



THE EFFECT OF PRETREATMENT AND VARIETY OF MICROORGANISMS TO THE PRODUCTION OF ETHANOL FROM COFFEE PULP

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ABSTRACT

Nowadays, the world is facing fossil energy problems such as the rarity of petroleum and natural gas because these kinds of energy cannot be renewed. Therefore, the discovery of renewable energy source is very important to fulfil the world's energy requirements that will always increase. Coffee pulp is one of the materials that can be used to produce glucose and xylose. The purpose of this study is to produce bioethanol from coffee pulp by utilized both cellulose and hemicellulose components. Coffee pulp is chosen because its production reached 743 kg/ha and has not been used properly. The compositions of coffee pulp are 63% cellulose; 2.3% hemicellulose; 17% lignin; 11.5% protein; 1.8 to 8.56% tannin and 6.5% pectin. Coffee pulp contains of high lignin, therefore pretreatment must be carried out to lower the lignin. Organosolv method is capable not only to produce large amount of high-quality and relatively pure lignin but also to dissolve most of the hemicellulose. The substrate of organosolv pretreatment has higher cellulose and hemicellulose contain than another alternative method. Alkaline method was used to compare the effectivity between alkaline and organosolv method. Cellulose and hemicellulose which are generated from chemical pretreatment, are being hydrolyzed using pure cellulase and xylene enzyme to be converted to glucose and xylose. Then, continue the hydrolysis to fermentation using variation of *Saccharomyces cerevisiae*, *Zymomonas mobilis* mutant (A3) and *Pichia stipitis* to ferment xylose and glucose to be ethanol. From this research, we got the best pretreatment process that was shown in Organosolv pretreatment using 50% (v/v) ethanol with the result 0.20% (w/w) of lignin; 52.24% (w/w) of cellulose and 11.48% (w/w) of hemicellulose. The best result of pretreatment was the one which produce the highest cellulose and hemicellulose has no effect relatively. Cellulose and hemicellulose from coffee pulp hydrolyzed by using mixture of pure cellulase and xylene enzyme at temperature 60 °C, pH 3 for 30 hours. Reduction sugar yield obtained from hydrolysis is 0,164 gram reduction sugar/gram coffee pulp. Hydrolysis using 50% ethanol with the mixture of pure enzymes gave the best result as productive as 3.480 reduction sugar/gram cellulose and hemicelluloses. High reduction sugar from enzymatic hydrolysis correlated with high concentration of cellulose and hemicelluloses. Then for the highest yield of bioethanol by fermentation using *Saccharomyces cerevisiae* is 0,065 gram ethanol/ gram glucose and xylose.

Keywords: bioethanol, organosolv method, alkaline method, hydrolysis, fermentation.

INTRODUCTION

The energy demands will always increasing is in inverse proportion to the reserves of fossil fuel. Therefore, it needs to discover new alternative energy that can be renewed. One of the alternative energy is ethanol. Bioethanol is a clean fuel because the combustion does not cause additional carbon dioxide to the atmosphere. The usage of ethanol as vehicle fuel can decrease the usage of fossil fuel and greenhouse effect significantly.

Bioethanol can be produced from biomass such as rice straw waste, sorghum stem, coconut coir dust, coffee pulp, etc (Widjaja, *et al*, 2015). The coffee productions produce 65% of coffee bean and 35% of coffee pulp. In most coffee industry do not use the coffee pulp and make it as a waste. The coffee pulp waste is chosen because it can reach almost 743 kg/ha and has not been processed properly. The compositions of coffee pulp are 63% cellulose; 2.3% hemicellulose; 17% lignin; 11.5% protein; 1.8 to 8.56% tannin and 6.5% pectin.

Cellulose is homo-polysaccharides that composed from anhydrous-glucopiranoside that binds to glucoside bond b-(1, 4) that form a linear glucan chain. The function of cellulose is for strengthening the plant's stem. Lignin is for protecting cellulose from chemical and biological reaction. Hemicellulose is for bonding the cellulose and lignin (Lee, 1992).

Cellulose is a linear hydrophilic glucose polymer that connected with glycoside bond. The amount of glucose molecule may vary. It lies between 15 and more than 10,000 in a molecule of cellulose. Cellulose polymer consists of crystalline and amorphous part. The amorphous part is easy to hydrolyze besides the crystalline part is hard to hydrolyze even in chemical or enzymatic method (Dahot and Noomrio, 1996).

Hemicellulose is a combination of short and branched polymers chain which consists of amorphous xylose monomer, arabinose, glucose, mannose and galactose (Bailey and Ollis, 1986). Hemicellulose is for strengthening and as an adhesive the cell walls.



