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# Sugar content improvement by sonication in the pretreatment of empty fruit bunch hydrolysis

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**Abstract**

The empty palm fruit bunches (EFB) has great potential as an alternative feedstock for bioethanol production due to its high content of cellulose and hemicellulose. However, besides cellulose and hemicellulose, EFB also contains lignin, which can hinder the hydrolysis process and therefore requires delignification. This study aims to determine the effect of sonication in alkali delignification on the sugar content of hydrolysis. Ultrasonic in 37 KHz was performed at a temperature of 80 °C. Sonication process durations ranged from 30 minutes to 150 minutes using a 10 % (w/v) NaOH solvent. The hydrolysis of EFB fibers was carried out in a water bath at 80 °C using a 0.5 N sulfuric acid solvent in a ratio of 1:20 (w/v) for 2 hours. The sugar content was measured using the phenol-sulfuric acid method with UV-Visible spectrophotometry. In this study found that the ultrasonic irradiation time length gave good results at a time limit not exceeding 90 minutes due to hemicellulose characteristics . The highest sugar content was obtained at a sonication duration of 90 minutes, measuring 20.60 mg/L, which was 38.5 % higher than alkali delignification without sonication for 150 minutes. SEM analysis indicated that EFB had undergone changes in the surface morphology and structure. Qualitative FTIR analysis showed that the hydrolysis solution contained glucose and pentose, which are products of hydrolyzed cellulose and hemicellulose.

**Keywords:** delignification, empty fruit bunches, sonication.

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## 1. INTRODUCTION

Empty Palm Fruit Bunches (EFBs) are solid waste generated from palm oil production. This waste belongs to the category of lignocellulosic materials, and has the potential to increase added value due to its high cellulose and hemicellulose content, ranging from 25 % to 45 %. Therefore, EFB can be utilized as an alternative raw material to produce glucose used in bioethanol production as a new energy source.

Lignocellulose consists of cellulose components surrounded by a layer of hemicellulose, and strongly bound with lignin polymers. Lignin is chemically bonded to specific polysaccharides in plant cell walls, creating what are known as lignin-

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carbohydrate complexes (Thoresen et al., 2023). However, this lignin layer is a barrier for enzymes and water to enter into the complex structure of lignocellulose, in order to break it down.

The lignin content in EFB ranges from 27.6 % to 32 %, which is a significant amount and can complicate the conversion process of cellulose and hemicellulose into glucose. Therefore, a pre-treatment process is required to reduce the lignin content before entering the hydrolysis process. This stage is known as the delignification process. Delignification is the process of chemically separating lignin from cellulose. The purpose of pretreatment is not only to delignify the biomass, which can inhibit enzymatic hydrolysis but also to increase the available surface area of cellulose exposed to enzymes. Similarly, the cellulose structure opens up due to the removal of lignin during pre-treatment so that the surface area available for enzymatic hydrolysis can be increased. (Subhedar, 2014). To produce optimal cellulose, the delignification process must be done well. The cellulose produced from this process will be the main raw material in the next stage, namely hydrolysis, where cellulose will be broken down into glucose monomers. Lignin is soluble in strong bases such as NaOH.

In recent years, the development of pretreatment methods for lignocellulosic materials has gained significant attention. Extensive research and technological advancements have focused on improving the process of cellulose hydrolysis into sugars, which can then be fermented into bioethanol or other products. Various pretreatment techniques, including physical, chemical, and combined methods, continue to be of great interest. These pretreatments, often referred to as delignification, aim to reduce or eliminate lignin content that hinders the hydrolysis and subsequent processes in bioethanol production.

Biological pretreatment, which leverages enzyme activity from bacteria, offers promising potential. These bacteria can be sourced from the biomass waste being processed. For instance, a study by Darliana et al. (2020) explored the fermentation of oil palm empty fruit bunches using a bacterial consortium of Bacillus coagulans, Micrococcus sp., and Bacillus circulans derived from the same biomass waste. They achieved the highest cellulase enzyme activity of 4.58 units/ml on the 21$^{st}$ day of fermentation at a 5 % dosage. However, a challenge with using bacteria for biodegradation, especially in cellulose degradation, is the potential decline in enzyme activity over time. This decrease may result from factors such as bacterial adaptation, nutrient competition, and glucose utilization for growth and maintenance.

