2024).

#### 2.2.1. Sampel Preparation

OPEFB was obtained from Bagan Kusik Village, Ketapang Regency, West Kalimantan. OPEFB was cleaned from dirt and then reduced in size using a chopper to reduce the surface area. Then the sampel was dried using a 50℃ cabinet dryer so that the water content was below 10% (wb). The dried OPEFB was milled using a disk mill (60 mesh), and the milled OPEFB powder was sieved using an electromagnetic shaker (7µm). The powder that passed the sieve was used as material in the pretreatment process. OPEFB powder was analyzed for lignin, hemicellulose, and cellulose to determine the percentage of content before pretreatment.

### 2.3. Pretreatment of OPEFB

#### 2.3.1. Physical Treatment

This study used steam explosion (SE) for pretreatment. A 100 g OPEFB powder was dissolved in 700 ml of water and put into the steam explosion. The steam explosion was heated according to the treatment which is 120℃ (pressure 2 bar), 140℃ (pressure 4 bar), and 160℃ (pressure 6 bar), and the explosion was carried out to produce a powder. The resulting powder was filtered using filter paper to produce filtrate. The last process was analyzing the lignin, hemicellulose, and cellulose content of the physical treatment OPEFB.

#### 2.3.2. Chemical Treatment

OPEFB powder was taken in as much as 28 g, then 150 ml NaOH was added with several concentrations of 2%, 4%, and 6%. Furthermore, the mixture was soaked for 104 hours with pH 9. After soaking for 104 hours, the pH was neutralized using HCl, and filtered using filter paper. The final process is testing the lignin, hemicellulose, and cellulose content of the chemical treatment OPEFB.

### 2.3.3. Biological Treatment

In the biological therapy, the *Trichoderma reesei* FNCC 6012 isolate was revitalized on PDA slant media inside a test tube, incubated at 30℃ for 7 days, subsequently refrigerated, and utilized for the pretreatment technique. In this study, 28 g of OPEFB solid waste was taken, put into a beaker, and nutrients were added consisting of 2% w/w wheat flour (2 g), 35% v/w distilled water (35 g), and 30% v/w nutrient solution (30 g). Then stirred until homogeneous and sterilized at 121℃ and 1 atm. After cooling, added 5 ml of *Trichoderma reesei* pore solution then incubated with variations of time for 5, 10, and 15 days. The last process was testing the content of lignin, hemicellulose, and cellulose.

## 2.4. Lignin, Hemicellulose and Cellulose Analysis Method

The first stage of this analysis was boiling the dry sample (a) with distilled water at a temperature of 150 ℃ for 1.5 hours and accompanied by reverse cooling using a condenser. After that, the sample was filtered to obtain filtrate and residue. This residue was then transferred into a porcelain cup, put in an oven for 24 hours, and weighed until its weight was constant (b). Furthermore, the hot water-soluble components can be calculated by calculating the difference in constant weight of the boiled sample with the residue from the boiling. The second stage was hydrolyzing the residue from stage 1 with 150 ml of 1 N sulfuric acid, accompanied by reverse cooling using a condenser at a temperature of 150 ℃ for 1.5 hours. After the boiling was complete, the sample was filtered, and the residue was washed with hot water. Then, the residue was dried in an oven for 24 hours (c). The constant weight obtained was used to reduce the residue from stage 1 of the sample so that the hemicellulose content was known. In the third stage, the sample in the cup was added with 10 ml of 72% (v/v) sulfuric acid concentration and left for 4 hours (shaking the cup carefully every 1 hour) at room temperature. Furthermore, the sample was hydrolyzed with 1 N $H_2SO_4$ for 1.5 hours at a temperature of 150 ℃. Furthermore, the residue was filtered using a crucible filter to obtain the residue and filtrate. The residue was then rinsed with hot water until the volume of water is 300 ml. After that, the sample and crucible filter are dried in the oven and weighed to a constant weight (d). The constant weight was then used to reduce the weight of the residue in stage 2 to determine the cellulose content of the material. The last stage was ashing. The sample was ashed at a temperature of 575±25 ℃ until it becomes ash. The ash was then constant in the oven and then weighed (e). The difference in constant weight from the ashing results with the weight of the residue in stage 3 becomes the lignin weight of the sample. Therefore, the lignin content in the sample can be calculated using equation (1), (2), (3), and (4). Where a is dry sample weight; b is dry weight (constant) stage 1; c is dry weight (constant) stage 2; d is the dry weight (constant) stage 3, and e is the dry weight (constant) stage 4.

