Enhancement the added value of sengon wood waste pulp as bioenergy raw material for bioethanol production

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Abstract. Lignocellulosic are renewable and abundant, and estimate to be produced in 10-50 billion tons/year as dry matter. Cellulose component in lignocelluloses can be converted into glucose as a feedstock for ethanol fermentation. This paper contains of research results of sengon waste pulp conversion became glucose and ethanol by enzymatic hydrolysis and the purpose of this study was to obtain information on the optimum conditions of treatment to produce high ethanol content of sengon waste pulp using the Response Surface Methodology (RSM) method and high loading substrate. Response surface methodology based on a three level, three variable Central Composite Rotatable Design (CCRD) was used to evaluate the interactive effect of Tween 20 concentration (0-2%), cellulase concentration (10-15 FPU/g substrate-dw) and substrate loading (20-35% dw). The results showed the highest ethanol content (15.2%) with addition 35% of substrate; 15 FPU/g substrate of enzyme and 1% Tween 20 with equations: \( Y = -66.551 + 0.491X1 + 10.794X2 - 0.025X12 - 0.472X22 - 1.549X32 + 0.065X1X2 + 0.234X1X3 - 0.086X2X3 \). So that the use of the RSM method can know quickly which combination of treatments can produce high ethanol levels through the resulting equation. Distinctive dosages of each variable within these ranges need to be matched to each other according to resulted mathematical formula from RSM to give optimum condition points. Several optimum condition points had been validated and ethanol concentration higher than 18%v in the fermentation broth had been achieved with acuration of equation \((R^2) 0.97\).

1. Introduction

Currently, bioethanol is one of the most important renewable fuels. It can be sustainable replacement of fossil fuels and produced from carbohydrate crops, a variety of starch and lignocellulose. Lignocellulose is classified as the second generation of bioethanol raw materials and abundant availability also absence of food vs fuels competition [1]. The residual biomass can also be converted into other value added platform chemicals in a well integrated biorefinery facility. According to [2] integrated biorefinery is an establishment where biomass is converted into fuels, power and value added chemicals with minimum waste generation. Lignocellulosics are renewable and abundant, and estimated to be produced in 10-50 billion tons/year as dry matter on the earth, which includes crop residues, grasses, sawdust, wood chips, etc.
The major components of biomass as lignocellulose materials are cellulose (about 45%), hemicellulose (ca 20% in softwood, and ca 30% in hardwood) and lignin (ca 30% in softwood, and ca 20% in hardwood) [4]. Sengon tree (Paraserianthes falcatoria (L) Nielsen) is a kind of fast growing species and the wood is used for raw materials for boxes, as building and construction materials or matches industries. According to [5], cellulose content of sengon wood is about 51.8%; 26.6% and 34.7% for the lignin and hemicellulose content, respectively. Indonesia is one of the countries that has a lot of wood processing industries. The final result of the sawmill industry is in the form of shale and wood powder which has been utilized optimally yet. According to the Ministry of Forestry [6], sawn timber production in 2011 reached 935,000 m³ and in 2012 could reached 1,218 million m³. Therefore, the potential of sawmill waste every year is very abundant; as well as for sengon wood waste, because 60% of the sawmill processing will produce wood waste [5].

Conversion of biomass to bioethanol mainly comprises of following steps: pretreatment, saccharification, fermentation, distillation, dehiddration and waste treatment [7]. For woody biomass, the enzymatic saccharification of the cellulose in the biomass without pretreatment is very low (<20%). On the other hand, a rapid conversion of cellulose substrates by enzymatic saccharification is difficult to obtain. The hydrolysis rate rapidly decreases during the time course of saccharification which leads to decreased yields and long process times [8]. Technical approach to overcome recalcitrance is the pretreatment of biomass feedstock to make a cellulose more accessible to hydrolytic enzymes for conversion to glucose [9]. The cell wall structure including lignin supresses accessibility of enzymes to substrate, cellulose. Therefore, the destruction of cell wall and/or extraction of lignin from woody biomass are one of the methods to improve saccharification, suggesting that pretreatments are required for the enzymatic saccharification [10]. Lignin, one of the major components of lignocellulose biomass is impediment to enzymatic saccharification. Hence, the delignification can substantially improve the enzymatic saccharification of the biomass [11]. However, pretreatment technology still becomes a bottleneck to make lignocelluloses materials more competitive with natural glucose syrups and starch as ethanol material [12].

