Effect of Organosolv Pretreatment on Delignification for Bioethanol Feedstock from Oil Palm Empty Fruit Bunch (OPEFB)

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Abstract. Lignin degradation of Oil Palm empty fruit bunch (OPEFB) as raw material for bioethanol production has been successfully performed by organosolv pretreatment. This paper presents the study and optimization of organosolv pretreatment on Delignification of OPEFB as raw material of bioethanol. Ethanol was used in organosolv pretreatment as the organic solvent. Organosolv pretreatment disrupted the lignocellulosic matrix to a considerable extent and increased hydrolysability of pretreated materials. Response surface methodology (RSM) was applied to determine the optimal condition operation of each significant variable, which included organic solvent concentration, reaction time, and particle size. The Box-Behnken Design (BDD) was employed to investigate the individual crucial component of the condition operation that significantly affected lignin degradation. The maximum lignin degradation was obtained 27.68% which correspond to 65% (v/v) of ethanol, with reaction time 65 min and 50 mesh of particle size. Therefore, it can be concluded that organosolv pretreatment can be used to degrade lignin from OPEFB as a bioethanol feedstock.

1. Introduction
Consumption value of fossil fuel and energy in Indonesia increased along with population growth. On the other hand, the availability of fossil resources yearly reduced. It brings the price of energy higher. Indonesia produced fossils fuel for 100 years and decreased by 5% each year. It causes Indonesia depends on imports of fossil fuels. Even in 2050 import of fossils fuels will be increased 8 times [1]. Therefore, alternative energy such as solar, wind, and biofuels must be developed to replace the gap in reducing fossil fuel production. Bioethanol is one of the biofuels that can decrease consumption of fossil fuels. Bioethanol is the renewable fuel that can be mixed with gasoline or can even be used in pure form. It generates very low gas emissions.

Lignocellulosic biomass is a potential raw material for alternative energy because of the potential volume and lowest cost for biofuel production [2]. The lignocellulosic materials are mainly composed of cellulose, hemicellulose, and lignin [3]. Currently, Indonesia is the largest exporter of palm oil in the world commercial market. In the processing of crude palm oil, oil palm empty fruit bunch (OPEFB) is generated as waste [4]. Lignocellulosic materials from palm empty fruit bunch are potential to produce bioethanol. OPEFB consist of cellulose 43.32%, hemicellulose 23.67%, and 22.1% lignin [5]. The cellulose and hemicellulose are formed together with lignin to the strong and rigid matrix in lignocellulosic biomass so are recalcitrant to enzyme attack [6]. Bioethanol produced by fermentation
of sugars. Sugars from lignocellulosic biomass can produce by two basic step namely pretreatment and hydrolysis. Pretreatment is the first step to produce bioethanol, by degrade lignin from lignocellulosic materials and to enhance the digestibility. An effective pretreatment must enhance enzyme efficiency, minimize carbohydrate losses, and inhibit by-product formation [7].

In this study, organosolv pretreatment was used to degrades lignin from OPEFB as raw materials to produce bioethanol. Organosolv pretreatment is a process to degrade lignin from lignocellulosic materials using organic solvent or their aqueous solutions [8]. Organosolv pretreatment is recognized as an apparent way ahead because of its inherent advantages, such as the ability to fractionate lignocellulosic biomass into three major components with high purity, as well as easy solvent recovery and reuse [9]. Ethanol was selected as a solvent in organosolv pretreatment because of low cost and easy to recover.

2. Material and Methode

2.1. Material preparation
Oil Palm Empty Fruit bunch was collected from PTPN VIII Cikasungka Bogor, Indonesia. Before used, OPEFB was sun dried, cleaned, milled up, and ground into small pieces, then sieved in 20, 50, and 80 mesh using Tyler sieve then oven dried at 105°C overnight [10]. C₂H₅OH (ethanol 99.5% by MERCK) H₂SO₄ (Sulfuric Acid 96%). OPEFB then analyzed to obtained cellulose, hemicellulose, and lignin using Chesson [11].

2.2. Organosolv Pretreatment
Organosolv pretreatment was performed chemically by dissolving OPEFB with aqueous ethanol at solid-liquid ratio 1:10 (10 OPEFB in 100 mL aqueous ethanol) in 250 mL Erlenmeyer flask. OPEFB was carried out in variation particle size (20, 50, and 80 mesh). Aqueous ethanol was carried out in variation concentration (55, 65, and 75% vol). The solutions then cooked at 160°C in variation reaction time (40, 65, and 90 min). The residue from pretreatment process then separated from ethanol by filtration process then washed with hot distilled water. The residue then dried at 105°C overnight to obtain the constant weight.

2.3. Lignin content analysis
Pretreated OPEFB was analyzed to measure lignin content after organosolv pretreatment. Lignin content after organosolv pretreatment measured by Chesson method [11].

