Utilization of coffee pulp cellulose for bioetanol production through simultaneous saccarification and fermentation (SSF) with cellulose enzymes

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Abstract. The bioetanol development from biomass bases on lignocellulose like pulp of coffee is one of alternative energy which has potential to be applied in Indonesia. Beside of raw material sources that are so many in our country, the process is also environmentally friendly. Conversion of coffee pulp becomes ethanol using Simultaneous Sacharification and Fermentation (SSF) technology by cellulase had been done on this research. Sacharification process or hydrolysis process, cellulase enzyme will break cellulose polymer becomes glucose. Then, glucose through fermentation is changed to ethanol by using yeast *Saccharomyces cerevisiae*. This study using Completely Randomized Design (CRD) with factorial pattern consisting of two factors. Factor I is the optimum pH (P) consists of three standards are: P1 = 4, P2 = 4.5, P3 = 5. Factor II is the incubation time (fermentation) (T) consisting of four standard that is T1 = 24 hours, T2 = 48 hours, T3 = 72 hours, and T4 = 96 hours. Pretreatment to break the lignin is done by soaking in 2% H_2SO_4 for 20 hours in the erlenmeyer. The analysis performed consists of the initial analysis and final analysis. Initial analysis consisted of analysis of water content, pH analysis, levels of cellulose and glucose levels, against the skin of the coffee pulp before the simultaneous saccharification and fermentation (SSF), whereas the final analysis included analysis of ethanol content, the analysis of cellulose and glucose from a solution of ethanol produced. The optimum pH treatment (P), incubation time (fermentation) (T) and interaction optimum pH and incubation time (fermentation) (PT) has very significant ($P \leq 0.01$), on levels of ethanol produced. The highest levels of ethanol obtained by fermentation of 96 hours and the optimum pH 4.5 and 5. Ethanol content is obtained that is equal to 6% and 6.07%.
Keywords : Bioethanol, coffee pulp, cellulase, and *Saccharomyces cerevisiae*

Introduction

Dependence on fuel derived from petroleum can be harmful because its potential to expire also causes air pollution is high. Therefore, it is necessary to find alternative fuels that are renewable, such as bioethanol as a biofuel. Bioethanol can be made from biomass-based starch, sugar or lignocellulose-based. However, starch and sugar-based biomass is generally used as food so that their utilization as raw material for bio-ethanol can disrupt the food supply. Therefore, based on the utilization of lignocellulosic biomass should be developed.

Advances in industrial biotechnology offer potential opportunities for economic utilization of agro-industrial residues such as coffee pulp. Coffee pulp waste including biomass containing lignocellulose is possible to be utilized as an alternative energy source bioethanol.Hydrolysis process can be done by using an enzyme that is often referred to enzymatic hydrolysis using cellulase enzyme type or other types. Advantages of hydrolysis by enzymes can reduce the use of acid so as to reduce the negative effects on the environment. Then after the hydrolysis process conducted fermentation using yeasts such as *S. cerevisiae* to convert into ethanol. Hydrolysis and fermentation process will be very efficient and effective if carried out in a sustainable manner without going through a long period, it is often known as Simultaneous Sacharificatian and Fermentation (SSF).

In the SSF process, cellulose hydrolysis and fermentation of sugars is not done separately or sequentially, but simultaneously. Microbes are used in the SSF process is usually a fungal cellulase-producing enzymes, such as *T. reesei, T. viride,* and the yeast *S. cerevisiae*. SSF optimal temperature process is 38° C (Sun and Cheng, 2002).

Materials and Methods

The material used in this study is Arabica coffee pulp waste obtained from Takengon-Central Aceh. *Tricoderma ressei* cellulose enzyme (cellulase) from Sigma Aldrigh imported from the United States and fermipan. The tools used in this study is an analytical balance, oven, grinder, sieve size of 60 mesh, hot plate, durant vials, petri dishes, erlenmeyer 100 ml and 250 ml, 50 ml beakers, thermometers 100°C, beaker 100 ml and 1000 ml, burette 50 ml, aluminum foil, filter paper, measuring cups, Pasteur pipette, test tube. Laminar flow cabinet,

autoclave, G-WON moisture, high temperature incubator, UV-Vis Spectrophotometer, pH meter.

