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ORIGINAL ARTICLE

Optimizing Raw Material Pre-Treatment for Bioethanol Production from Empty Fruit Bunches: A Comparative Study

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Abstract

Bioethanol (C_2H_5OH) is an organic chemical widely used as a solvent. It is also applied in producing germicides, beverages, antifreeze, fuel, and depressants as an intermediate in producing other chemical substances. Generally, bioethanol is generated from the fermentation of glucose (sugar) accompanied by a distillation process. This study aimed to optimize bioethanol production against variations in pre-treatment and solvent concentrations through enzyme hydrolysis and fermentation of oil palm empty fruit bunches. The pre-treatment is an important stage in the conversion process of lignocellulosic biomass, as it eliminates lignin, reduces the crystallinity of the cellulose, and increases the porosity of the substance, thereby facilitating the hydrolysis process and glucose fermentation. The process was carried out with different concentrations of acid, base, and organolsov solvents, after that, it was hydrolyzed using cellulase enzymes for 24 hours and fermented using *Saccharomyces Cerevisiae* yeast for 5 days. Furthermore, the bioethanol produced was separated through a rotary vacuum evaporator at 180 rpm and a temperature of 78 °C. The highest ethanol content produced was 7.8% with 4% NaOH and 90 minutes of pre-treatment residence time.

Introduction

Indonesia has abundant renewable energy potential from sources such as geothermal [1–3], wind, solar, hydropower, and biomass from agricultural waste [4–6]. To prevent the total depletion of the oil reserves in the form of gasoline and kerosene, the government initiated the use of various biofuels, including bioethanol from oil palm empty fruit bunches. Bioethanol is a promising alternative fuel for potential use in Indonesia. Ethanol is a renewable plant-based fuel and represents a promising alternative for liquid and gaseous fuels, utilizing renewable and environmentally friendly feedstocks [7].

Bioethanol (C_2H_5OH) is a liquid biofuel produced through the fermentation of sugars by microorganisms [8]. It can be blended with gasoline in any ratio to improve fuel quality. Bioethanol can be produced from feedstocks containing fermentable sugars such as molasses, palm sap, sugarcane juice, and fruit juices [9]. It can also be made from starchy and cellulosic materials including wood fibers, oil palm empty fruit bunches, banana fibers, bagasse, and pineapple fibers. These starch and cellulose sources require pre-treatment to break them down into fermentable sugars before undergoing fermentation to produce bioethanol [10,11].

Oil palm empty fruit bunches (OPEFB) are the largest solid waste product from oil palm plantations [12]. For every 1 ton of oil palm fruit processed, 220-230 kg of OPEFB is obtained [13]. This lignocellulosic waste has not been utilized optimally so far, with applications limited to boiler fuel, compost, and road hardeners in plantations. OPEFB contains high fiber content - 44.2% cellulose, 33.5% hemicellulose, and 20.4% lignin [14]. The base and tip sections of OPEFB bunches, which are usually pointy and hard, were found to be especially rich in cellulose

[15]. Cellulose can be hydrolyzed into glucose and further fermented to produce bioethanol. With around 45% cellulose content, OPEFB is a prime candidate feedstock for bioethanol production and could be prioritized as a renewable source for this biofuel [16].

The Indonesian agricultural sector, especially oil palm plantations, is seeing increasing foreign exchange revenue. Riau Province has the highest oil palm supply, with a total plantation area of 1,732,748 hectares in 2022 [17]. Oil palm provides diverse products including edible oil, industrial oil, and biodiesel fuel. As oil palm plantations continue expanding, the availability of these products in the market also increases. The widespread expansion of oil palm cultivation in Indonesia has led to greater product supply and foreign exchange earnings, with Riau Province being the leading producer with over 1.7 million hectares of plantations.

Pretreatment is a critical step in converting lignocellulosic biomass like oil palm empty fruit bunches (OPEFB) into bioethanol. It helps remove lignin, decrease cellulose crystallinity, and increase biomass porosity to facilitate hydrolysis and fermentation. This study aims to synthesize bioethanol from OPEFB through different pretreatment and hydrolysis processes. Further research to build on these findings could help expand the scientific knowledge and economic value of this abundant biomass resource, especially in Indonesia. Investigating optimal pretreatment approaches and developing efficient hydrolysis methods can help improve OPEFB's potential as a sustainable feedstock for bioethanol production.

