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Production of Biobuthanol from Various Lignocellulose Waste with *Clostridium Acetobutylicum* Bacteria using ABE (Acetone-Buthanol-Ethanol) Fermentation

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Abstract. Biobuthanol is produced by fermentation of lignocellulose waste from Clostridium acetobutylicum by the ABE (Acetone-Butanol-Ethanol) fermentation method. In this research on biobuthanol production, variations in the type and composition of lignocelluloses raw materials (rice husk and corn husk) were carried out. The steps of this research include delignification, hydrolysis, fermentation, and yield analysis. The results found in this study are that in the comparison of types and composition mixture of raw materials, the highest production of biobuthanol is achieved in samples with a variation of the composition of the raw material mixture of 25% rice husk and 75% corn husk, this is due to the discovery of the highest levels of cellulose with the least lignin in the sample, so that the hydrolysis process can take place quickly and produce more glucose as the main substrate in biobuthanol fermentation.

INTRODUCTION

Indonesia still faces various problems in achieving its energy sector development targets to date. This condition requires the discovery of renewable and environmentally friendly energy sources [1]. One of the liquid fuels that can be formed from material that can be found in the surrounding environment is biobutanol. Biobutanol is produced by fermentation of lignocellulosic waste from Clostridium acetobutylicum by the fermentation method of ABE (Acetone-Butanol-Ethanol) [2].

Butanol (C_4H_9OH) is an intermediate compound from the production of butyl acrylate, coatings and sealants such as glycol or butyl acetate ethers. Butanol is often used as a solvent in the production of antibiotics, hormones, and vitamins. Butanol production is usually done by the chemical process Oxo. Where propylene is reacted with carbon monoxide and hydrogen in the presence of a catalyst, followed by hydrolysis of the aldehyde formed into a mixture of iso and n-butanol. Biobutanol can also be produced from waste material through the fermentation process. This kind of fermentation process was introduced in the 1910s and penetrated on an industrial scale until it was rivaled by other chemical processes in the 1960s. At the end of the decade, interest in this process again increased due to knowledge of the potential of biobutanol as vehicle fuel [3].

Biobutanol can be produced from a variety of raw materials. When selecting raw materials for industrial-scale processes, stock availability, reasonable shipping costs, and the convenience of feed conversion should be considered. Molasses, potatoes, corn, starch, and sweet potatoes are the basic ingredients in industrial scale biobutanol production. In addition, agricultural waste containing lignocellulose also has the potential to become the main raw material for biobutanol production [3].

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Bioutanol can be obtained using several chemical technologies. It is also possible to produce butanol through the fermentation process by using the Clostridium bacteria. This process takes place in anaerobic conditions. The resulting butanol is referred to as bio-butanol. The bacteria most often used for fermentation is Clostridium acetobutylicum. This fermentation method is called ABE (acetone-butanol-ethanol) [4].

ABE fermentation is divided into acidogenesis, and solventogenesis phases as presented in the Figure 1. In the acidogenesis phase there is an exponential growth of cells, a decrease in pH, and accumulation of acetate and butyrate. The solventogenesis phase starts from the formation of endospores and the cell enters the stationary phase. The products of the acidogenesis phase are acetate and butyrate [5].

The accumulation of organic acids causes a decrease in pH during fermentation. This leads to a change in the phase of acidogenesis to solventogenesis. Acetate and butyrate are re-assimilated and joined to the solvent formation. Under the catalysis of the enzyme CoA transferase, acetate and butyrate are converted to acetyl-CoA and butyryl-CoA. This alcohol formation has the same key enzyme, namely aldehyde/alcohol dehydrogenase bound to NAD (P) H. In addition, butanol is produced by the enzyme butanol dehydrogenase [6].