$$Hot\ water\ soluble\ materials = \frac{a-b}{a} \times 100\% \tag{1}$$

$$Hemicellulose = \frac{b-c}{a} \times 100\% \tag{2}$$

$$Cellulose = \frac{c-d}{a} \times 100\% \tag{3}$$

$$Lignin = \frac{d-e}{a} \times 100\% \tag{4}$$

# 3. RESULT AND DISCUSSION

## 3.1. Pretreatment

The pretreatment process was carried out because the lignin and hemicellulose content in the lignocellulose material of OPEFB forms a strong structure through covalent bonds that protect plant cells from microorganism attacks. The structure formed from the covalent bonds between lignin and hemicellulose protects cellulose so that cellulose is difficult to hydrolyze. Based on the OPEFB powder analysis before pretreatment, lignin, hemicellulose, and cellulose components can be seen in Figure 1. Based on the analysis of lignin, hemicellulose, and cellulose showed results of 20.05%, 30.49%, and 35.32%, respectively. This result is higher than another research. The OPEFB lignin content was 25%, hemicellulose was 30%, and alpha-cellulose was 45% (Koesoemadinata et al., 2021).

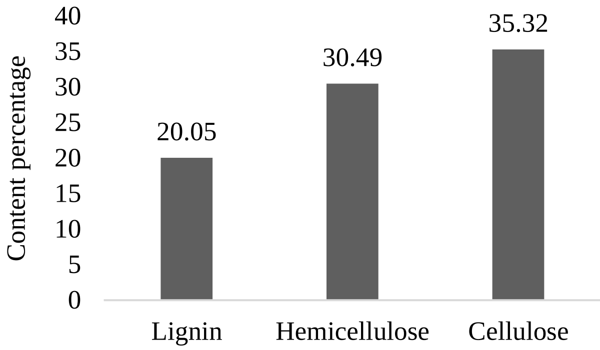



Figure 1. Content of lignin, hemicellulose and cellulose components.

### 3.2. Lignin

Lignin is a part of the plant cell wall with the most polymer after cellulose (Goshadrou, 2019). Lignin is insoluble in water and stable as a cellulose and hemicellulose adhesive (Watkins et al., 2015). Alkaline and acid solvents have disadvantages in extracting lignin because of low purity and yield levels (Ma'ruf et al., 2017). Pretreatment using steam explosion (SE), *Trichoderma reesei* (T.r), and NaOH can be seen in Figure 2.

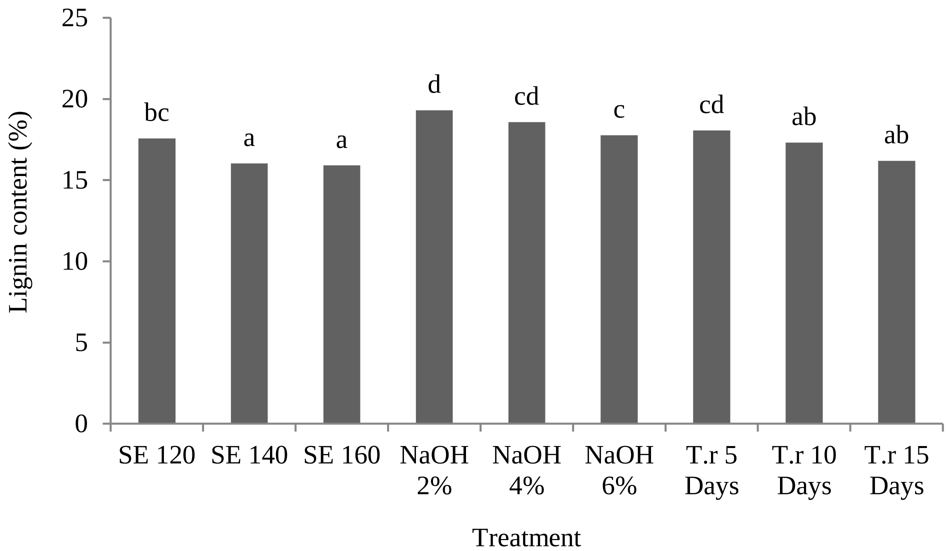



Figure 2. Lignin component content.