Hemicellulose will degrade first then cellulose because it only has 200 of polymerization degree. The main component of hemicellulose in plantation is xylene which bonded to cellulose, pectin, lignin, and the other polysaccharides to form the cell wall. The amount of xylene is the second biggest after cellulose (Subramariyan and Prime, 2002).

Lignin is an aromatic polymer which is associated with the polysaccharide in the secondary plant's cell wall. Compared to cellulose and hemicellulose, the fission of lignin by fungi and bacteria is very slow (Aderemi *et al.*, 2008).

Organosolv pretreatment is a separation process of fiber using organic solvent with or without acid or base catalyst to hydrolyze lignin in biomass (Amiri and Keihosro, 2014). This pretreatment can increase the cellulose and it is effective to degrade hemicellulose or lignin and decrease the cyrtzalline. The greater the solvent concentration the greater the reduction of lignin and it will dissolves small part of hemicellulose and increase the cellulose. Organosolv pretreatment is carried out at 100-250 °C with acidic catalyst. With the addition of acidic catalyst, high temperature will decompose the xylose to be furfural, hydroxylemthyl furfural and polyphenol which will inhibit the pretreatment. Another disadvantage of this method is the requirement of high amount of solvent and high energy recovery consumption. Ethanol is used as the solvent because of its cheap price (Geng and Fengxue, 2012).

Compared to another chemical pretreatment, the advantages of Oganosolv pretreatment are a relatively pure lignin will be produced and less energy consumption. From environmental point of view, the separation of lignin will decrease waste water treatment problem. The increasing of cellulose from the removal of lignin will increase the maximum glucose concentration (Amiri and Keihosro, 2014).

Continue fermentation process with an integrated extraction process has been discussed in the previous study (Widjaja, *et al.*, 2014). This study is expected to improve the ethanol production technology by using cellulose and hemicellulose with enzymatic degradation to be glucose and xylose by cellulose and xylanase enzyme. Moreover, glucose and xylose will be fermented in the batch system to be ethanol by using *Saccharomyces cerevisiae*, *Zymomonas mobilis*, *Pichia stipitis*, mixture of *Saccharomyces cerevisiae* and *Pichia stipitis*, and mixture of mutated *Zymomonas mobilis A3* and *Pichia stipitis*.

METHODS

Coffee Pulp Pretreatment

Coffee pulp pretreatment separated to mechanical and chemical pretreatment. For mechanical pretreatment, the coffee pulp was dried under the sunlight for 12 hours then dried coffee pulp was milled and sifted with 100-120 mesh sieves. The coffee pulp hydrolyzed with 1:16 (w/w)

citric acid at 80 °C for 75 minutes then the solid and liquid were separated. The solid part was added with various ethanol concentration from 10%-50% (v/v) and stirred with 500 rpm stirrer for 2 hours at 50 °C then the liquid was separated from the solid by using paper sieve with the help of vacuum jet pump, the rinsed it with distilled water until neutral. The coffee pulp solid was put in the oven for 2 hours at 60 °C. The solid that have been in the oven was analyzed by Chesson method to get the cellulose, hemicellulose and lignin concentration.

Enzyme Preparation

Pure cellulose and xylene enzyme were used in this study. A gram of cellulose enzyme which has been known the enzyme activity was diluted in 100 mL 0.1 M citric buffer at pH 5.5. The mixture of crude cellulose and xylene enzyme was made by inoculate fungi (*T. reesei* and *A. niger*) in a fermentation media and incubated at 32 °C for 8 days. 100 mL pH 3 citric buffer solution which contains of 0.1% Tween-80 was added to fermentation media that was grown with fungi then homogenized the solution and then separating the filtrate from the mycelia and sediment media in it using filter paper, which the supernatant containing enzyme.

Coffee Pulp Hydrolysis

5 grams of 100-120 mesh of coffee pulp that was gone to pretreatment process was added with various variables of enzyme as many as 93 units each 5 grams of coffee pulp. The solution of enzyme and coffee pulp was added with 0.1 M pH 3 citric buffer solutions until 150 mL. Hydrolysis was done at 60 °C and was stirred until 30 hours of hydrolysis. DNS method and HPLC (High Performance Liquid Chromatography) method were used to measure the glucose concentration every 2 hours

Coffee Pulp Fermentation

The fermentation was done at 32 °C and used various kinds of microbes such as *Saccharomyces cerevisiae*, mutated *Zymomonas mobilis A3*, *Pichia stipitis*, mixture of *Saccharomyces cerevisiae* and *Pichia stipitis*, and mixture of mutated *Zymomonas mobilis A3* and *Pichia stipitis*.

