

LOW-COST CELLULOSIC BIOETHANOL PRODUCTION OF OIL PALM EMPTY FRUIT BUNCHES USING HYDROTHERMAL-ALKALINE PRETREATMENTS SYNERGETICLY

M. Nurdin^{1,✉}, S.P. Simanungkalit², A.S. Muis¹, D. Wibowo³, Z. Arham⁴,
L.O.A. Salim¹, I. Irwan¹, M. Maulidiyah¹ and H. Abimanyu²

¹Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Halu Oleo, Kendari, 93231-Southeast Sulawesi, Indonesia.

²Research Center for Chemistry, Indonesian Institute of Sciences (LIPI), Kawasan PUSPIPTEK, Serpong, 15314-Tangerang Selatan, Indonesia.

³Department of Environmental Engineering, Faculty Engineering, Universitas Muhammadiyah Kendari, Kendari 93117-Southeast Sulawesi, Indonesia.

⁴Department of Mathematics and Natural Science, Institut Agama Islam Negeri Kendari, Kendari 93116-Southeast Sulawesi, Indonesia.

✉Corresponding Author: mnurdin06@yahoo.com

ABSTRACT

The second generation of bioethanol has been developed by utilizing the residual products of crops such as oil palm empty fruit bunches (OPEFB). In this study, the OPEFB was carried out synergistically by applying two methods, namely hydrothermal and alkaline. This pretreatment is intended to improve the ability of the OPEFB delignification process. Hydrothermal pretreatment was carried out by annealing at 220°C for 30 minutes, while alkaline pretreatment was carried out by varying the concentration of NaOH, pretreatment time, and temperature. Based on the research results, it was found that hydrothermal pretreatment with the addition of DI water was effective in reducing hemicellulose levels from 21.54% to 1.11%. The combination of alkaline pretreatment using NaOH 2.0 M at 25°C for 60 minutes effectively reduced lignin levels from 47% to 26.6%, and increased cellulose levels to 83.04%. NaOH solution has been shown to dissolve lignin without affecting the stability of cellulose.

Keywords: Pretreatment, Hydrothermal, Alkaline, OPEFB, Bioethanol

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INTRODUCTION

Currently, bioethanol production is being developed by several countries to reduce dependence on fossil fuels. Unfortunately, this effort was stopped due to intense competition in identifying sources of raw materials for bioethanol production. It is well known that the first generation of bioethanol relies heavily on main food ingredients such as cassava, rice, sugar cane, sago, and corn. To avoid this sustainable food conflict, the identification of raw materials for bioethanol transformation is enhanced by switching to second-generation bioethanol through the utilization of lignocellulosic biomass waste (LBW) which contains several main chemicals such as cellulose, hemicellulose, lignin, and other organic materials.^{1,2} Based on previous research, it has been reported that lignocellulosic biomass such as corn cobs³, rice straw⁴, sago pulp fiber⁵, wood⁶, cocoa pods⁷, coffee skins⁸, grass⁹, and OPEFB^{2,10,11} is a viable bioethanol raw source. Various studies have reported that the increase in bioethanol yield depends on the substance of cellulose in the lignocellulosic biomass. Bioethanol is produced in four important stages including pretreatment, hydrolysis, fermentation and purification.¹² Pretreatment and hydrolysis must be able to increase the cellulose content so that the fermentation process can be optimized. The problem during the pretreatment process is that there is competition between cellulose and lignin content which are difficult to separate. The pretreatment process is the first step to break down the lignocellulose structure and

reduce the lignin content and break lignin bonds with other sugar content. In addition, it is used to remove or reduce hemicellulose content, break down the crystalline structure of cellulose, and increase its porosity. Based on these reasons, cellulose is an essential substrate in the production of bioethanol yield.

In this study, a synergic pretreatment process between hydrothermal and alkaline was studied in OPEFB biomass waste pretreatment. Several OPEFB pretreatment techniques have been studied such as hydrothermal¹³, ionic liquids¹⁴, biological fungi¹⁵, hot compressed water¹⁶, deep eutectic solvents¹⁷, acid and alkaline solvents¹⁸, etc. However, hydrothermal is easier to prepare, low cost, and environmentally friendly, so it has the potential to be applied in the pretreatment of OPEFB. In addition, it can be combined with alkaline pretreatment to reduce lignin or hemicellulose, thereby increasing cellulose.¹⁹ Previous studies have shown that the optimum conditions for alkaline-thermal (AT) pretreatment of *Spectinomycin mycelium* residues occur at pH 12, with temperature and pretreatment time of 90°C and time of 120 minutes.²⁰ Another study showed that AT pretreatment had a high performance in destroying the lignin content of lignocellulosic biomass. Significant in the alkaline technique occurs when using 7% NaOH, the temperature of 120°C, and time of 1 hour.