The major factors that affect the efficiency of the enzymatic saccharification of lignocellulose are substrate concentration, enzyme loading and surfactant concentration. The current study by [13] showed that increasing of addition Span 85 and cellulose concentration can improve the amount of resulted reducing sugar of palm oil EFB pulp.

Experimental design and data analysis are important to improve the process ability. Response Surface Methodology or RSM is a collection of mathematical and statistical techniques that are useful for modeling and analysis of problem in which a response of interest is influenced by several variables and the objective is to optimize this response [14]. The main advantage of RSM is the reduced number of experimental runs needed to provide sufficient information for statistically acceptable result. It is faster and less expensive method for gathering research result than the classical method [15]. Among the different types of central composite design (CCD), the central composite rotatable design (CCRD) has shown great potential to target the stability region of the design around a central point determined by its properties of rotatability and orthogonality [16]. The CCRD approach has several applications, especially in studies aiming the optimization of experimental conditions or containing several treatments of variables [17]. Several studies has been applied the CCRD to optimize the concentrations of substrates, enzymes loading and surfactants for bioethanol production [11,15].

The objective of this research was to study the effect of substrate loading, enzyme and surfactant concentration in increasing ethanol production of wood waste sengon pulp. Tween 20 was the chosen commercial surfactant and its concentration in cellulase saccharification reaction will be observed by using RSM method. It is expected that the results of this study can increase the added value of sengon wood waste as raw material for bioethanol production.
2. Materials and Methods

2.1 Materials

Sengon wood waste pulp was prepared by pilling of the bark of “sabetan” and was cut into 3-5 cm length as a wood chips before the pulping process. Then, wood chips digested using kraft pulping method (NaOH and Na₂S 25% as active alkali, with cooking liquor 1:5) decrease the lignin content in the sample. Digestion was kept at 170ºC for 2.5 hours. The digestion results was filtered and washed using hot and cold water thoroughly until it reaches normal pH.

Cellulase from Novozyme was used in all enzymatic saccharification in this research. Cellulase activity was 50 FPU/mL and Saccharomyces cerevisiae as yeast was used in fermentation part. Has a chemical name polyoxyethylene sorbitan monolaurate or also known as Tween 20 were obtain from Merk; with molecular formula C₅₈H₁₁₄O₂₆ and molecular weight of 1227.51 g / mol. Surfactant chemical structures were shown in Figure 1.

![Figure 1. Chemical structure of Tween 20](image)

2.2. Methods

2.2.1. Enzymatic saccharification sengon wood waste pulp

Several concentration of Tween 20 (0-2%v) was dissolved in 100 mL of 0.05 M citric acid (C₆H₈O₇) buffer at pH 4.8. Cellulase enzyme (10-15 FPU/g substrate) was added to the solution, and the mixture was stirred for 1 h in room temperature. Finally, several concentration of substrate (20,27,35% dw) were added to the solution, and the suspension was shaken at 50ºC for 48 h. After saccharification, as much as 5 mL of saccharification product from each experiments were filtered. The reducing sugar concentration in the filtrate was measured by DNS (3,5-dinitrosalicylic acid) method. The rest of saccharification product of each experiment was fermented by adding yeast (1% dw substrate). The fermentation process was performed in room temperature for 96 h. The resulted fermentation broth was distilled twice with water then distilled until the distillate volume reached 100 mL. Ethanol concentration in distillate was determined using gas chromatography (GC).