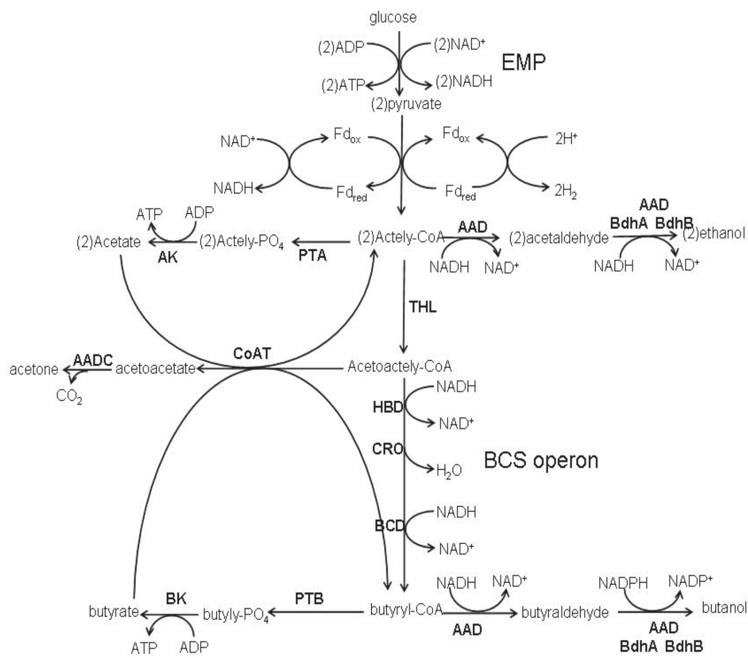

Figure 1. Scheme of metabolic reaction of biobutanol fermentation from glucose

MATERIALS AND METHODS

Rice Husk

Rice husk is one of agricultural waste. Rice husk waste is abundant in rural areasAbout 20% by weight of rice is rice husk. The main composition of rice husk consists of cellulose 33-34% by weight, lignin 19-47% by weight, if burned with oxygen will produce 13-29% by weight of husk ash, rice husk containing silica is quite high ie 87 - 97% by weight of rice husk ash [7].

Rice husk is composed of various metal and non-metal elements. The content of carbon, oxygen, and silicon in rice husk is more dominant than the other elements, as shown in Table 1.

Table 1. Rice husk nutrition content

Component	Value (%Weight)
Carbon	41.44
Hydrogen	4.94
Oxygen	37.32
Nitrogen	0.57
Sillicon	14.66
Potassium	0.59
Sodium	0.035
Phospor	0.3
Sulfur	0.07
Calcium	0.06
Iron	0.006
Magnesium	0.003

As one of the lignocellulosic biomass, rice husk contains lignin, cellulose, and hemicellulose. Cellulose and hemicellulose compounds are polysaccharides that can be broken down into monosaccharides to be further utilized for the production of useful compounds, one of which is ethanol. Ethanol production from a renewable natural resource (hereinafter referred to as bioethanol) is in line with the government program through Presidential Instruction No. 1 of 2006 dated January 25, 2006 concerning the Provision and Utilization of Biofuel as an alternative fuel. In addition, the use of rice husks for bioethanol production contributes to the handling of agricultural waste [8].

Corn Husk

Biomass from plants consists of lignocellulose compounds with its components lignin, cellulose, and hemicellulose. The availability of biomass from plants is quite abundant, so that the potential as a source of energy through the conversion process is quite large. The conversion process can be carried out biologically, chemically, or physically. One of the processes of converting biomass into a fuel source that is quite widely studied, is the process of converting lignocellulose into bioethanol. Bioethanol is used as a substitute material on premium or gasoline which is used as fuel for motor vehicles.

One of the biomass that can be processed into glucose is corn. Based on Central Sulawesi Statistics Agency (BPS) data, the amount of Central Sulawesi corn production in 2013 was 139,265 tons of dry shelled. Based on these data, it was concluded that corn husk waste was produced in large quantities.