The fermentation process was separated to two steps, make of the starter and fermentation process itself. In the make of starter, the hydrolysate was added with nutrition (0.3315 gram of yeast extract; 0.0663 gram of $MgSO_4 \cdot 7H_2O$; 0.3315 gram of $(NH_4)_2SO_4$; and 0.828 gram of KH_2PO_4) and then the solution was sterilized in an autoclave. The sterile solution was cooled in room temperature and inoculated with microbes in certain variables. The make of starter was done in a shaker incubator at 32 °C. In the fermentation process, the solution of starter was mixed with the solution that would be fermented. The mixture was incubated in a shaker incubator at 35 °C for 2 days and the speed of the shaker was 125 rpm. Gas chromatography was used to analyze



concentration of ethanol and DNS method was used to analyze concentration of reduction sugar.

RESULT AND DISCUSSIONS

Pretreatment

Pretreatment was needed to break the structure of lignocellulose in the coffee pulp which has firm structure. Lignin can reduce the enzyme activity to degrade the cellulose. Concentration of cellulose, hemicellulose and lignin were analyzed using Chesson method and shown in the Figure-1.

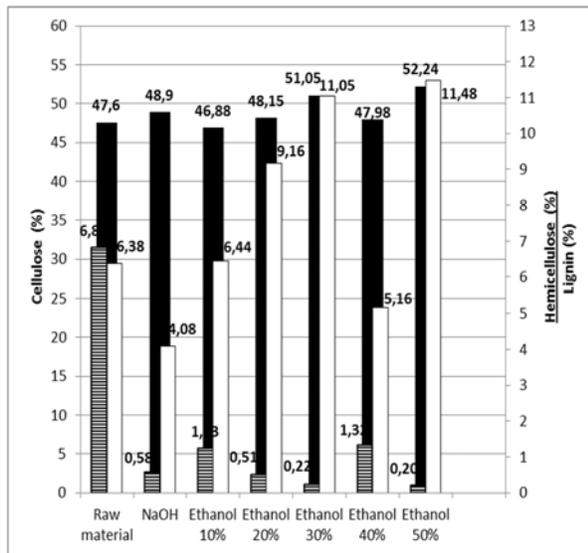


Figure-1. The Concentration of Cellulose, Hemicellulose, and Lignin after the Pretreatment. Symbols: (■) cellulose; (▨) lignin and (□) hemicellulose.

The concentration of lignin was smaller after the pretreatment than before the pretreatment. This case was shown that some of the lignin was diluted at the pretreatment process. This result agreement with the aim of the pretreatment which is to reduce the lignin to make it easier for enzyme to hydrolyze the cellulose and hemicellulose. Besides, based on the literature, Organosolv method can dilute most of the hemicellulose (Mesa *et al*, 2010). The increasing of the temperature

affected the reduction of lignin and xylene (Amiri and Keihosro, 2014). In our study, hydrolysis temperature was 80°C which is lower than that of Amiri *et al*. study. It means that the degradation of hemicellulose in our study does not occur.

Hydrolysis Result

Hydrolysis was done by using the mixture of pure cellulose and xylene enzyme. The relation of time (hour) and concentration of sugar reduction (g/L) can be seen at Figure-1. Then, for the yield of coffee pulp after done pretreatment by organosolv at each variables and NaOH can be seen at Table-1.

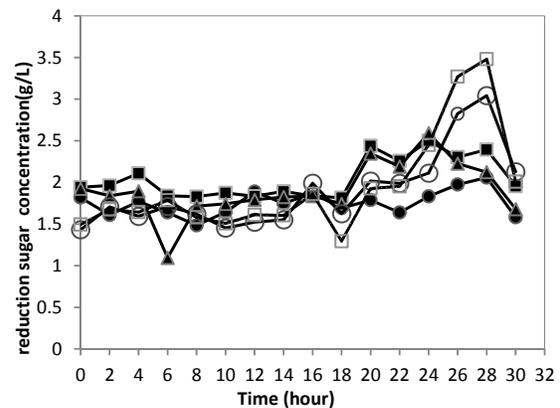


Figure-2. Concentration of Reduction Sugar from Hydrolysis by Using Mixture of Pure Cellulose and Xylene Enzyme Symbol: (●) ethanol 10%, (■) ethanol 20%, (▲) ethanol 30%, (○) ethanol 40%, and (□) ethanol 50%.