Studies on the pretreatment effect have generally been evaluated to optimize cellulose content, this is based on the fact that each raw material has a different chemical content, so pretreatment studies need to be investigated. Therefore, in this study: (1) the synergistic role of alkaline-hydrothermal pretreatment on OPEFB delignification, (2) the effect of hydrothermal-alkaline pretreatment through optimization of the OPEFB biomass pretreatment process including alkaline concentration, contact time, and temperature.

EXPERIMENTAL

Hydrothermal Pretreatment

Firstly, the preparation of OPEFB biomass was carried out by grinding and sieving under 18 mesh to obtain biomass powder. Subsequently, it was dried in an electric oven for 24 h at 105°C to reduce the moisture content below 10%. The hydrothermal has been applied in the reactor autoclave (stainless steel, maximum of 280 mL, with a diameter length of 5.4 cm) with inserting of 10-gram OPEFB powder and 100 mL DI water (equivalent to 1:10, solid: liquid ratio). During the hydrothermal pretreatment, it was rinsed with nitrogen gas 3 times and given pressure of 7 bar. After that, the reactor was stirred at a speed of 60 rpm to obtain a homogeneous sample and increased temperature from 25°C to 220°C for 30 minutes. The sample is cooled at ambient temperature and separated by using a vacuum pump. Subsequently, the black powder was dried in an oven at 105°C for 24 h and analyzed chemical constituents were, then it continues alkaline pretreatment step.

Alkaline Pretreatment

The black powder was continuously applied in alkaline pretreatment using NaOH solution by considering factors including the effect of alkaline concentration, time duration, and temperature. In experimental, the black powder was weighed as much as 5 gram put into a beaker glass, and added 50 mL NaOH (1:10) by varying concentrations of 2.0 M, 1.0 M, and 0.5 M. It was stirred using a magnetic stirrer under 60 rpm for 60, 120, and 240 min at 25°C (ambient temperature), respectively. Meanwhile, the effect of temperature was applied to temperature variations of 50°C and 100°C. The results were filtered to separate liquid and residue using a vacuum filter. The liquid is collected in a small glass to identify pH, while the residue is rinsed with distilled water to a neutral pH (pH=7) and separated using a vacuum filter. Finally, it was analyzed for their chemical composition using a UV-Vis spectrophotometer (UV2550, Shimadzu, Japan) for lignin, while the cellulose and hemicellulose contents are used high-performance liquid chromatography (HPLC-Shimadzu) instrument.

Lignocellulosic Compounds Analysis

The cellulose and hemicellulose were identified using a procedure established by the National Renewable Energy Laboratory (NREL). Meanwhile, the lignin was measured by combining the gravimetric method and the UV-Vis spectrophotometer in the 205 nm wavelength range. In detail, the dried OPEFB sample after pretreatment was weighed as much as 0.3 gram and stirred every 15 minutes. It was then transferred to a Schott bottle with a capacity of 100 mL containing 42 mL of distilled water. It is hydrolyzed in an

autoclave reactor at 121°C for 2 h and cooled under ambient temperature $\pm 24^\circ\text{C}$ until it becomes a clear liquid and forms a black precipitate. The determination of lignin content was carried out using a UV-Vis spectrophotometer. Meanwhile, in the determination of cellulose and hemicellulose constituents, it is necessary to neutralize them using CaCO_3 up to pH 7 and filter using a syringe with a pore size of 0.2 μm . It accommodated in the HPLC autosampler vial to identify the chemical constituents using HPLC instrument with Aminex HPX 87H column (300 x 7.8 mm) at a temperature of 65°C with a mobile phase of 5 mM H_2SO_4 and a flow rate of 0.6 ml min⁻² and a Refractory Index detector (waters 2414 T: 40°C).