Materials and Methods

Materials

The materials used in this study were OPEFB obtained from PT. Perkebunan Nusantara (PTPN) V Sei Pagar, sulfuric acid (H_2SO_4) at 3%, 3.5%, and 4% concentrations, sodium hydroxide (NaOH) at 3%, 3.5%, and 4% concentrations, 50%, 60%, and 70% ethanol solvent, distilled water (aquadest), cellulase enzyme, *Saccharomyces cerevisiae* yeast, sodium phosphate, potassium, and urea. Some chemicals were purchased from Aldrich products. The equipment utilized included an autoclave, Erlenmeyer flasks, magnetic stirrer, shaker, incubator, analytical scale, beakers, measuring flasks, pipettes, petri dishes, separating funnel, aluminum foil, filters, stirrers, blender, oven, containers, pH meter, test tubes, 100 ml measuring cups, and an evaporator set.

OPEFB Pre-treatment Process

The dried OPEFB was first physically pretreated by milling to reduce the size to approximately 2-4 mm. Chemical pretreatment was then done using several processes - acid pretreatment with 3%, 3.5%, and 4% H_2SO_4 base pretreatment with 3%, 3.5%, and 4% NaOH; and organosolv pretreatment with 50%, 60%, 70% ethanol and cellulase enzyme as catalyst.

Saccharomyces cerevisiae rejuvenation

Saccharomyces cerevisiae yeast was revived by preparing a medium inoculated with 1 ose of yeast. The yeast was streaked onto PDA medium, cultured, and then incubated for 24 hours at 30°C to grow the inoculum.

Saccharomyces cerevisiae Inoculum Production

The inoculum helps adapt the yeast cells to the fermentation medium and was added at 10% of the total substrate volume in this study. The inoculum was prepared by adding 0.3 g/L urea and 0.4 g/L sodium phosphate potassium to the Erlenmeyer flask with the fermentation medium. This was sterilized by autoclaving at 121°C for 15 minutes, then allowed to cool. Next, 5 ml of Potato Dextrose Broth (PDB) was added to a test tube with 1 ose of *Saccharomyces cerevisiae* inoculum per 50 ml. This was incubated for 24 hours. The inoculum was then transferred to the fermentation medium at room temperature and agitated using a shaker for 24 hours.

Bioethanol separation was carried out using a rotary vacuum evaporator, which evaporates solvents below their boiling point under low pressure. This avoids damaging components at high temperatures. The process was performed at 180 rpm and 78°C. Finally, the bioethanol concentration was measured using an alcohol meter.

Bioethanol Fermentation Stage

The fermentation was carried out at room temperature and pH 5, maintained at a stirring speed of 150 rpm. The process was conducted by the addition of 0.3 g/l urea and 0.4 g/l NPK with a media volume of 1000 ml. All these were then sterilized at 121 °C for 15 minutes in the autoclave and cooled at room temperature. Then, 70 ml of the inoculum was added into the fermentation medium, which was sampled once every 24 hours for five days.

Results and Discussion

OPEFB Composition before Pre-treatment

Before pretreatment, the composition of OPEFB was characterized to determine the percentages of cellulose, hemicellulose, lignin, and other components. As shown in Figure 1, OPEFB contained approximately 36% cellulose, 25% hemicellulose, 18% lignin, and 21% ash and other constituents. This composition aligns with typical biomass components, which generally comprise 35-50% cellulose, 20-35% hemicellulose, 15-20% lignin, and 15-20% ash and other materials [18]. Characterizing the OPEFB composition provided baseline values for cellulose, hemicellulose, and lignin content before conducting the various pretreatment processes.