The combination of chemical and irradiation methods for pretreatment is also advancing. Kristen et al. (2015) combined 10 % NaOH chemical pretreatment with irradiation at 150 °C and 4-7 kg/cm² pressure for 30 minutes on oil palm empty fruit bunches (OPEFB). This treatment reduced lignin content from 35.94 % to 5.86 %, while cellulose increased from 30.41 % to 71.96 %, and hemicellulose changed from 20.70 % to 15.20 %. These results demonstrate that combining chemical and irradiation methods significantly improves the chemical composition, crystallinity, surface morphology, and hydrolysis properties of OPEFB, enhancing its suitability for bioethanol production.

Another study by Akhtar et al. (2016) investigated the potential of rice straw for ethanol production using a microwave-alkali-acid pretreatment method. This approach increased the crystallinity index of the biomass, which positively impacts enzymatic hydrolysis efficiency and overall sugar yield during fermentation. Nissa et al. (2017) examined the structural changes in OPEFB fibers using microwave-assisted oxalic acid at temperatures between 160 and 200 °C, finding optimal delignification at 180 °C. Li et al. (2023) concluded that microwave treatment should be complemented with

other pretreatment techniques to avoid localized hot spots that could cause thermal degradation of certain biomass components.

In principle, the use of ultrasonic in the delignification process aims to accelerate the process and produce optimal cellulosic feedstock for hydrolysis. The sonication process, can propagate through various media such as solid, liquid, and gas that have elastic properties. In a liquid medium, the sonication of sound waves causes acoustic cavitation effects in the solution. When the solution is stretched, the sound waves break the intermolecular bonds in the solution, and the gases formed during this process are trapped when the solution is compressed.

Ultrasonic-assisted pretreatment methods generally employ acid, alkali, or thermal solutions. Research examples include sonication with a 50 Hz frequency and 2 M NaOH solution on palm fronds (Sugiarto, 2014), and combined autoclave-based and ultrasonication pretreatments on palm empty fruit bunches (Abdullah et al., 2016). Wu et al. (2017) used ultrasound-assisted alkaline (NaOH) on rice straw, while Quek et al. (2019) evaluated ultrasound-assisted deep eutectic solvents (DESs) on OPEFB, identifying choline chloride-lactic acid (ChCl-LA) as the most effective due to its acidic properties.

Hermansyah (2019) reported a combination of acid and alkali solutions with ultrasonics that yielded hemicellulose, cellulose, and lignin contents of 14.13 %, 77.27 %, and 8.6 %, respectively. This represented a 68.73 % reduction in lignin and 121.85 % increase in cellulose compared to untreated EFB, though the process took over 10 hours. Despite these advancements, the combination of ultrasonic treatment with alkaline NaOH solution for empty fruit bunch remains limited, necessitating further research. Therefore this research is aimed at compare delignification pretreatment using alkaline solutions only versus combined alkaline and ultrasonic methods on lignocellulosic materials with the same initial composition, aims to find the most effective time for delignification assisted by sonification in order to achieve the highest sugar content in the hydrolysis process.

## 2. MATERIALS AND METHODS

This study adopted an experimental design with a completely randomized design involving two varied variables. The first variable was the delignification time with five different levels: 30 min, 60 min, 90 min, 120 min and 150 min. The second variable was the presence or absence of sonication, with one group using sonication and one group without sonication. The stages of this study can be seen in Figure 1.

### 2.1. Research Tools

In this study, the equipment used included crusher, blender, mesh size sieve 40, ultrasonic waterbath (Elmasonic S30H), waterbath, analytical balance, 500 mL beaker, magnetic stirrer, hotplate, oven, desiccator, thermometer, measuring cup, boiling flask, soxchlet cooler, filter paper, 500 mL erlenmeyer and hot mantle, UV-Vis spectrophotometer.

