Bioethanol Production from Bamboo Pulp using Enzymatic Sacharification with Several Concentration of Surfactant

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Abstract. Bioethanol is one of new alternative and renewable energy to decrease energy crisis and negative effect of fossil fuels usage. Lignocellulosic 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 the results of study on the conversion of lignocellulose to glucose and ethanol in the process of enzymatic sacharification. Bamboo is lignocellulosic raw materials that can be used for bioethanol production. This research was conducted to utilize bamboo waste to make bioethanol through enzymatic hydrolysis process and determine the effect of surfactant and substrat on the produced bioethanol. Surfactants Tween 20 that were used in this research were 0-4% (v) and the amount of enzyme which was added were 10 FPU/g substrate. On the other hand, substrate concentration were 20, 25, and 30 % (dw) added to the sacharification. Addition of 1% (v) surfactant could increase the reducing sugar up to 90% until 3% concentration and subsequently decrease. Enhancement of reducing sugar level was not followed by the increasing of ethanol level at some treatment so that the linear trendline was different between reducing sugar level and ethanol level.

1. Introduction

Currently, fuel is a primary need for human needs both to full fill daily needs. From the end of 19th century, we are highly dependent on various fossil resources, such as petroleum, coal, natural gas and so on. Excessive consumption of fossil fuels, particularly in large urban areas, has resulted generation of CO₂ and high level of pollution during the last few decades. Developing biomass energy is considered to be the main strategy to solve this problem and one of liquid biofuels receiving the most interest is bioethanol [1]. Bioethanol can be produced from cellulose component; especially unused lignocellulosics have never competed to food production.

Lignocellulosics are renewable and abundant and estimated to be produced in 10-50 billion tons/year as dry matter on the earth, which includes grasses, sawdust, wood chips and waste [2]. Cellulose fibers are covered by lignin, which plays roles as reinforced material to cellulose and protecting agent againsts pathogenic attacks of the fungi and bacteria [3]. Utilization of lignocelluloses as biomass and potential raw materials in bioenergy processes (second generation) has been studied extensively. Enzymatic saccharification of cellulose into soluble sugars is one og effective conversion cellulose to ethanol process for liquid fuel application i.e ethanol. On the other hand, the hydrolysis rate rapidly decreased during the time saccharification which leads to decreased yields and long process time [4].
Surfactants are amphiphilic compounds that usually contain a hydrophilic head and a hydrophobic tail. Surfactants are able to self-assemble into micelles and will absorb onto the surface of the guest molecule. The assembly and degree of adsorption depends on the surfactant structure and polarity of the guest surface. Enhancement of cellulose hydrolysis by adding surfactants to the saccharification mixture has been reported by several authors [5], [6], [7]. Such surfactants have been reported to have a positive effect on enzymatic saccharification of lignocelluloses, resulting in a faster saccharification rate and enabling lower enzyme dosage [8], [9]. The positive effect of surfactants has been observed not only for enzymatic saccharification of cellulose, but also for simultaneous saccharification and fermentation (SSF) [10]. The effect of surfactant on the enzymatic saccharification of steam pretreated spruce (SPS) was the object of intense research for use in an ethanol producing process [11], [12], wheat straw pretreated with various technique and PEG was also found to be efficient in the increase of cellulose conversion [13]. Another research showed that Tween 20 plays an important role in the saccharification of crystalline cellulose and that Tween 20 disturbs the adsorption of endoglucanase on cellulose, i.e. varies the adsorption balance of endo- and exoglucanase, resulting in enhancing the reaction [5].

The objective of this research was to study the effect of surfactant and substrate concentration addition in the enzymatic saccharification of bamboo pulp in increasing ethanol production and cellulose conversion. Tween 20 was the chosen commercial surfactant and several concentration of surfactant and substrate to evaluate the efficient of the saccharification and we declare that this manuscript are our original work and research.

2. Research Method

2.1 Materials

The bamboo waste was cut into 3-5 cm length and then it was digested using kraft process (NaOH and Na2S 25% as active alkali, with cooking liquor 1:5). Digestion was kept at 170° C for 2.5 hours. The pulp was filtered and washed until it reach normal pH. The resulted bamboo pulp has 0.56% lignin. Has a chemical name polyoxyethylene sorbitan monolaurate or also known as Tween 20 were obtain from Merk; with molecular formula C_{58}H_{114}4O_{26} and molecular weight of 1227.51 g / mol. Surfactant chemical structures were shown in Figure 1.

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

Cellulase and β-glucosidase from NOVOZYMЕ (cellictec 2) 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.
2.2. Chemical compound analysis

Chemical compound analysis of raw materials and pulp, i.e moisture content, lignin content using Klason method and cellulose content by Norman and Jenkis method before and after pulping process (delignification).

2.3. Procedures for cellulase saccharification

Several concentration of Tween 20 (0, 1, 2, 3, and 4% v) was dissolved in 100 mL of 0.05 M citric acid (C₆H₈O₇) buffer at pH 4.8. Cellulase (10 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, 25, 30% dw) were added to the solution, and the suspension was shaken at 50°C for 48 h. After saccharification, as much as 5 mL of saccharification product from each experiment was filtered. The reducing sugar concentration in the filtrate was measured by DNS (3,5-dinitrosalicylic acid) method.