Corn husk is part of the plant that protects corn kernels. Corn husk waste has been used as animal feed by the community, but its utilization has not been maximized. Both of these wastes still have low economic value and will cause environmental pollution when burned. Corn husk has a high cellulose fiber content. The chemical composition of corn husk includes 15% lignin; 5.09% ash; 4.57% alcohol-cyclohexane; and 44.08% cellulose. The results of the chemical analysis of corncobs contain 30.91% hemicellulose; alpha cellulose 26.81%; lignin 15.52%; carbon 39.80%; nitrogen 2.12%; and water content of 8.38% [9].

ABE Fermentation Method

In this study, biobutanol will be made with variations of lignocellulosic waste raw materials: rice husk and corn husk. The fermentation process changes raw material into biobutanol using the bacterium Clostridium acetobutylicum. This research was conducted at the Laboratory of Bioprocess Chemical Engineering, Faculty of Engineering, Diponegoro University. The main stages of research are:

1. Pre-treatment stage
   This stage consisting of raw materials extraction, delignification of raw materials, manufacturing the work of Clostridium acetobutylicum bacteria, making starters, and making cellulase enzymes
2. Treatment stage

At the treatment stage, the hydrolysis of raw materials resulting from delignification results is carried out for 3 days. Continued are the division of variables and fermentation for 16 days in the incubator.

3. Data Analysis

The analysis conducted is glucose analysis. Glucose analysis was carried out before, during and at the end of fermentation using the Fehling titration method. The glucose data found is then processed using a conversion table to produce biobutanol yield. The table is taken from: "Bioconversion of Butyric Acid to Butanol by Clostridium saccharoperbutylacetonicum N1-4 (ATCC 13564) in a Limited Nutrient Medium" journal by Al-Shorgani et al. [10].

As for formulation of the variabels used in this experiment, are listed in Table 2. below.

Table 2. Variable formulation for ABE fermentantion process

Sample	Corn husk extract (%V)	Rice husk extract (%V)	Bacterial Starter (%V)	pH
1	-	100	10	5
2	25	75	10	5
3	50	50	10	5
4	75	25	10	5
5	100	-	10	5

RESULTS AND DISCUSSION

The producing of biobutanol from rice husk and corn husk begins with cellulose hydrolysis to produce glucose. The glucose produced is then fermented to produce biobutanol. The fermentation process is carried out for 16 days with the testing time is every 4 days. The glucose level of the sample was tested using fehling titration. The data obtained is processed in order to obtain glucose samples. The rate of reduction of the glucose level in the sample was converted to biobutanol levels produced in each fermentation time span. Conversion value are taken from table listed in the references.

Biobutanol can be produced biochemically from a variety of raw materials. When selecting raw materials for industrial-scale processes, stock availability, reasonable shipping costs, and the convenience of feed conversion should be considered. Lignocellulosic waste is a type of raw material that can be found anywhere. One of them comes from the pulp of agricultural crops. Corn husk and rice husk are examples of agricultural plant pulp. Both of them contain lignin and cellulose which are quite large, so they can be used as raw material for biobutanol fermentation. It is possible to mix the two ingredients in a biobutanol fermentation medium, resulting in greater biobutanol conversion. However, variations in the composition of the mixture of the two, can also produce different levels of biobutanol produced. The phenomenon of the influence of a mixture of different raw materials, is presented in Table 3. and Figure 2.

Table 3. Biobutanol contents of all samples during fermentation

Variable	Biobutanol content (g/L)			
	Day 4	Day 8	Day 12	Day 16
1	6.786	6.786	7.691	8.55
2	6.786	6.786	6.786	9.955
3	6.786	6.786	8.142	9.573
4	7.238	7.464	7.464	11.917
5	3.167	5.427	5.879	7.883