RESULTS AND DISCUSSION

Hydrothermal Pretreatment

Determination of the lignocellulose content of OPEFB raw materials was carried out to obtain preliminary information on the levels of cellulose, hemicellulose, and lignin which will be used to determine the success of the pretreatment process. The amounts of cellulose, hemicellulose, and lignin in OPEFB before and after hydrothermal pretreatment are shown in Table-1. Based on the table, the cellulose content before pretreatment was quite high when compared to hemicellulose and lignin. However, hemicellulose and lignin must be removed, so pretreatment is needed to remove them. Hemicellulose can interfere with the glucose fermentation process²¹, while lignin will interfere with the hydrolysis process, where hydrolysis is slower because lignin has a complex chemical structure.²²

Table-1: The Lignocellulosic Content of the OPEFB Raw Materials

Lignocellulosic Compounds	Raw Material (%)	Hydrothermal Pretreatment (%)
Cellulose	40.51	47.07
Hemicellulose	21.54	1.11
Lignin	36.76	47.21
Ash	3.03	2.66

In this study, hydrothermal is applied to remove lignin and hemicellulose. The success of this process is marked by a reduced mass of OPEFB raw materials.^{23,24} If we look at Table-1, it can be concluded that hydrothermal pretreatment was effective in reducing hemicellulose levels from 21.54% to 1.11%. However, this pretreatment was not effective in reducing lignin levels. Lignin levels increased from 36.75% to 47.21%. This increase occurs due to damage to the hemicellulose structure at 220°C heating. Apart from lignin, decreased hemicellulose levels caused OPEFB cellulose levels to increase from 40.51% to 47.05%. In general, this process shows that hydrothermal pretreatment with the addition of water is effective at removing hemicellulose, but not for removing lignin. Based on these results, further research is needed on the use of water in increasing the capability of hydrothermal pretreatment for lignin removal. The hydrothermal pretreatment capability can be done by adding alkaline such as NaOH and CaO. Both are reported to exert influence during hydrothermal pretreatment.¹⁹

Alkaline Pretreatment

The increased lignin levels after hydrothermal pretreatment led to optimization of NaOH concentration, pretreatment time and temperature to be important parameters in alkaline pretreatment. Optimization of alkaline pretreatment in OPEFB has been reported by many researchers.^{23,24,25} However, the different lignocellulose content of OPEFB is the reason for the importance of optimization of the alkaline species used. The use of NaOH in pretreatment is because NaOH is effective in reducing lignin levels²⁸, stable, safe, and odorless.^{27,29,30} Several studies have reported that NaOH acts as hydrolysis in lignin degradation. Theoretically, NaOH degrades lignin by breaking the lignin ester cross-linking. Hydrolysis results in the form of alcohol derivatives such as coniferyl alcohol, p-coumaryl alcohol, and synapyl alcohol.³¹ The delignification mechanism in the hydrothermal-alkaline pretreatment is shown in Fig.-1.

Figure-2 shows the results of alkaline pretreatment optimization in both NaOH concentration (Fig.-2a), pretreatment time (Fig.-2b), and pretreatment temperature (Fig.-2c). The optimization of the NaOH concentration was carried out at 25°C for 60 minutes. Based on Fig.-2a, it can be seen that the use of 2.0

M NaOH resulted in higher cellulose levels and lower lignin levels compared to the other two NaOH concentrations. This condition is then used to optimize the pretreatment time and temperature. Fig.-2b is the result of the optimization of the pretreatment time carried out at 25°C with a NaOH concentration of 2.0 M. The optimization results show that 60 minutes of pretreatment is the optimum time to produce high cellulose levels in a 2.0 M NaOH solution. In addition, these results explain that high cellulose and low lignin levels occur at low temperatures (<100°C). The results of NaOH concentration optimization (2.0 M) and pretreatment time (60 minutes) were then used to explain the optimization process for the pretreatment temperature of OPEFB. The greatest cellulose and lowest lignin levels were achieved at a temperature of 25°C, as shown in Fig.-2c. The cellulose levels obtained were 83.04% and lignin was 25.60. This cellulose level is then expressed as the highest level in the synergistic application of the hydrothermal-alkaline pretreatment methods. Although the yield of cellulose content is high, the presence of different levels of lignin from each treatment is an interesting phenomenon to be studied further.