The rest of saccharification product from each experiment was fermented by adding yeast 0.1% substrate and urea 0.3% substrate. The fermentation process was performed in room temperature (926-28°C) for 96 hours. The resulted fermentation broth was diluted twice with water then distilled until the distillate volume reached 100 mL. Ethanol concentration in distillate was determined based on alcohol meter measurement.

Meanwhile, the suspension was filtered and washed with buffer solution, and weighed after complete drying at 105°C. The conversion cellulose was calculated using the following equation:

\[
\text{Cellulose conversion (\%)} = \frac{\text{initial cellulose (g)} - \text{cellulose residue (g)}}{\text{initial cellulose (g)}} \times 100
\]

3. Result and Discussion

3.1 Chemical compound analysis

Water content is the amount of water present in a wood or sample. It is defined as the weight of water expressed as the weight percentage of the water free timber or dry oven. Bamboo has thick fibers so that the ability to bind water is greater. Water in woods with a certain amount of wood allows easy attacked by fungi and wood destructive organisms [14]. The result showed that the water content in Chinese bamboo waste pulp is 10.6% (Table 1.). Production of bioethanol is preferably wood/sample with low moisture content the manufacture of bioethanol because it is an impurity substance that can decrease the speed of hydrolysis and fermentation [14].

Table 1. Chemical compound analysis of bamboo pulp

| No  | Parameter (Parameter) (%) | Bahan baku (Raw materials) | Pulp (Pulp) |
|-----|--------------------------|-----------------------------|-------------|
| 1.  | Water content            | 15.2                        | 10.6        |
| 2.  | Ash content              | 2.19                        | 0.47        |
| 3.  | Hot water solubility     | 18.7                        | 12.7        |
| 4.  | Cold water solubility    | 13.0                        | 12.5        |
| 5.  | NaOH solubility          | 26.1                        | 11.1        |
| 6.  | Lignin content           | 22.7                        | 0.56        |
| 7.  | Cellulose content        | 53.7                        | 90.6        |

Compounds that act as binder components of the constituent plants (hemicellulose and cellulose) is lignin. Lignin is formed from aromatic compounds that are interconnected with aliphatic chains that are resistant to hydrolysis. The result of experiment showed that lignin content in bamboo chinese is 22.7%. Because the cell wall structure including lignin suppresses accessibility of enzymes
to the substrate, cellulose. Therefore, the destruction of cell wall of lignin from woody biomass are one of the methods to improve saccharification, suggesting that preteratments or delignification are required for enzymatic saccharification [15]. After delignification process, the lignin content of bamboo waste pulp showed 0.56%; that results indicated the success of the delignification process in the preliminary treatment. Wood that has high lignin content is not desired in the raw material of bioethanol manufacture. And enzymatic saccharification of cellulose in the biomass without pretreatment is very low (< 20%). Thus, delignification must be performed with the aim of reducing lignin levels, reducing cellulose crystallinity, and shrinking the size of polysaccharides to make it more easily hydrolyzed thus increasing the ethanol content [16].

The main ingredient of making second generation of bioethanol is cellulose. High cellulose levels indicate that the pulp has the potential to be further processed into bioethanol, with pretreatment, saccharification and fermentation. Wood/sample with a higher cellulose content will give the mass of sugar C (sugar with 6 carbon atoms ) higher than wood/sample with lower cellulose content [14]. The result showed that cellulose content of bamboo waste pulp after delignification is 90.6%, it is means that high cellulose content as raw material of bioethanol manufacture. High levels of cellulose will produce high glucose in the enzymatic hydrolysis process. Saccharification involves polysaccharide breakdown process in lignocellulose biomass, ie cellulose and hemicellulose into its constituent sugar monomer. Cellulose-perfect hydrolysis produces glucose, whereas hemicellulose produces some pentose (C5) and hexose (C6) monomers.

3.2. The Effect of several Tween 20 and substrate concentration

Analysis of reducing sugar content in bamboo pulp is to know the content of glucose monomer produced after the saccharification process. The addition of surfactant may increase the reducing sugar and ethanol content. In the previous study, enzymatic saccharification for bioethanol production using 10 FPU/ g substrates of cellulase concentration and Tween 20 could produced reducing sugar before fermentation was higher than without surfactant (control) [17].