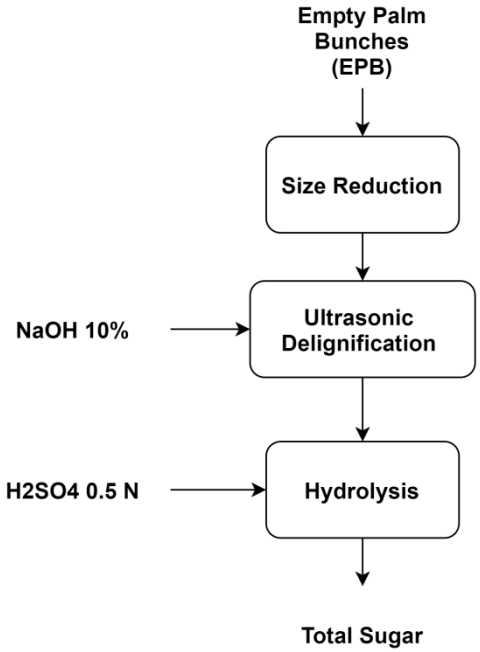



**Figure 1.** Research Flowchart

## 2.2. Research Materials

The empty fruit bunches (EFB) used in this study are EFB used in this study come from a CPO Mill located in the Agam area, West Sumatra. The process of reducing the size of the EFB starts with drying it for 2 to 3 days until it is completely dry. After that, the EFB is chopped into smaller parts and then crushed using a crusher. Next, the EFB is pulverized using a blender until the size reaches 40 mesh size. The processed EFB samples were stored in a dry state and placed in vacuum plastic bags to ensure that they were well preserved during storage.

**Table 1.** Composition of research oil palm empty bunches

| Component | % Weight |
|---|---|
| Hemicellulose | 29.88 |
| Cellulose | 33.84 |
| Lignin | 17.74 |

## 2.3. Delignification and Hydrolysis Method

A total of 20 grams of EFB sample was mixed with 10 % NaOH solution in a ratio of 1:10 (weight:volume). The mixture was stirred evenly and then sonicated in an ultrasonic bath type Elmasonic S 30 H which had been heated to 80 °C. The 37 KHz (Ofori-Boateng & Lee, 2014) sonication process was carried out for a time range from 30 minutes to 150 minutes, with increments every 30 minutes. Using UOP/H2O2 And ultrasound frequency of 37 kHz was able to improve the SSF process for high bioethanol yields. After the delignification process is complete, the delignified EFB fibers are separated from the brown-black lignin solution by washing. For large-scale applications, Singhal et al (2021) recommended EFB washing conditions are 10 min washing with a 1:15 S:L ratio at 50 $^{o}$C.

The sample that has been separated from the lignin is then resuspended with the addition of 0.5 N $H_2SO_4$ solution in a ratio of 1:20 (weight: volume). The mixture was placed in a waterbath heated to 80 °C, and incubated for 2 hours. Afterwards, the hydrolysis solution was separated from the precipitate using a vacuum pump and Whatman No. 42 filter paper. The solution was then transferred into glass bottles and stored in the refrigerator.

### 2.4. Analysis of glucose content

In this study, glucose levels were analyzed using the phenol-sulfuric acid method in UV-Visible spectrophotometry (Wiyantoko, 2017). The chemicals used for the analysis included glucose, which was used to prepare a standard solution with a concentration of 100 mg/L. Deionized distilled water served as the solvent for creating glucose standard solutions with concentrations of 0, 20, 40, 60, 80, and 100 mg/L. An 80 % phenol solution was prepared by mixing 20 grams of deionized distilled water with 80 grams of phenol crystals. Concentrated $H_2SO_4$ with a concentration of 95.5 % was also used.

To perform the glucose content test, start by taking 0.5 ml of the supernatant. Evaporate the ethanol using airflow at room temperature. Next, dissolve the resulting extract to a final volume of 100 ml with the appropriate solvent. Using a pipette, take 2 ml of the diluted extract and add 0.1 ml of 80 % phenol solution, followed by 0.5 ml of concentrated $H_2SO_4$. Allow the mixture to react for 10 minutes, then stir and incubate it at a temperature between 25 to 30 °C using a heater. Finally, read the absorbance of the solution at a wavelength of 490 nm using a spectrophotometer.