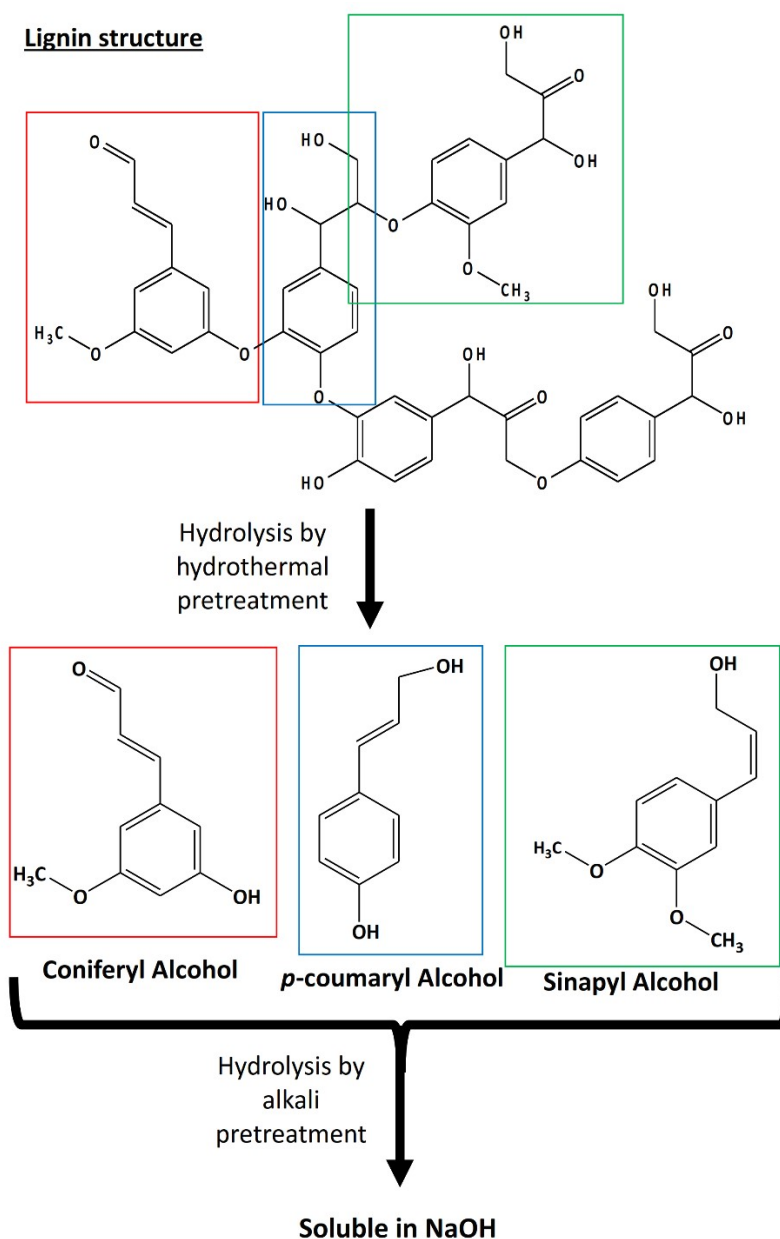


Fig.-1: Schematic of Delignification Process by Hydrothermal-alkaline Pretreatment