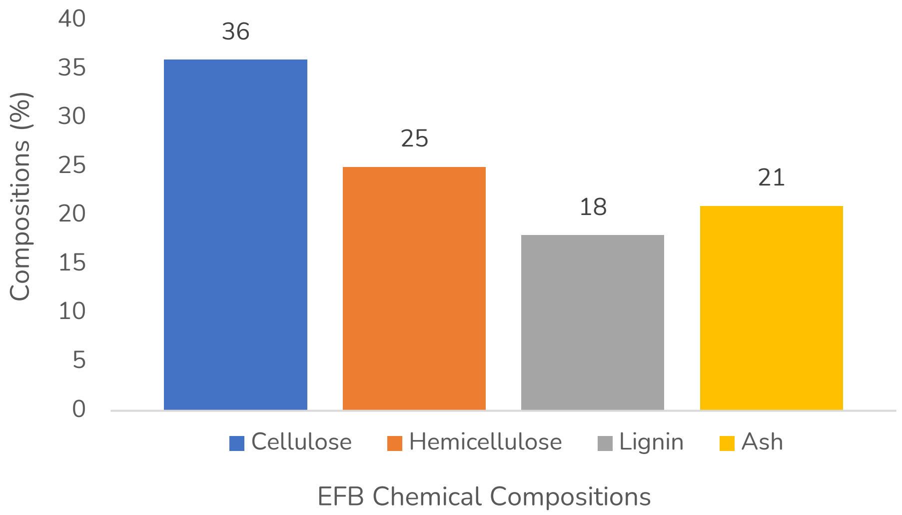

Figure 1. Chemical composition of empty fruit bunches before pre-treatment.

OPEFB after Acid Pre-treatment

The various concentrations of H_2SO_4 added to the pre-treatment process were 3%, 3.5%, and 4%. Figure 2 shows that the higher the concentration, the higher the levels of bioethanol produced. This is due to the higher concentrations of solvents, leading to more degradation of lignin compounds. Hence, cellulose is hydrolyzed more into glucose with the help of the cellulase enzymes. Based on the figure above, it can be concluded that the highest level of bioethanol obtained was 4.3% with 4% H_2SO_4 at a residence time of 90 minutes during the space of fermentation for five days using *Saccharomyces cerevisiae* yeast. In this phase, the yeast uses the substrate as a nutrient for the growth process, multiplying very quickly and increasing the concentration of the bioethanol produced.

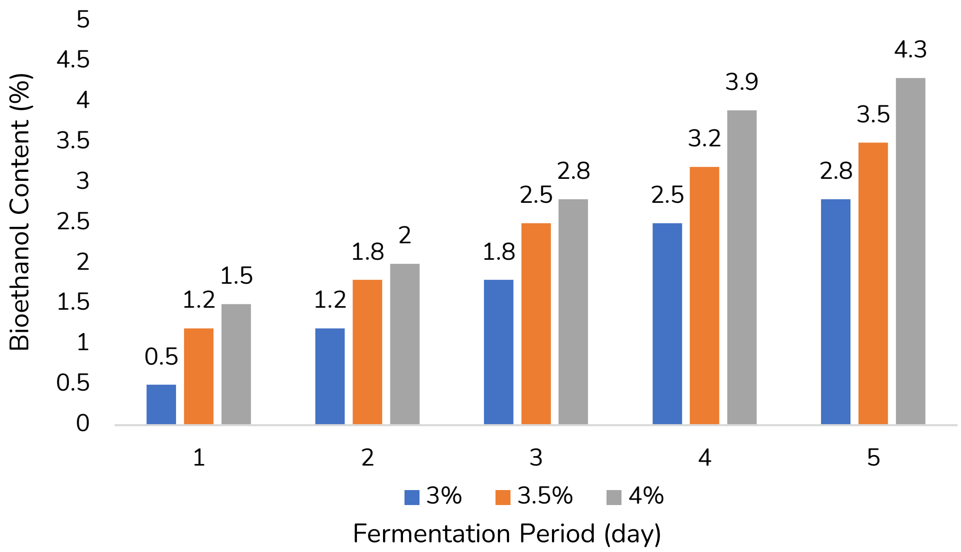

Figure 2. Effect of H_2SO_4 concentration variation on bioethanol content.

OPEFB after Base Pre-treatment

The NaOH pretreatment was tested at concentrations of 3%, 3.5%, and 4%. As shown in Figure 3, higher NaOH concentrations resulted in increased bioethanol production. The higher alkali levels likely caused more extensive degradation of lignin compounds, enabling greater hydrolysis of cellulose into glucose with the cellulase enzyme. Based on these results, pretreatment with 4% NaOH for 90 minutes gave the maximum bioethanol yield of 7.8% after 5 days of fermentation with Saccharomyces cerevisiae. The 90 minute residence time allowed sufficient lignin removal and cellulose exposure under the tested NaOH concentrations. During fermentation, the yeast utilizes the cellulosic substrates as nutrients to increase growth rate and bioethanol production. In conclusion, pretreatment with 4% NaOH for 90 minutes, followed by five day fermentation enabled optimal bioethanol yields.