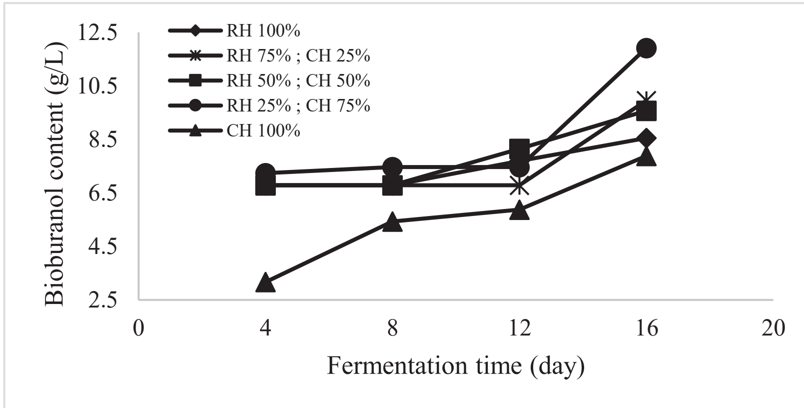

Figure 2. Comparison graph of the mixture composition of different raw materials to the biobutanol content of the sample

Figure 2 presents a graph of the phenomenon of changing levels of biobutanol variables with raw materials 100% rice husk, a mixture of 75% rice husk and 25% corn husk, a mixture of 50% rice husk and 50% corn husk, a mixture of 25% rice husk and 75% husk corn, and 100% corn husk over fermentation time. All five variables have increased levels of biobutanol each time the test. Overall variables with variations in the mixture of raw materials 25% rice husk and 75% corn husk, resulting in higher levels of biobutanol. Followed by variables with variations in the mixture of raw materials 75% rice husk and 25% corn husk, 50% rice husk and 25% corn husk, then rice husk and corn husk individually.

Rice husk contains 42.22% cellulose; 18.47% hemicellulose and 19.4% lignin [8]. The content or composition of corn husk is hemicellulose 67%, cellulose 23%, and lignin 0.1% [11]. The content of lignocellulose in raw materials, can be hydrolyzed using the cellulase enzyme into glucose which will be fermented using Clostridium acetobutylicum bacteria, into biobutanol. So that the more cellulose content of a material, the more biobutanol levels produced will be.

From the results of the research, the levels of biobutanol in samples with a mixture of 25% raw material of rice husk and 75% of rice husk during fermentation were greater than samples with other variations of raw materials. This is due to the mixing of raw materials with the composition, making the levels of lignin in the sample less and less. So that with the same delignification process, lignin in the sample is more easily released until it runs out. The absence of lignin in the delignification results, will facilitate the cellulase enzyme to process cellulose into glucose, which will later be converted by the bacterium Clostridium acetobutylicum into biobutanol. This factor causes the butanol content in the sample variations in the composition of the mixture of raw materials 25% rice husk and 75% corn husk is greater than the whole.

When compared with the sample variation of the composition of the mixture of raw materials 75% rice husk and 25% corn husk, the total cellulose content is more. However, this comparison also results in total lignin levels which are also more due to greater rice husk lignin content than corn husk. In the same delignification process, the amount of lignin released was less than that of a variation of 25% rice husk and 75% of corn husk. This can inhibit the cellulase enzyme hydrolyze cellulose, so that glucose for biobutanol fermentation produced less.

This result has the same tendency with the results of Qin's research [12]. The tendency is the highest biobutanol yield found in samples with raw materials that have the highest cellulose and glucose content. The study used samples with variations in raw materials of starch, cassava, and pure glucose. Final biobutanol levels were found in all three samples of 5.23 g / L, 3.21 g / L, and 5.14 g / L. Where is known starch has the highest cellulose content compared to the other two samples, which is as much as 60 g / L. So that biobutanol products that are converted from glucose from starch cellulose hydrolysis are also numerous.

CONCLUSION

Biobutanol can be produced from lignocellulosic waste from rice husks and corn husk with the help of the bacterium Clostridium acetobutylicum. The process used is the ABE (Acetone-Butanol-Ethanol) fermentation method. In the variation of the type and composition of the mixture of raw materials, the most biobutanol production was achieved in the sample with a variation of the composition of the raw material mixture of 25% rice husk and 75% corn husk.

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