The results of this study indicate that the treatment using Steam explosion with a temperature of 140 °C and 160 °C has the lowest lignin content of 16.03% and 15.90%. This is because the longer the biomass is in the reactor, the longer the contact between the Steam explosion and biomass so that the rigid lignin structure in the biomass can be decomposed more. The higher the temperature in the pretreatment, the more lignin is depolymerized. This is because, at an increase in temperature, thermal softening occurs in the lignin polymer, which causes the rate of lignin depolymerization to increase (Jędrzejczyk et al., 2019). Steam explosion treatment with a temperature of 190 °C for 5 minutes with the addition of $SO_2$ reduced the lignin content of corn litter by 48% (Öhgren et al., 2007).

### 3.3. Hemicellulose

Modified hemicellulose is usually earned by esterification, etherification, cross-linking and so on (Hu et al., 2020). Hemicellulose obtained from various plant sources and places has different microstructure and molecule (Huang et al., 2021). Pretreatment of OPEFB using steam explosion (SE), *Trichoderma reesei* (T.r), and NaOH can be seen in Figure 3.

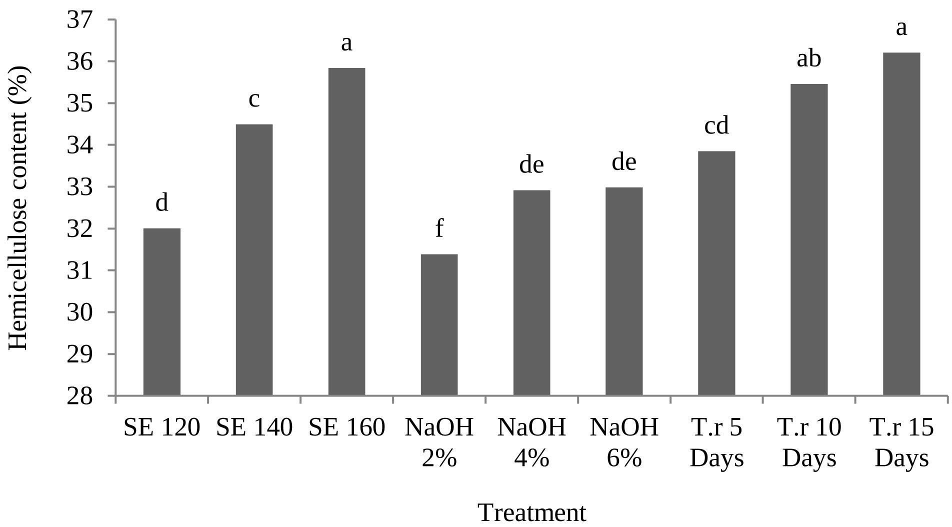



Figure 3. Hemicellulose component content.

The graph above shows the increase in hemicellulose of OPEFB. Pretreatment using steam explosion at a temperature of 160 °C and *Trichoderma reesei* for 15 days has the highest hemicellulose content of 35.84% and 36.21%. This is because pretreatment using steam explosion is a tool that is assembled using high pressure and temperature, so that it can break down lignin and hemicellulose to produce high cellulose and increase the porosity of the material, break down hemicellulose and polymerize hemicellulose (Sun & Cheng, 2002). The higher the temperature, the higher the hemicellulose content obtained. This is because during the steam explosion, xylan is depolymerized into xylose, then dehydrated into purfural (Damay et al., 2018). In the treatment using *Trichoderma reesei* for 15 days, 36.21% was the highest treatment. This is because hemicellulose components such as acetyl groups are dissolved during pretreatment. The dissolution of acetyl groups will reduce steric hydrance which is an inhibitor of xylanase enzyme activity so that the longer the fermentation time, the greater the degradation of hemicellulose (Gaikwad & Meshram, 2020; Sikder et al., 2023).