| No. | Substrate (%dw) | Tween 20 (%v) | Cellulase (10 FPU/g substrate) | Reducing sugar (mg/mL) | Ethanol content (%) |
|-----|----------------|---------------|-------------------------------|------------------------|---------------------|
| 1.  | 20             | 0             |                               | 2.003                  | 8                   |
| 2.  | 1              |               |                               | 2.915                  | 9                   |
| 3.  | 2              |               |                               | 3.627                  | 10                  |
| 4.  | 3              |               |                               | 4.387                  | 10                  |
| 5.  | 4              |               |                               | 3.274                  | 8                   |
| 6.  | 25             | 0             |                               | 1.915                  | 11                  |
| 7.  | 1              |               |                               | 2.995                  | 12                  |
| 8.  | 2              |               |                               | 2.475                  | 13                  |
| 9.  | 3              |               |                               | 2.995                  | 12                  |
| 10. | 4              |               |                               | 2.030                  | 12                  |
| 11. | 30             | 0             |                               | 1.490                  | 7                   |
| 12. | 1              |               |                               | 2.650                  | 14                  |
| 13. | 2              |               |                               | 2.656                  | 14                  |
| 14. | 3              |               |                               | 6.566                  | 13                  |
| 15. | 4              |               |                               | 3.805                  | 13                  |

Table 2 shows the value of reducing sugar and ethanol content for several concentration of tween 20 and substrate in the cellulose saccharification (10 FPU/g substrate). Without tween 20, the
value of reducing sugar shows lower values level than others in all treatments. The research data showed that the increase in reducing sugar levels occur in addition of 1 mL surfactant compared without surfactant (control) of the amount from substrate 20 and 30% (dw). The addition of tween 20 as much as 1% (v) on the 20% (dw) of substrate concentration can increase reducing sugar; i.e. 2.0034 mg / ml to 2.915 mg / ml. This is in accordance with the research that has been conducted by [18], [19]. Surfactants could cause substrate structural changes and make it more accessible for enzymatic saccharification. However, after addition 3% (v) of tween 20 in the enzymatic saccharification, all treatments resulted a decreased of the value of reducing sugar. This is due to the toxicity of tween 20 as a nonionic surfactant in the fermentation process. Non ionic surfactant Triton X-100 solution alters the cell membrane structure of E.coli and enables intracellular components to be released, which has been applied for the release of intracellular proteins [20].

In principle, fermentation is a change of 1 mole of glucose (sugar) to 2 moles of ethanol and 2 moles of carbon dioxide. In the ethanol fermentation process, the yeast will perform the metabolism of glucose and fructose to form pyruvic acid through the reaction stage of the Embank-Meyerhof-Parnas, while the resulting pyruvic acid will be decarboxylated into dehydrogenated acetaldehyde to ethanol. In the Table 1. Shows the more substrate concentration added to the saccharification process, the higher the ethanol content produced or the higher the concentration of cellulose at the time of saccharification, the higher the ethanol produced. This is in accordance with the results of research conducted by [19]. Meanwhile, ethanol content without surfactant show lower values in all treatments. This is accordance to the result of research done by [17]. Similar with the reducing sugar value, after addition 3% (v) of tween 20, all treatments resulted a decreased of ethanol content. Similar study of surfactant effect in fermentation for ethanol production was used series of Triton X was conducted [21] (Dhamole et al, 2012). It was shown similar result which 6% (v) is optimum concentration of surfactant.

### 3.3. Cellulose conversion

In the saccharification process, all samples were added with several concentration of tween 20, except on the controls (without surfactant) (Figure 2.). The value of cellulose conversion of control shows the lowest levels compared with all other samples with the addition of tween 20. This is due to the one positive effects of surfactant that can help the performance of cellulase enzymes so that the cellulase function could optimally converting cellulose into glucose. The highest cellulose conversion (90.67%) was showed in the 20% (dw) of substrate concentration and 4% (v) of tween 20 concentration. On the other hand, high substrate loading and addition more concentration of surfactant in the cellulase saccharification could not increase the cellulose conversion level. This is probably due to the lack of cellulase, because there is no more addition of enzyme concentration in the saccharification process, so there are still many cellulose that have not been converted to glucose. This result is in accordance with research that has been conducted by [17].

In the previous studies, there are several different explanation of surfactant effect on enzymatic saccharification were proposed. Surfactant may increase enzyme stability and prevent denaturation of enzymes during saccharification. Thermal deactivation and shear deactivation were mostly two factors that concerned in enzyme denaturation. In previous study, addition of Tween can increase the saccharification temperature of corn stover from 40-50 °C which become a strong argument that surfactant addition increase cellulase thermal stability [18]. Another previous study suggested that surfactants adsorb at the air-liquid interface and prevent enzyme denaturation during agitation in the saccharification process [22].
4. Conclusions

Bamboo pulp is a potency raw material for bioethanol production due to its cellulose content and it ease in lowering its lignin level. Results in this study showed that reducing sugar value can be improved by increasing surfactant (Tween 20) concentration until 3% (v). This result supported the explanation the surfactant mechanism in enhancing enzymatic saccharification. On the other hand, fermentation of saccharification product without any treatment (control) for reducing the effect of surfactant prior fermentation showed that there is an optimum value of Tween 20 to get the optimum resulted ethanol and cellulose conversion. This results was due to the Tween 20 toxicity. And the addition of high substrate loading concentration in the cellulase saccharification could decrease the cellulose conversion, due to the lack of cellulase concentration in the saccharification process.

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Figure 2. Cellulose conversion of bamboo pulp (%)
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