Figure-2 was shown that the reduction sugar concentration tend to increase during the hydrolysis. In Organosolv pretreatment using 50% of ethanol was tend to have bigger increasing to produce the reduction sugar. This means that the enzyme can hydrolyze the coffee pulp. The reduction sugar concentration at 28 hours was 3.48 gram/L and at 30 hours the reduction sugar concentration was decreased to 2.01 gram/L.

**Table-1.** Yield of coffee pulp hydrolysis after pretreatment.

Pretreatment Method	Lignin (%)	Cellulose (%)	Hemicellulose (%)	Result of reducing sugar (g/L)	Yield (g/g reducing sugar/Cellulose and hemicellulose)
Raw material	6.81	47.60	6.38	0.408	0.023
NaOH	0.58	48.90	4.08	2.147	0.122
etanol 10%	1.23	46.88	6.44	2.058	0.116
etanol 20%	0.51	48.15	9.16	2.394	0.125
etanol 30%	0.22	51.05	11.05	2.124	0.103
etanol 40%	1.32	47.98	5.16	3.042	0.172
etanol 50%	0.20	52.24	11.48	3.480	0.164

The yield that generated was relatively small as can be seen on Table-1. The biggest yield is 0, 17. It caused by inhibitor that reduce the hydrolysis process. In this study, a mixture of cellulose and xylene enzyme has been used. It made the hydrolysis process produces higher reduction sugar concentration. In the Table-1, it can be seen that for ethanol 50% the result of sugar reduction is the biggest one, 3.48 g/L. However, its yield is not the biggest ones. This result is caused by the calculation of yield is by dividing reducing sugar with the total of cellulose and hemicellulose and the total amount of it when the raw material is ethanol 50% is the biggest one. That is why ethanol 50% resulting not the biggest yield

eventhough it has the biggest result of reducing sugar. From Table-1 also we know that pretreatment by organosolv at concentration etanol 20% gave better result at total sugar reduce rather than pretreatment by NaOH at pH 3.

Fermentation Result

Fermentation was done for 48 hours with certain microbe variables: *Saccharomyces cerevisiae*, mutated *Zymomonas mobilis* A3, *Pichia stipitis*, mixture of *Saccharomyces cerevisiae* and *Pichia stipitis*, and mixture of mutated *Zymomonas mobilis* A3 and *Pichia stipitis*.

Table-2. Ethanol Concentration for Each Microbe Variable.

Strain	Ethanol (% v/v)	Weight of Ethanol (g)	Weight of Reducing Sugar (g)	Yield (g/g ethanol/glucose and xylose)
S. cerevisiae	0.029	0.022	0.104	0.065
Z. mobilis	0.028	0.021		0.061
(SC + PS)*	0.018	0.014	0.106	0.039
(ZM + PS)**	0.013	0.009		0.029
P. stipitis	0.013	0.009		0.028

*(SC + PS) : mixture of *S. cerevisiae* and *P. stipitis*

** (ZM + PS) : mixture of *Z. mobilis* A3 and *P. stipitis*

Table-2 was shown the result of ethanol concentration by gas chromatography analysis. The ethanol concentration for the usage of mutated *Zymomonas mobilis* A3, *Saccharomyces cerevisiae* and *Pichia stipitis* were 0.061 gram ethanol/gram glucose; 0.065 gram ethanol/gram glucose and 0.028 gram ethanol/gram glucose respectively. From the data, the highest ethanol concentration was produced by using

Saccharomyces cerevisiae. This microbe was vulnerable to high sugar concentration (>5g/L) (Ndaba and Chiyanzu, 2014). In this study, the reduction sugar that produces was 3.48 gram/L.

Saccharomyces cerevisiae could decompose glucose to be ethanol. This microbe was difficult to disentangle xylose to ethanol because of less production of xylose reduction enzyme. The change of xylose to xylitol could reduce the fermentation. The small amount of yield could be caused by some kind of sugar that could not be fermented by *Pichia stipitis*.



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CONCLUSIONS

From this study can be concluded that:

1. The best result of pretreatment was the one which produce the highest cellulose and hemicellulose has no effect relatively.
2. The best pretreatment process was shown in Organosolv pretreatment using 50% (v/v) ethanol with the result 0.20% (w/w) of lignin; 52.24% (w/w) of cellulose and 11.48% (w/w) of hemicellulose.
3. High reduction sugar from enzymatic hydrolysis correlated with high concentration of cellulose and hemicellulose.
4. Hydrolysis using 50% ethanol with the mixture of pure enzyme gave the best result as productive as 3.48 reduction sugar/gram cellulose + hemicelluloses.
5. Fermentation using *Saccharomyces cerevisiae* produce the highest ethanol yield as high as 0.065 gram ethanol/ gram glucose.

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