2.4. Experimental Design
The aim of the experiment is to determine optimal condition of organosolv pretreatment. The input variables for organosolv pretreatment were ethanol concentration, reaction time, and particle size of the substrate. The aim of optimization process was to determine the parameters of organosolv pretreatment which maximize lignin degradation of OPEFB. The experiment designed using a Box-Behnken Design (BBD) and Response Surface Methodology (RSM) planned in the Design Expert software version 10.0.2. Independent variables were selected based on the preliminary experiment (Table 1). Evaluation of organosolv pretreatment was conducted based on 17 runs generated by the program presented in table 2. Design Expert software version was used for regression analysis and analysis of variance (ANOVA). The experimental responses obtained were analyzed with the second-order polynomial equation below:

\[ Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i<j}^{k} \beta_{ij} x_i x_j + \epsilon \]

\( Y \) is the response, \( X_i \) and \( X_j \) are coded independent variables, and \( \beta_0, \beta_i, \beta_{ii}, \) and \( \beta_{ij} \) are the intercept, linear, quadratic, and interaction constant coefficients, respectively [12]. In the equation, positive signs indicate synergetic effects while the negative signs indicate antagonistic effects [13]. Response surfaces and contour plots were developed using the fitted quadratic polynomial equation obtained from
regression analysis, holding one of the independent variables at a constant value corresponding to the stationary point and changing the other two variables [14].

**Table 1.** Coded and actual values of organosolv pretreatment for three-factor Box-Behnken Design

| Independent variables                  | Coded and actual levels |
|----------------------------------------|-------------------------|
| Ethanol Concentration (% v/v)          | -1 0 1                  |
| Reaction Time (min)                    | 40 65 90                |
| Particle Size of Substrate (mesh)      | 20 50 80                |

**Table 2.** Experimental design of organosolv pretreatment and result

| Run no. | Ethanol Concentration (% v/v) | Reaction Time (min) | Particle Size of Substrate (mesh) | Lignin degradation (%) |
|---------|-------------------------------|---------------------|-----------------------------------|------------------------|
| 1       | 65                            | 65                  | 50                                | 23.96                  |
| 2       | 65                            | 65                  | 50                                | 30.47                  |
| 3       | 75                            | 65                  | 20                                | 16.72                  |
| 4       | 75                            | 65                  | 80                                | 22.37                  |
| 5       | 75                            | 90                  | 50                                | 31.81                  |
| 6       | 65                            | 40                  | 80                                | 23.05                  |
| 7       | 65                            | 90                  | 80                                | 34.79                  |
| 8       | 75                            | 40                  | 50                                | 23.95                  |
| 9       | 65                            | 40                  | 20                                | 12.56                  |
| 10      | 55                            | 65                  | 80                                | 22.42                  |
| 11      | 65                            | 90                  | 20                                | 25.64                  |
| 12      | 65                            | 65                  | 50                                | 25.74                  |
| 13      | 55                            | 40                  | 50                                | 7.43                   |
| 14      | 55                            | 90                  | 50                                | 24.41                  |
| 15      | 65                            | 65                  | 50                                | 32.24                  |
| 16      | 55                            | 65                  | 20                                | 7.97                   |
| 17      | 65                            | 65                  | 50                                | 26.00                  |

3. Result and Discussion

3.1. Effect of organosolv pretreatment on lignin degradation

Pretreatment process was performed to break lignin bond by bonding the ether chain of molecule lignin. It also breaks microfibril crystal chains of cellulose to elevate the degradation into its monomers during hydrolysis process [15]. Large hemicellulose and lignin polymers split into small fragments that dissolve in hot liquor when pretreatment was performed. Lignin, acetic acid, furfural, and lipophilic extractive emerge in hot liquor. Meanwhile, solids particles termed as dignified cellulose.

In this study, the effect of organosolv pretreatment on lignin content showed by lignin loses after pretreatment. Lignin content of pretreated PEFB was analyzed by Chesson method [11]. Table 3. shows the statistical analysis of variance (ANOVA). The significance of the model, individual terms, and their interaction with lignin degradation was investigated through ANOVA. Table 3 shows that the models are significant with a p-value less than 0.05. Meanwhile, the value of R-square is 0.92. The high R-squared value represents that the model obtained will be able to give a sufficiently good estimate of the
response of the system within the range studied. This finding signifies that the correlation between experimental and the predicted value could be accepted.

Table 3. Analysis of variance of the experimental result

| Source            | Sum of Squares | Df | Mean Square | F Value | p-value | Prob > F |
|-------------------|----------------|----|-------------|---------|---------|----------|
| Model             | 924.73         | 9  | 102.75      | 9.22    | 0.004   | significant |
| A-Ethanol Concentration | 133.00         | 1  | 133.00      | 11.93   | 0.01    |           |
| B-Reaction Time   | 308.34         | 1  | 308.34      | 27.66   | 0.001   |           |
| C-Particle Size   | 197.57         | 1  | 197.57      | 17.73   | 0.004   |           |
| AB                | 20.84          | 1  | 20.84       | 1.87    | 0.21    |           |
| AC                | 19.30          | 1  | 19.30       | 1.73    | 0.23    |           |
| BC                | 0.45           | 1  | 0.45        | 0.041   | 0.84    |           |
| A^2               | 162.41         | 1  | 162.41      | 14.57   | 0.006   |           |
| B^2               | 0.78           | 1  | 0.78        | 0.07    | 0.79    |           |
| C^2               | 70.76          | 1  | 70.76       | 635     | 0.04    |           |
| Residual          | 78.02          | 7  | 11.25       |         |    |           |
| Lack of Fit       | 29.01          | 3  | 9.67        | 0.79    | 0.56    | not significant |
| Pure Error        | 49.01          | 4  | 12.25       |         |    |           |
| R-square          |                |    |             | 0.92    |        |           |