2.2.2. Experimental design for RSM

A three-level-three-factor central composite rotatable design (CCRD) was employed in this study, requiring 9 experiments. The variable and their level selected for the cellulase saccharification were substrate loading (20-35% dw), cellulose concentration (10-15 FPU/g substrate-dw) and Tween 20 concentration (0-2%v). The data obtained were fitted for a second-order polynomial equation.

\[ Y = b_0 + \sum b_i x_i + \sum b_{ii} x_{ii} + \sum b_{ij} x_i x_j \]

In this study, two equation will be obtained with Y were resulted concentration of alcohol (%v) and in % cellulose conversion. On the other hand, b₀, bᵢ, bᵢᵢ, bᵢⱼ are constant coefficients and xi the uncoded independent variables. Table 1 shows the independent factors (xᵢ), levels and experiment
design. After the polynomial equations were obtained, nine series of independent variables were performed in cellulose saccharification. Then for validation of the prediction, cellulose saccharification was conducted at those conditions and the actual values were compared to the predicted ones.

3. Result and Discussion

3.1. Chemical analysis of sengon wood waste

Chemical composition analysis is a method of chemical analysis to complement the chemical composition of a material. This analysis is carried out on the raw material before pulping and after being pulped as a substrate in the process of saccharification and fermentation to produce ethanol. Pentosan content of raw materials is 15.4%; holocellulose 73.2%; alpha cellulose 47.4%; hemicellulose 25.8%. After delignification process (kraft pulping) alpha cellulose content will increase (82.2%) with the decreasing of the lignin content (1.6%). The results of cellulose and lignin contents are in similar with the results of research on cellulose and lignin contents carried out by [18], that is equal to 51.8% and 26.6%.

3.2 Model fitting equation of Response Surface Methodology

The predicted values were obtained from model fitting technique using SPSS version 16 software. Fitting of the data were conducted to quadratic polynomial model and were seen to be sufficiently correlated to actual values. The quadratic polynomial equations are given below:

\[
Y_1 = 10.441 + 2.975 X_1 + 4.092 X_2 - 0.057 X_1^2 - 0.047 X_2^2 - 2.743 X_3^2 - 0.031 X_1 X_2 + 0.554 X_1 X_3 - 0.368 X_2 X_3
\]

\[
Y_2 = -199.758 - 1.822 X_1 + 42.907 X_2 - 0.030 X_1^2 - 1.815 X_2^2 - 4.688 X_3^2 + 0.160 X_1 X_2 + 0.658 X_1 X_3 - 0.187 X_2 X_3
\]

\(Y_1\) is resulted ethanol yield (in %), \(Y_2\) is resulted ethanol (in %v). For the independent variables, \(X_1\) is the substrate loading (in %dw), \(X_2\) is the enzyme concentration (in FPU/g substrate) and \(X_3\) is Tween 20 concentration (in %v).

Table 1. Central composite rotatable quadratic polynomial model of wood waste sengon pulp

| Independent variables | Dependent variables |
|-----------------------|---------------------|
| Substrate loading (%dw) | Ethanol conversion (%dw) | Resulted ethanol concentration (%v) |
| X₁ | X₂ | X₃ | actual | predicted | actual | predicted |
| 35 | 12 | 0 | 20.01 | 20.33 | 8.80 | 8.70 |
| 27 | 12 | 1 | 41.66 | 45.32 | 14.20 | 13.84 |
| 20 | 12 | 2 | 46.56 | 46.78 | 11.70 | 11.53 |
| 20 | 10 | 1 | 35.69 | 37.94 | 9.01 | 9.28 |
| 20 | 12 | 1 | 54.12 | 49.92 | 13.60 | 12.53 |
| 20 | 15 | 0 | 33.43 | 34.99 | 8.40 | 8.48 |
| 20 | 15 | 2 | 36.81 | 36.96 | 9.25 | 9.06 |
| 27 | 10 | 1 | 33.04 | 31.09 | 11.21 | 10.68 |
| 35 | 15 | 1 | 34.40 | 34.39 | 15.20 | 15.19 |

Coefficient of determination (R²) 0.94 0.97

In predicting resulting ethanol yield and ethanol concentration, the quadratic polynomial model was highly sufficient to represent the actual relationship between the response and the independent
variables with coefficient of determination $R^2 = 0.94$ and 0.97, respectively. It’s implying that most of the experimental results are very close in agreement with the model predicted results.