This research used Completely Randomized Design (CRD) with factorial pattern consisting of 2 factors. The first factor is the pH (P) consists of three levels, namely: P1 = 4, P2 = 4.5, P3 = 5. Factor II is the incubation time (fermentation) (T), which consists of four standard, namely T1 = 24 hours, T2 = 48 hours = 72 hours of T3, and T4 = 96 hours. Combination treatment is 3x4 = 12 treatments with 2 replications, thus obtained 24 experimental units.

Research Procedures

The research was conducted based on Samsuri *et al*. (2007), Afrida, (2010) and Gozan *et al*. (2007) as follows:

Preparation of Raw Materials

Coffee pulp waste from wet processing of coffee is then dried in the sun for 1-2 days. Performed using advanced drying oven with a temperature 65°C for 3 hours. Coffee pulp that have been dried, then ground using a grinder. Rind coffee grinding results then sieved using 60 mesh sieve.

Pretreatment

Coffee pulp powder already ground 60 mesh size is taken as 1 gram. Analyzed for levels of cellulose before pretreatment. Coffee pulp powder soaked with 2% H_2SO_4 (15 ml) for 20 hours in the erlenmeyer. In an autoclave at a temperature of 129°C for 10 minutes. The result is a mix of solids and liquid filtrate analyzed for levels of cellulose. Filtrate was filtered using filter paper and solids screening results are stored at a temperature of 4°C.

Breeding Saccharomyces cerevisiae

Potato Dextrose Agar (PDA) 3.9 grams diluted using distilled water of 100 ml. Put into the durant bottle, then in an autoclave at a temperature of 121°C for 15 minutes, PDA is then poured into a cup and cooled to harden. Fermipan weighed as much as 1 gram and then diluted with 10 ml distilled water, then take 1 ml of fluid fermipan first dilution and further diluted with 10 ml distilled water. Taken 1 ml liquid fermipan result from second dilution and poured into petri dishes containing PDA and leveled using the scapula, and then incubated for 2-3 days.

Preparation of yeast inoculum

Diluted 10 grams of glucose, 0.1 g KH_2PO_4, 0.1 g $MgSO_4.7H_20$ and (NH_4) $2SO_4$ as much as 0.1 grams in 1 liter of distilled water. In an autoclave at a temperature of 121°C for 20 minutes, then cooled. Distreaking *S. cerevisiae* culture on PDA using an ose, and then diluted with distilled water as much as 10 ml. Then pipetted by 2 ml of inoculums dilution into a solution, then stirred until the solution became homogeneous. Incubated for 24 hours.

Making nutrition to the media

Diluted 1 g (NH_4) $2PO_4$, and 0.05 g $MgSO_4.7H_2O$ in 1 liter of distilled water. In an autoclave at a temperature of 121°C for 20 minutes, then cooled. Distreaking *S. cerevisiae* culture on PDA using an ose, and then diluted with distilled water as much as 10 ml. Then pipetted by 2 ml of dilution into a solution of nutrient medium, then stir until the solution became homogeneous.

Simultaneous saccharification and fermentation process (SFS)

Sample results of the pretreatment used subsequently for simultaneous saccharification and fermentation process. Sample pretreatment results in adding 15 ml of distilled water and analyzed pH (4, 4.5, and 5), if the solution is too acidic, then add 10% KOH to achieve the desired pH. Added yeast inoculums and nutrients to the media, each of 2.5 ml. Further added cellulase enzyme (powder) of 0.032 grams. The next sample mixed with a homogenizer at 100 rpm for 30 min. Samples were inserted into the simple fermenter (anaerobic fermentation). Incubated with a temperature of 38°C at high temperature incubator for 24 hours, 48 hours, 72 hours, and 96 hours. Then do the analysis of cellulose, pH, ethanol levels every 24 hours, 48 hours, 72 hours, and 96 hours. Also analyzed glucose every 24 hours, 48 hours, 72 hours, and 96 hours.

Analysis of the product

Analysis conducted consisted of preliminary analysis and the final analysis. preliminary analysis consisted of analysis of water content, pH analysis, cellulose and glucose levels, the

coffee pulp prior to SFS, while the final analysis includes analysis of ethanol content, pH of the final analysis, the analysis of cellulose and glucose levels of a solution of ethanol produced.

Results and Discussion

Analysis of Raw Materials

The water content

From the results of measurements of initial moisture content of coffee pulp powder is obtained 12.10%. Therefore, the samples used have a low water content or material classified as dry. This is consistent with the statement of Braham and Bressani (1979) which states dried coffee pulp moisture content of 12.60% was classified as dry.