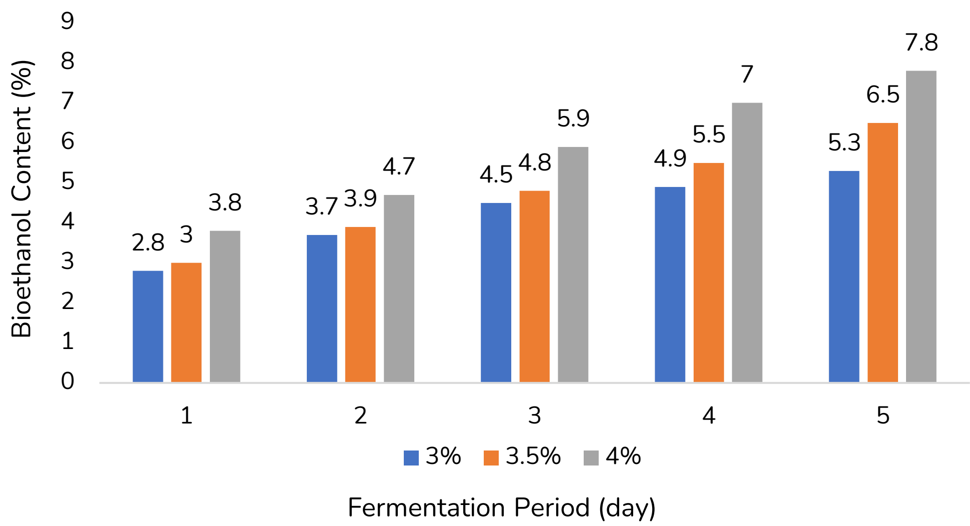

Figure 3. Effect of NaOH concentration variation on bioethanol content.

OPEFB after Organosolv Pre-treatment

Ethanol was tested at concentrations of 50%, 60%, and 70% for organosolv pretreatment. Similar to the acid and base pretreatments, Figure 4 shows that higher ethanol concentrations resulted in increased bioethanol production after fermentation. The higher solvent levels likely caused more extensive lignin degradation, enabling greater hydrolysis of cellulose to glucose by the cellulase enzymes. Based on the results, pretreatment with 70% ethanol for 90 minutes gave the maximum bioethanol yield of 6.1% after five days of fermentation with *Saccharomyces cerevisiae* yeast. The 90 minute residence time allowed sufficient lignin removal and cellulose exposure under the tested ethanol concentrations. During fermentation, the yeast utilizes the cellulosic substrates as nutrients, increasing growth and bioethanol production. In summary, pretreatment with 70% ethanol for 90 minutes, followed by five day fermentation enabled the optimal bioethanol yield.

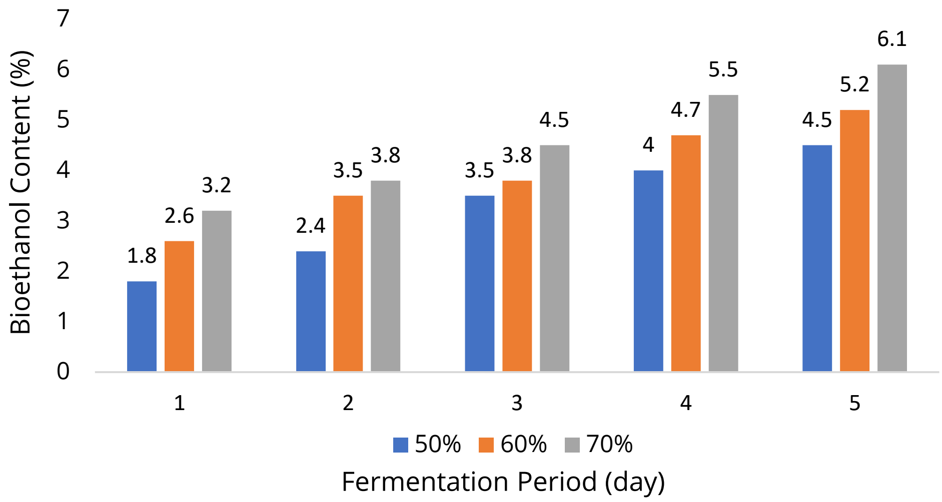

Figure 4. Effect of ethanol concentration variations on bioethanol content.