## 3. RESULTS AND DISCUSSION

The delignification process is aimed at removing the lignin that coats the hemicellulose and lignocellulose structures, thereby increasing the concentration of cellulose and hemicellulose. Alkaline delignification of EFB was carried out using a 10 % NaOH alkaline solution in a ratio of 1:10 (weight:volume). The process temperature was 80 °C. Meanwhile, alkaline sonication delignification is carried out with a ratio of raw materials and alkaline solvent and the same operating temperature as the previous delignification but assisted by ultrasonic irradiation with certain time variations. The product of alkaline delignification was hydrolyzed with the solution results shown in Fig. 2. There is a change in color that gets darker as the delignification time increases. Likewise, the results of hydrolysis with alkaline sonication delignification in Fig. 3.


**Figure 2.** Hydrolysis product of alkaline delignification EFB


**Figure 3.** Hydrolysis Product of Alkaline Sonication EFB

The color of the solution indicates an increase in the amount of lignin released from cellulose and hemicellulose. As seen in Figure 2, samples A to E from left to right show a change in the color of the solution from clear yellow to slightly brownish as well as the sonication pre-treatment results in a much darker color level.

The sugar content of the hydrolysis results through the ordinary delignification process continued to increase from 12 mg/L to 14.87 mg/L as the delignification process lengthened. Whereas in sonication delignification, the sugar content increased rapidly but decreased rapidly at irradiation duration of more than 90 minutes. This is due to the fact that palm empty fruit bunches are lignocellulosic and contain not only cellulose but also hemicellulose. Hemicellulose is non-crystalline, non-fibrous, and expands easily, making it more soluble in alkaline solvents. The ease with which hemicellulose dissolves is further enhanced by ultrasonic irradiation, which, through mechanical effects and cavitation, breaks the bonds within the hemicellulose. As a result, hemicellulose degrades into pentose and subsequently into other substances that do not hydrolyze as efficiently as cellulose. Therefore, in this study, ultrasonic irradiation for more than 90 minutes led to a decrease in total sugar concentration. The highest sugar content of 90 min sonication-assisted delignification reached 38.5 % higher than 150 min regular delignification.

**Table 2.** Effect of sonication irradiation in delignification on glucose content

| Delignification Process Time (minutes) | Total Sugar Content by Ordinary Delignification (mg/L) | Total Sugar Content by Ultrasonic Delignification (mg/L) |
|---|---|---|
| 30 | 12.00 | 16.73 |
| 60 | 12.09 | 20.01 |
| 90 | 12.23 | 20.60 |
| 120 | 14.70 | 8.05 |
| 150 | 14.87 | 9.18 |

Hemicellulose has properties that make it more soluble in alkaline solvents due to its non-crystalline, non-fibrous and fluffy characteristics. The longer the delignification process is given to hemicellulose. The solubility of hemicellulose is also influenced by sonication, where the mechanical and cavitational effects of sonication break the bonds in hemicellulose. During sonication, ultrasonic waves propagate through the solvent and form bubbles that assist the breakdown of solid materials so that lignin and hemicellulose can diffuse out of the matrix. (Kanani, 2018)

The alkali pre-treatment method does leave some hemicellulose. An alternative is to perform acid hydrolysis on this fraction. Hemicellulose breaks down into pentoses in a continuous manner and then turns into other substances that are not hydrolyzed, as well as cellulose. As a result, there is less cellulose and pentose to be hydrolyzed into glucose and xylose. This phenomenon is one of the factors causing the decrease