According to the analysis of variance that had been done in data processing, optimization in actual value for predicting lignin degradation from ethanol concentration (A), reaction time (B), and particle size of substrate (C) factors was obtained. Optimization of lignin degradation model is represented by following scheme:

\[ \text{Lignin degradation} = -357.96 + 9.44A + 0.77B + 1.12C - 9.13 \times 10^{-5}AB - 7.32 \times 10^{-3}AC - 4.49 \times 10^{-4}BC - 0.06A^2 + 6.88B^2 - 4.55 \times 10^{-3}C^2 \]

Where:
A : Ethanol concentration (% v/v)
B : Reaction time (min)
C : Particle size of Substrate (mesh)

The equation shows that ethanol concentration (A), reaction time (B) and particle size of substrate have a synergetic effect on lignin degradation. The effect of each factor presented by figure 1.
The effect of organosolv pretreatment variables is also presented in 3D surface contour. The effect of organosolv pretreatment on lignin degradation presented by figure 2.

**Figure 1.** The effect of Ethanol Concentration (a), reaction time (b), and particle size of substrate (c) on lignin degradation.

The effect of organosolv pretreatment variables is also presented in 3D surface contour. The effect of organosolv pretreatment on lignin degradation presented by figure 2.
Figure 2. The effect of Ethanol Concentration (a), reaction time (b), and particle size of substrate (c) on lignin degradation.

Figure 2. shows that ethanol concentration (A), reaction time (B), and particle size of the substrate (C) have a significant effect on lignin degradation of OPEFB. Figure 2(a) shows the interaction between ethanol concentration (A) and reaction time (B) which are presented with 55-75% of ethanol and 40-90 min reaction time. It represents that lignin degradation increase along with increasing of ethanol concentration and reaction time. However, higher ethanol concentration and longer reaction time do not bring significant effect on lignin degradation in large particles. The effect of ethanol concentration and reaction time is more obvious at small particle size. The particle size of substrate will affect on porosity of the substrate [16]. Large substrate porosity will make ethanol easier to degrade lignin. Therefore, higher lignin degradation will be obtained in small particle size.

Figure 2(b) shows the interaction between ethanol concentration (A) and particle size of substrate (C) which are presented with 55-75% of ethanol concentration and 20-80 mesh particle size of substrate. It represents that lignin degradation will increase along with increasing of ethanol concentration (A) and
a decrease of particle size of substrate (C), but a high concentration of ethanol and smaller particle size of does not bring significant effect on lignin degradation when reaction time is too short. Reaction time is important variables on organosolv pretreatment. The fibrous structure of OPEFB which makes it more resistant to an organic solvent or thermal attacks without giving sufficient reaction time [14].

Similarly, figure 2(c) shows the interaction between reaction time and particle size of substrate. It represents that lignin degradation will increase along with increasing of reaction time and decreasing particle size of substrate. However, longer reaction time and smaller particle do not bring significant effect on lignin degradation at low ethanol concentration. Lignin degradation increased by the increase of ethanol concentration as an organic solvent in organosolv pretreatment. [15]. Therefore, higher ethanol concentration will generate high lignin degradation.

3.2. Model validation

The aim of model validation was to the confirmed optimum condition of organosolv pretreatment. The RSM model was suitable if the predicted value was close with actual value on optimum condition. Optimum condition of RSM model showed in figure 3.

![Graph](image1)

**Figure 3.** Optimum condition of organosolv pretreatment based on RSM model and desirability.

Figure 3. shows that optimum condition of organosolv pretreatment based on RSM model was obtained at 65% vol ethanol concentration, reaction time 65 min, and particle size of substrate 50 mesh. Lignin degradation on optimum condition based on RSM model is 27.68%. Optimum condition and validation presented in table 4.
Table 4. Optimum condition and validation of organosolv pretreatment

| Ethanol concentration [% v/v] | Reaction time [min] | Particle size [mesh] | Lignin degradation | Validation |
|-------------------------------|---------------------|----------------------|--------------------|------------|
| 65                            | 65                  | 50                   | 23.962             | 27.6847    | 86.55%    |

Table 4. shows that validation between the observed value and RSM model value of lignin degradation is 86.55%. It shows that RSM model is suitable for organosolv pretreatment condition.

4. Conclusions

The effect of organosolv pretreatment using ethanol solvent, respectively to degrade lignin were successfully evaluated in this study. Optimum condition of organosolv pretreatment was in ethanol 65% vol, reaction time 65 min and 50 mesh particle size of substrate with lignin degradation is 27.68% and validation value 86.55%.

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