The fermentation was carried out over five days using *Saccharomyces cerevisiae* yeast. During fermentation, the yeast utilized the cellulosic substrates as nutrients to facilitate rapid growth and increase bioethanol production. As fermentation time increased, allowing for more yeast growth, and with higher glucose concentrations, greater yields of bioethanol were achieved. In conclusion, extended fermentation times and higher initial glucose levels enabled higher concentrations of bioethanol to be produced through the bioconversion process.

Analysis of Bioethanol Synthesis Results

As shown in Figure 5, the type of pretreatment solvent significantly impacted bioethanol yields. Base pretreatment with NaOH produced the highest bioethanol levels compared to acid and ethanol pretreatments. NaOH acts by directly attacking and disrupting the lignin, crystalline, and amorphous structures of the biomass. It helps remove portions of lignin and hemicellulose, and also causes swelling of the cellulose [19]. NaOH has proven effective at degrading lignocellulose for bioethanol production. It increases biomass degradation, reduces lignin content, and enables conversion to glucose [20]. In summary, NaOH was the optimal pretreatment solvent as it enabled effective delignification and cellulose exposure for maximum bioethanol yields from the OPEFB biomass.

Pretreatment with concentrated acid has some drawbacks for bioethanol production. It can increase formation of inhibitor compounds, cause equipment corrosion, and make bioethanol recovery difficult. Hence, acid and ethanol are less suitable solvents compared to bases. While acids like H_2SO_4 are effective for delignification, they tend to overly degrade cellulose and hemicellulose. This reduces glucose yields from the hemicellulosic pentosans needed for bioethanol formation. Therefore, although NaOH is theoretically less effective than acid at removing lignin, it helps retain more fermentable sugars. In summary, H_2SO_4 and ethanol are useful for delignification, but NaOH is a better choice to maximize bioethanol yields. Both solvent concentration and fermentation time positively impact yields, with higher levels and longer durations leading to increased bioethanol production.

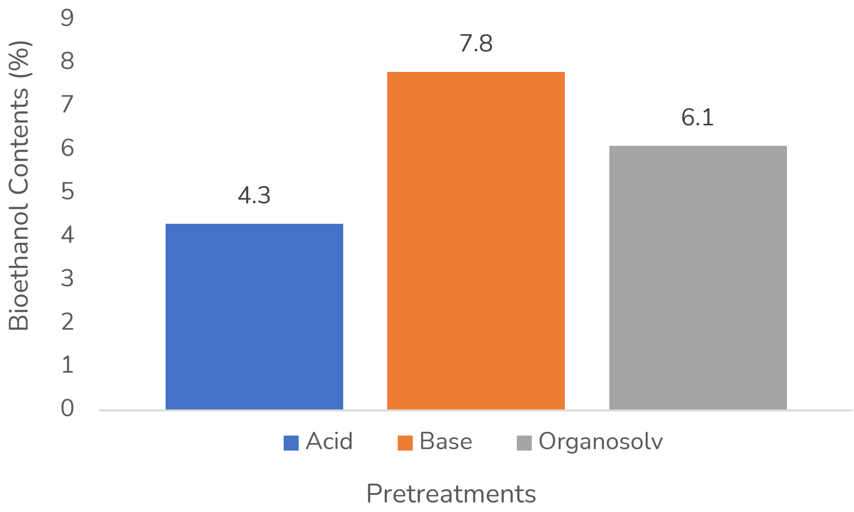

Figure 5. Comparison of pre-treatments.

Conclusions

This study demonstrated that alkaline pretreatment is effective at delignifying OPEFB to make cellulose more accessible for enzymatic hydrolysis and fermentation. The data indicates NaOH removes lignin while retaining cellulose and hemicellulose sugars better than sulfuric acid or ethanol. Further optimization of parameters like NaOH concentration, residence time, and fermentation duration could improve OPEFB bioethanol yields beyond 7.8%. Overall, this investigation provided valuable insights into pretreating this underutilized oil palm biomass to produce renewable bioethanol fuel. The findings can guide future large-scale bioethanol production from OPEFB as a sustainable feedstock.

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