Cellulose Levels

Levels of cellulose coffee pulp waste are 12.89% and cellulose content after pretreatment increased to 13.80%. According to Braham and Bressani (1979), the composition of crude fiber in the dried coffee pulp was 21.5%. Crude fiber composed of cellulose, gum, hemicellulose, pectin and lignin (Muchtadi, 1992). The increase in cellulose content after pretreatment is thought to occur because of hemicellulose and lignin soluble in water (solvent pretreatment) when filtering to separate the filtrate and solids (pulp), resulting pulp / cellulose is not dissolved purer / cleaner than the other components (hemicellulose and lignin).

Pretreatment resulted in amorphous substances that are lost, and the rising degree of crystalinity. This is a result of the dissolution of lignin and hemicellulose that are amorphous component. Reducing lignin content resulted in an increase in enzyme effectiveness by eliminating the non-productive adsorption and increase access to cellulose (Pahlevi, 2010). Cellulose in the residue levels did not decrease. This is due to the crystal structure of cellulose is hydrolyzed by making it difficult for water (Pahlevi, 2010). Pretreatment process aimed at breaking the bonds between lignin and hemicellulose that eases access of cellulase enzymes to hydrolyze cellulose into sugar monomers. The presence of lignin and hemicellulose will reduce the rate of hydrolysis due to the adsorption of cellulase to lignin (Polanen, 2004).

Glucose Levels

Glucose level of coffee pulp which has been analyzed by the method of Nelson Somogyi (Sudarmadji 1989) is 12.11% and glucose levels after pretreatment decreased to 3.07%. Glucose levels were obtained in accordance with Braham and Bressani (1979), which states that the levels of glucose in the fruit skin material (pulp) is 12.4% coffee. After pretreatment, the glucose level decreased presumably because glucose is decomposed by dilute acid (H_2SO_4) at high temperatures into other compounds. According Mussatto and Roberto (2004), higher temperatures will facilitate the decomposition of simple sugars and lignin compounds.

Analysis of Ethanol Levels

Catabolism of glucose to ethanol by yeast, is an attempt to obtain the necessary energy in growth. Adams (1985), states that the amount of ethanol produced depends on the amount of glucose available in the substrate. Produced ethanol levels ranged from 2.12 to 6.07%. The results of analysis of variance for the data analysis showed that treatment of alcohol pH (P), incubation time (T) and the interaction of pH and incubation time (PT) was highly significant ($P \leq 0.01$), the levels of bioethanol produced.

From Figure 1 it can be seen that the high levels of ethanol obtained at pH 5 treatment with incubation time of 96 hours were not significantly different from the treatment of pH 4.5 and incubation time of 96 hours, but significantly different from all other treatments. This is presumably because the PH 4.5-5 is optimum pH for growth of yeasts *S. cereviseae*. Optimal growth temperature for *S. cereviseae* is 28-36°C and pH optimum for the growth of yeast cells from 4.5 to 5.5 (Moat and Foster, 1988). According to Frazier and Westhoff (1978), which states that the yeast can grow well at pH 5, therefore the concentration of ethanol produced is higher. According to Mustika *et al*. (2008), incubation time gives a different effect on the levels of ethanol produced by the yeast *S. cereviciae*. From Figure 1 also can be seen that the ethanol content increased with increasing incubation time. This is in line with research Samsuri *et al*. (2007), which states that the highest

concentration of ethanol produced at pH 5 with an incubation time of 96 hours is equal to 5.62 g / L. This suggests that pH 5 is more optimum conditions than pH 4 and 4.5. According Hikmiyati and Yanie (2007), the longer the fermentation, the ethanol content produced increasingly large and tend to be constant after a fermentation time of 96 hours.