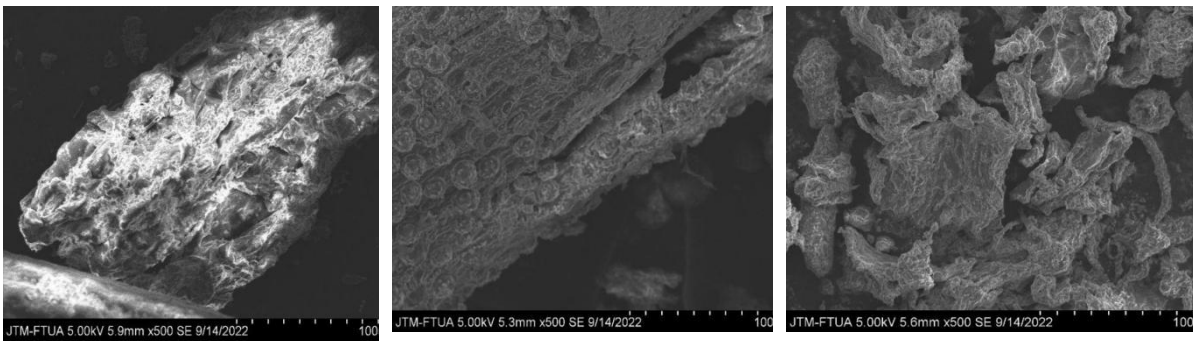
of sugar concentration in the sonication delignification process with a duration of more than 90 min. According to Kristiani (2015), a higher cellulose content in the sample can lead to an increased production of glucose. Additionally, hemicellulose can break down into xylose, and lignin can generate phenolic derivative compounds.

(a) (b)

**Figure 4.** Alkali Delignified EFB Solution (a); Alkali-Sonicated Delignified EFB Solution (b)

The delignification process that took place at 80 °C accompanied by sonication caused evaporation so that the solution became more shrinkable and formed a highly concentrated leachate that hardened on its surface at room temperature shown by fig.4. Delignification that takes place for a long time causes more lignin that has been freed from its lignocellulose structure, this leachate is blackish brown in color. Black leachate contains lignin, hemicellulose and residual NaOH from the process (Muryanto, 2016).

Cellulose and hemicellulose from delignification cannot be completely separated from lignin through washing due to the thick and concentrated physical properties of the leachate in the solution. This condition makes it difficult to separate the lignin from the hemicellulose and cellulose and potentially carry it over to the hydrolysis process, thus affecting the hydrolysis which should be in an acidic atmosphere. Lignin carried over to the hydrolysis process is shown in the slightly yellow-brown colored hydrolysis solution and produces lower glucose levels in the same series of sonication delignification treatments. This situation is indicated by the presence of lignin precipitate in the acidic hydrolysis solution which can be seen in Fig. 2 to fig. 3.




(a) (b) (c)

**Figure 5.** SEM Test Results on Raw EFB (a), EFB After 90 Min Alkali Delignification (b), EFB After Alkaline Sonication 90 min (c)

The structure changes of the EFB can be observed through SEM imaging results before and after delignification with various treatments showed in fig.5. The EFB structure before pre-treatment, looks dense and tight, with fibers bound together and small gaps. After delignification using 10 % NaOH solvent, the structure of the EFB changed. There is a combination of lignin chunks and fine fibers, with few voids. Some fiber surfaces look damaged or torn. Kristiani (2015) also reported similar findings in SEM micrographs of OPEFB treated with 10 % NaOH and a combination of 10 % NaOH and irradiation. The images showed clear differences in the OPEFB structure before and after treatment. Before treatment, OPEFB had a solid, intact, rough, and rigid structure, whereas after treatment, the structure became brittle and flaky. This is due to the dissolution of hemicellulose in the alkali, which causes the formation of disconnected or torn parts.

Meanwhile, when EFB underwent sonication delignification employing a 10 % NaOH solvent, a more pristine surface was observed, featuring extended and larger fibers, along with noticeable large and profound voids or gaps. This suggests a degradation of the lignin structure, signifying that the ultrasonic waves have effectively disrupted the cell wall and inflicted damage to the secondary cell wall within the middle layer.