### 3.4. Cellulose

Cellulose is the most available source of natural polymers (Acharya et al., 2021). Cellulose has advantages in terms of non-toxicity, bioversatility, and biodegradibility (Chopra & Manikanika, 2022). Cellulose is associated with hemicellulose and lignin, which form the framework of plant cell walls. Cellulose is difficult to degrade either chemically or mechanically. Various microorganisms are able to hydrolyze cellulose for energy sources (Goshadrou, 2019). Enzyme activity will contribute to the conversion of cellulose slabs

(Alfarisy & Rahmadhia, 2022). Pretreatment of snack fruit fronds using steam explosion, *Trichoderma reesei*, and NaOH, can be seen in Figure 4.

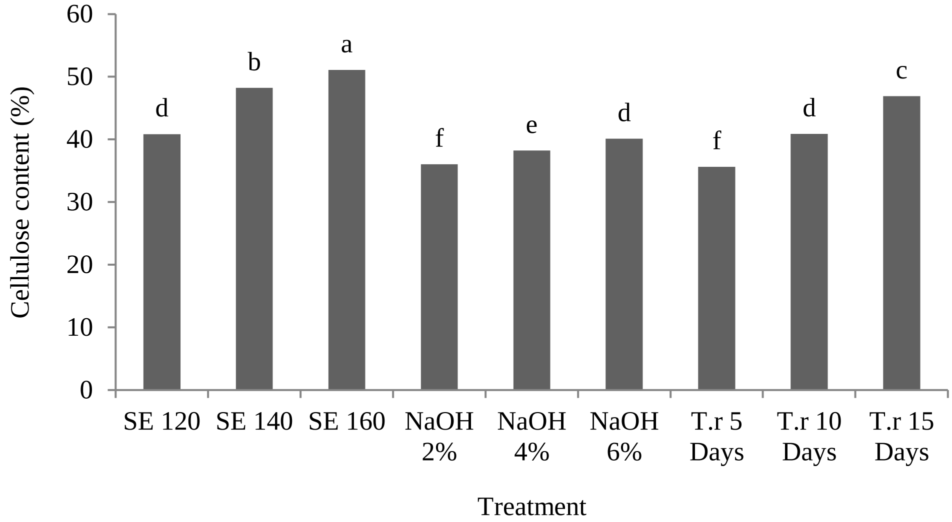



Figure 4. Cellulose component content.

Pretreatment using steam explosion at 160℃ has the highest cellulose content of 51.09%. This is because cellulose fibers are soft and shorter fibers, so they are very easily degraded in the rapid steam release process; steam and hot water in the material come out quickly, resulting in the degradation of the structure of the material (Yu et al., 2012). This process also provides a modification effect on the physical properties of the material (specific surface area, water retention capacity, color, cellulose crystallinity level), hydrolysis of hemicellulose components, and modification of the chemical structure of lignin, the higher the solubility, the pressure increase. Higher pressure can accelerate the rate of hydrolysis reaction so that in the steam explosion process, it reduce pKw so that the water can be acidic (Sui & Chen, 2016) and increase the pressure in the steam explosion reactor so that during the rapid pressure release process it produces a higher cutting force so that the biomass can be cut (Risanto et al., 2023; Rizal et al., 2018).

## 4. CONCLUSIONS

Pretreatment treatment using steam explosion at temperatures of 140 ℃ and 160 ℃ can reduce lignin levels by 16.03% and 15.90%. Treatment using steam explosion at a temperature of 160 ℃ and *Trichoderma reesei* for 15 days can increase hemicellulose levels by 35.84% and 36.21%. Treatment using steam explosion at a temperature of 160 ℃ has the best effect on cellulose by 51.09%.

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