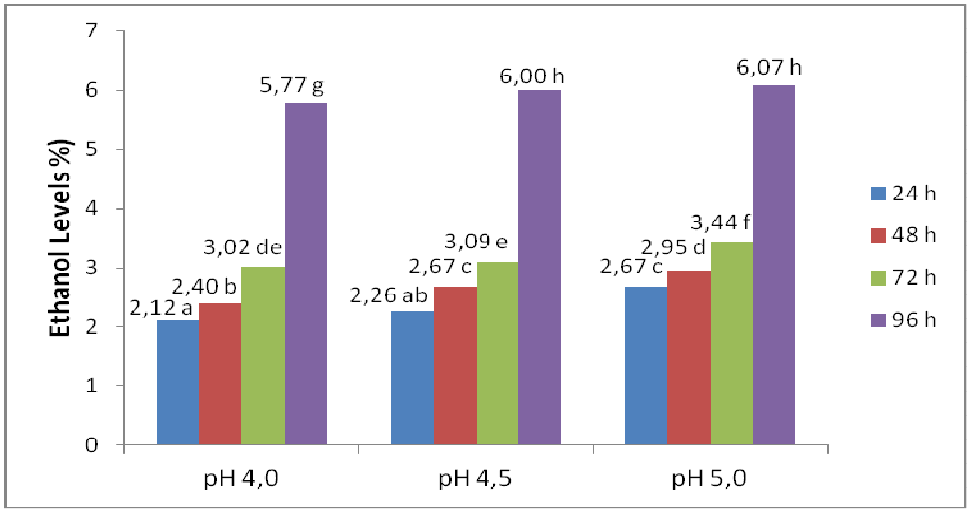

Figure. 1 The interaction of pH and incubation time (PT) on levels of bioethanol

Analysis of Glucose Levels

Ethanol produced glucose levels ranged from 1.03 to 2.08%. The results of analysis of variance for glucose analysis data showed that the treatment pH (P), incubation time (T) and the interaction of pH and incubation time (PT) was highly significant (P ≤ 0.01), the glucose levels of bioethanol produced.

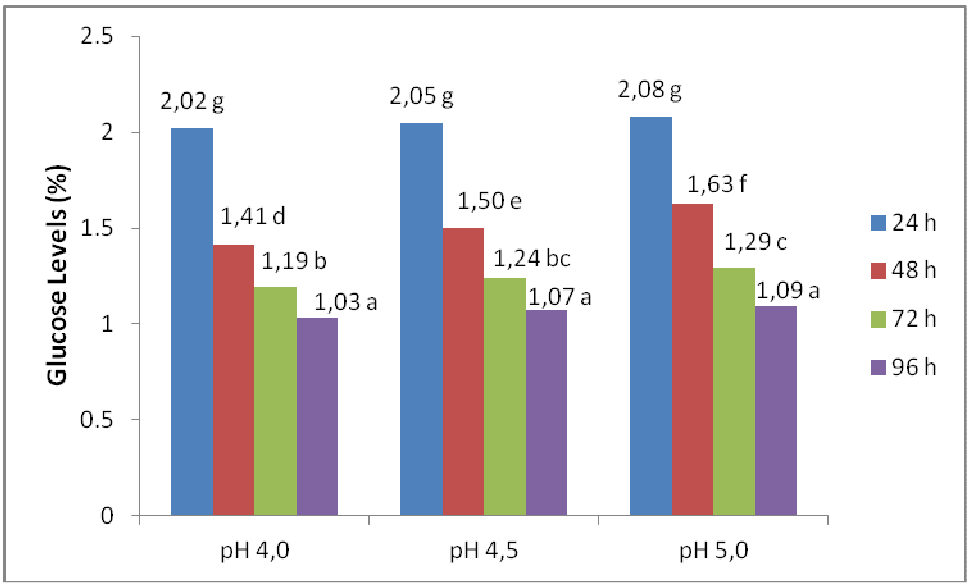

Figure. 2 The effect of the interaction of pH and incubation time (PT) of the glucose levels of bioethanol

From Figure 2 it can be seen that the longer the incubation time, glucose levels decreased. It is thought to occur during the process of fermentation of glucose as a substrate reduction. Glucose is used as food for microbial growth and the formation of bio-ethanol as fermentation products. The greater the amount of ethanol that forms the greater the reduction in the amount of glucose. From Figure 2 it can be seen that the high glucose levels

obtained at pH 5 treatments with a 24-hour incubation period at 2.08% which was not significantly different by treatment at pH 4 and pH 4.5 with an incubation time of 24 hours, but significantly different from all other treatments. Glucose levels by 2.08% after incubation for 24 hours and continues to decrease with longer incubation time. This shows *S. cerevisiae* yeast works well convert glucose into ethanol.