There are no lignin chunks as in ordinary delignification. This indicates that the lignin structure has been degraded, marked by a change in particle size that resembles fibers. Possibly, the lignin has been exposed and is able to break down the carbohydrates contained in the EFB, including cellulose and hemicellulose. Hemicellulose also appears to be reduced, indicated by the lack of fibers attached to the large fibers. Lee et al (2020) also reported that the pretreated Empty Fruit Bunches (EFB) exhibited notable structural alterations characterized by the rupture of hemicellulose and lignin bonds, resulting in improved enzymatic saccharification.

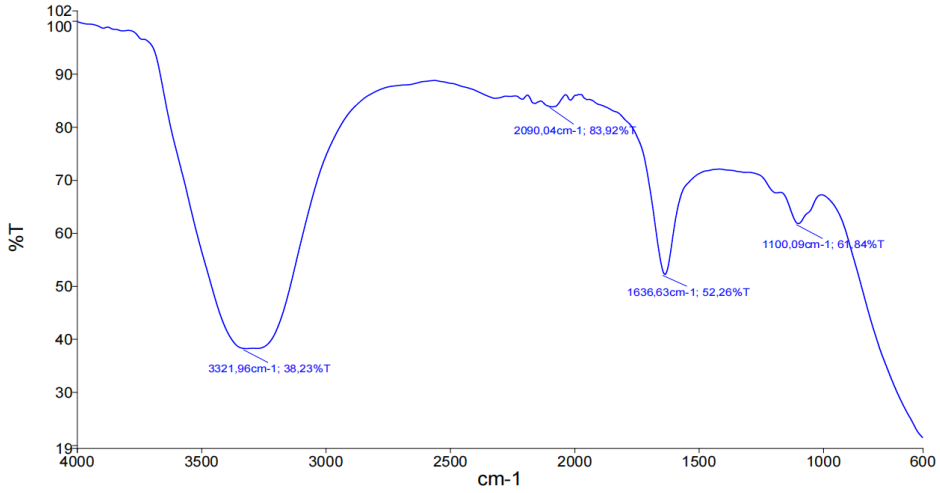



**Figure 6.** FTIR Spectrum Results of Alkaline Sonication EFB Hydrolysis Solution

Glucose in this acid solution was tested using infrared light because almost all organic groups have typical absorption wave numbers in certain regions. The spectrum graph shows that the hydrolysis solution has glucose content as evidenced by the presence of peaks at a wavelength of 3300 $cm^{-1}$, peak 1 in Fig.6 with a wave number of 3332.21 $cm^{-1}$ indicates the presence of -OH groups, The absence of other peaks after a wavelength of 3000 $cm^{-1}$ in addition to -OH groups and single bonds (C-

H, C-O) is indicated by peak 4 which is in the range of 1500 $cm^{-1}$ to 500 $cm^{-1}$ region indicating the presence of glucose. The wavelength of 1637.81 $cm^{-1}$ shows carbonyl groups (C=O) and the presence of -OH groups from wavelengths over 3300 $cm^{-1}$ which indicates the presence of xylose, a simple sugar formed from hemicellulose hydrolysis. The image also shows the absorption of wave number 2090.91 $cm^{-1}$ which indicates the presence of carbon group of double bond 3 or alkyne group. Possibly, this peak comes from other compounds or contaminants in the solution.

## 4. CONCLUSION

Sonication in delignification of palm empty fruit bunches can increase the sugar content of hydrolysis results. The highest sugar content of 90 min alkali sonication-assisted delignification reached 38.5 % higher than 150 min delignification with alkali only. The physical changes in the fibers of palm empty fruit bunches (EFB) are indicated. SEM (Scanning Electron Microscope) test results of EFB before pre-treatment, the structure looks dense and tight, with fibers bound together and small gaps. After the delignification process, large and deep voids or gaps began to form. Qualitative FTIR (Fourier Transform Infra-red Spectroscopy) test results show that the hydrolysis solution contains glucose and pentose which are the result of cellulose and hemicellulose being hydrolyzed. There is an opportunity to scale up the delignification reactor to accommodate a larger amount of raw materials for sugar production. This research further highlights the effectiveness of using a combination of methods for the pretreatment of lignocellulosic materials.

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