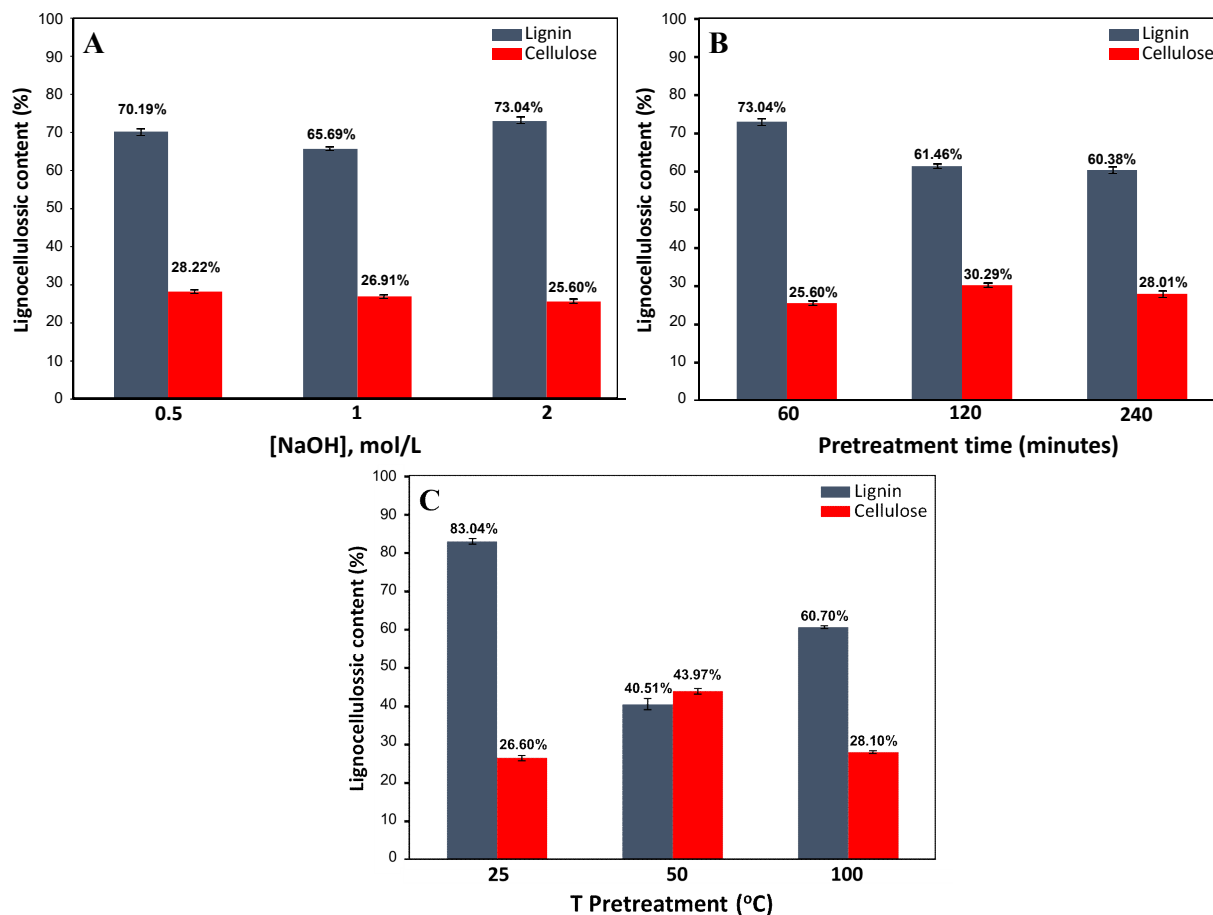


Fig.-2. Optimization Results of Alkaline Pretreatment: (a) NaOH Concentration, (b) Pretreatment Time, and (c) Pretreatment Temperature

The release of lignin during the alkaline pretreatment was also observed through changes in the pH of the OPEFB extract solution. Figure-3 shows the relationship between NaOH concentration and changes in pH at 25°C. Based on this figure, the pH increases with increasing NaOH concentration. The increase in pH is identical to the number of lignin compounds that dissolve together with NaOH. Lignin is reported to contain phenol hydroxyl groups which are easily ionized to form salts and have polar properties that will affect changes in pH.

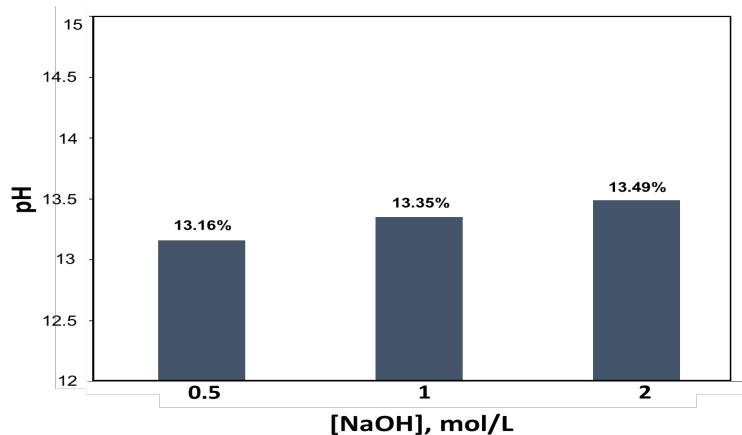


Fig.-3: The Relationship between NaOH Concentration and Changes in OPEFB Ph

CONCLUSION

This study reports an economical pretreatment process for OPEFB biomass through the application of the hydrothermal-alkaline method. The results show the good effects of this method. Hydrothermal pretreatment was effective at removing hemicellulose, while alkaline pretreatment was effective at removing lignin and increasing cellulose levels.

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