According Hikmiyati and Yanie (2007), by the levels of ethanol and reducing sugar. Explained that at the time 96 hours *S. cerevisiae* has the greatest activity or in the logarithmic phase. Logarithmic phase is the phase for the formation of the largest ethanol product. Then after 96 hours of microbes will have stationary phase, where the number of microbes that grow as much as microbes die so that there is no increase in the number of microbes that will transform the substrate into ethanol so ethanol is formed relatively constant. Once the microbes undergo stationary phase it will continue to be a death phase. This is consistent with microbial growth curve. At 24; 48 and 72 hours of ethanol produced is not optimal because the yeast *S. cerevisiae* is the stage lag phase and exponential phase. Phase lag phase is the stage adaptation of microbes to the environment and the exponential phase is the stage where microbial growth began. Thus, the activity for the formation of ethanol products is not optimal.

Cellulose Content Analysis

Levels of residual cellulose bioethanol produced ranged from 0.78 to 7.05%. The results of analysis of variance for the data analysis showed that the levels of cellulose pH treatment (P) and incubation time (T) and the interaction of pH and incubation time (PT) as highly significant ($P \leq 0.01$) on levels of residual cellulose bioethanol produced. From Figure 3 it can be seen that the high levels of cellulose were obtained in the treatment of pH 4, 4.5 and 5 with an incubation time of 24 hours, which is significantly different from all other treatments.

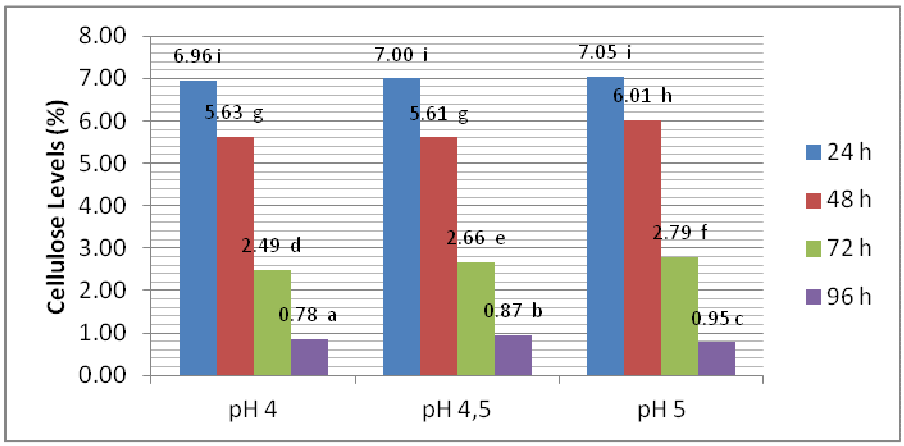

Figure 3. Interaction effect of pH and incubation time (PT) on levels of cellulose bioethanol.

According Frazier and Westhoff (1978), fermentation conditions were very influential one is pH. Necessary for the growth of mold different pH with the production of enzymes. In general, to grow mold (*Tricoderma sp*) requires a higher pH is above 4.0 and for the production of enzymes needed to lower the pH below pH 4.0. From Figure 3 can also be seen that the cellulose levels decreases with increasing time of incubation. This is presumably because the activity of fungi that degrade cellulosic material to produce glucose. To meet the needs of these carbon sources molds will synthesize the enzymes that degrade carbohydrates (lignocelluloses) contained in the substrate. According to Moore (2003) molds utilize carbohydrates contained in food as an energy source. Fungi can degrade cellulose in the form of carbohydrates. Cellulose is degraded by the enzyme cellulase producing glucose.

Conclusions

Based on the research results of bioethanol fermentation to the treatment of solution pH on fermentation, it can be concluded:

1. From the analysis of coffee pulp powder had cellulose levels at 12.89%, which can be converted into bioethanol.
2. Treatment pH (P), incubation time (T) and the interaction of pH and incubation time (PT) was highly significant ($P \leq 0.01$), on the levels of ethanol and glucose produced.
3. High levels of ethanol obtained at fermentation time of 96 hours and pH 4.5 and 5. Ethanol levels obtained in the amount of 6% and 6.07%.
4. Treatment pH (P) and incubation time (T) as well as the interaction of pH and incubation time (PT) was highly significant ($P \leq 0.01$), to the levels of cellulosic bioethanol produced.
5. Cellulose and glucose levels continued to decrease with longer incubation time because of an overhaul of chemical compounds made by microbes.

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