3.3. Effect of parameters of Response Surface Methodology

The effect of loading substrate is explained by the Figure 2A. At low concentration of substrate loading (20%) a very low ethanol concentration is observed because low concentration of enzyme. As the concentration of substrate increased there is a significant increase in the resulted ethanol (35%), indicating a synergistic effect of the enzyme on the saccharification process. Figure 3B. Explained the optimum addition of enzyme concentration was used during saccharification to produce high resulted ethanol. For example, to produce 12%v ethanol, we could use only 10 FPU/ gr substrate for the enzyme, but with 33.6 g of loading substrate in the saccharification, or we could added initial substrate (20%dw) and higher enzyme concentration (12.7 FPU/g substrate). Figure 2C. showed that increasing Tween 20 which was not followed by increasing of resulted ethanol. This result was similar to the result of [13]. Increasing of Span 85 concentration which was not followed by increasing the resulted ethanol of palm oil empty fruit bunch (EFB) pulp due to the Span 85 toxicity. Tween 20 is a polysorbate-type nonionic surfactant, its mean Span 85 and Tween 20 were the same group of surfactant. The toxicity of nonionic surfactant to microbial cell is attributed to an extraction of lipids in the cell membrane. Nonionic surfactant Triton X-100 solution transforms the cell membrane structure of *E. coli* and enables intracellular components to be released [19]. In categorizing surfactant toxicity, it was follow the order ionic surfactant > nonionic surfactant > polymeric nonionic surfactant [20].

**Figure 2.** Surface contour plot of resulted ethanol content (in %v) that predicted by polynomial model
In the earlier studies, there were proposed three different explanations to the surfactant effect on cellulose saccharification. Such surfactants have a positive effect on enzymatic saccharification of lignocellulose, resulting in a faster saccharification rate and enabling lower enzyme dosage [21,22,23,24]. In addition, surfactants make re-use of cellulose possible [25]. The positive effect of surfactants has been observed not only for enzymatic saccharification of cellulose, but also for a process of simultaneous saccharification and fermentation (SSF)[26]. Recently, the effort to elucidate the positive effect of surfactants on the improvement of enzymatic saccharification is being made intensively [27]. The proposed mechanism are summarized as follows : 1) Surfactants may act as enzyme stabilizers and prevent denaturation; 2) Surfactants may have an effect on substrate structure; 3) surfactants may effect enzyme-substrate interactions, in particular by preventing non-productive adsorption on enzymes [26,28].

Figure 3. Surface contour plot of resulted ethanol conversion (in %)

Ethanol conversion curve were shown in Figure 3. There was an optimum value in the curve which is specific for each substrate loading, enzyme-Tween 20 composition in the saccharification and followed by fermentation shows significant effect roles in ethanol conversion. Effect of Tween 20 was decreased in high substrate loading and shows no significant effect in substrate loading higher than 35% (green line). Increasing substrate loading reduced the ethanol conversion. At substrate loading higher than 27% the change of ethanol conversion was insignificant. This result similar with the research resulted by [13] that proposed there was no increasing ethanol conversion in substrate loading higher than 35% of oil palm EFB pulp on enzymatic saccharification.

4. Conclusions
The highest production of ethanol content of pulp sengon waste (15.2%) could achieved from treatment with addition 35% of substrate; 15 FPU/g substrate of enzyme and 1% Tween 20 with equations : \( Y = -66.551 + 0.491X_1 + 10.794 X_2 - 0.025 X_1X_2 + 0.472 X_2^2 - 1.549 X_3^2 + 0.065 X_1X_2 + 0.234 X_1X_3 - 0.086 X_2X_3 \) from RSM equation. Several optimum condition points had been validated and ethanol concentration higher than 18%v in the fermentation broth had been achieved with acuration of equation \( (R^2) 0.97 \). Its conclude that, the sengon waste pulp is a potential raw material for bioethanol production.

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