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RESEARCH ARTICLE

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Utilization sugarcane waste (*Saccarhum officinarum* L.) as a bioetanol basic material through a bioprocess engineering approach based microbes

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Abstract

Sugarcane (*Saccharum officinarum* L.) is a plant that is able to produce a lot of sugar content in its stem organs. The amount of sugar production from sugar cane, of course, produces and leaves bagasse waste. A number of studies have shown that bagasse waste still contains a lot of important materials, one of which contains lignocellulose substrates that have the potential to be converted into bioethanol raw materials. The purpose of this study is to determine, see and assess the production of bioethanol produced by utilizing sugarcane waste as raw material through a microbial-based bioprocess engineering approach. This research is an experimental type with the treatment of free cells and immobilized cells in bioethanol production by utilizing sugarcane waste as raw material with the stages of processing waste into flour, dried, hydrolyzed, and analyzed sugar content before ethanol production test. Based on the results of research on ethanol testing using free cells, the highest alcohol content was obtained at a concentration of 3% and 4%. For the test of ethanol content using immobilized cells, the highest ethanol content was obtained at a concentration of 4%.

Keywords: Sugarcane waste, bioetanol, bioprocess engineering

Introduction

Sugarcane (*S. officinale* L.) is a type of herbaceous plant with a growing period of approximately one year. This plant is indirectly often used as a basic ingredient in making sugar[1]. From the results of making this sugar, sometimes it leaves and produces waste from the processed sugar cane mill after being processed into sugar water. Some of the waste generated after the sugarcane milling process, the waste produced is three types of waste, namely, liquid waste, gas waste, and solid waste. Sugar cane

solid waste is often found to be reprocessed for use, usually this waste is produced from sugar cane bagasse which is formed naturally to form fibers from milling called *bagasse*[2].

Bagasse or known as bagasse is one of the solid waste products after milling sugarcane stalks (*S. officinarum* L.). This waste product is widely reused as the main ingredient of boiler fuel by the factory itself for combustion needs by the factory[3]. However, with the excessive amount of bagasse waste, it will certainly be neglected. In fact, some of the biomass material content such as lignocellulose, which consists of the remaining sugar components in bagasse, can be processed back into one of the raw materials for fermented sugar with special treatment. Lignocellulose material components have quite complex substrates and are quite potentially beneficial with the content of lignin, polysaccharides, extractive substances, and other organic compounds produced[4]. With this favorable potential, of course the compounds contained in sugarcane waste that still contain sugar; become one of the advantages utilized by immobilized cells for bioethanol production process[5].

Bioethanol (C_2H_5OH) is a biochemical liquid from the fermentation process of sugar using carbohydrate sources with the help of microorganisms. Ethanol is also known as one of the biofuels that is present as an alternative fuel that is more environmentally friendly and renewable[6]. The utilization of starch from cassava, *gembili*, arrowroot, sago, and corn into ethanol has been widely done. One of the starchy materials that can be utilized as an ingredient in ethanol production is molasses. Molasses has a high content of sugar compounds, ranging from 50-65%. This sugar compound is the basic component that can be converted by yeast (*Saccharomyce cerevisiae)* into ethanol. Molasses is an alternative substrate source that can be used because it can save production costs, besides the sugar compounds in molasses can also be directly converted into ethanol[7]. The effectiveness of the fermentation process is something that needs to be considered to obtain optimal ethanol levels. The level of ethanol is determined by the level or concentration of *S. cerevisiae*. Nasrun et al (2015), have tried to make ethanol from papaya skin using baker's yeast. According to the results of his research, the amount of yeast and the length of fermentation affect the level of ethanol produced[8].

The fermentation process using microorganisms can produce ethanol. *S. cerevisiae* is a commonly used microbe for various industries such as food, beverage, and biofuel industries[9]. This yeast in ethanol production is very commonly used because it has an ethanol fermentation pathway. *S. cerevisiae* can also be used by making it into immobilized cells or bound cells. Cell immobilization is a way to use starter cells repeatedly. *S. cerevisiae* in the fermentation of glucose to ethanol can be immobilized in a matrix to entrap the yeast cells before being used for ethanol production. One of the commonly used carriers for S. cerevisiae immobilization is calcium alginate[10–12]. The use of calcium alginate can help the cell immobilization process so that ethanol production can run well. The aim of the research is to produce bioethanol by utilizing bagasse (*S. officinarum* L.) as raw material through an immobilized cell-based bioprocess engineering approach.

Material and methods

Study design and tools

The tools used in the study were Erlenmeyer, measuring cup, goblet, scale/reader, hotplate, waterbath, spatula, pipette, distillator, alcohol meter, goose neck glass, hand-refractometer, pH meter,

incubator, shaker incubator. While the materials used are solid waste in the form of bagasse, distilled water, 0.5 M H_2SO_4, 0.2 M $CaCl_2$, alginate 2%, and instant yeast.

The method used in this research is experimental method with the treatment of free cells and immobilized *S. cerevisiae* cells in bioethanol production by utilizing sugarcane waste as raw material. Data collection was carried out after the sample was incubated for 24 hours. Measurement of yield parameters includes ethanol content, residual sugar and pH. Ethanol concentration is obtained by distilling a sample of 100 mL to obtain distillate as much as 70 mL. Then, distillate is analyzed for ethanol concentration using an alcohol meter, residual sugar using a hand-refractometer and pH using a pH meter.

Procedure

The research procedure consists of sample preparation, namely *bagasse* is pulverized into flour and dried in an oven for 1 hour at a temperature of 100–120 ºC. After that, hydrolyzed with 1 liter of 0.5M H_2SO_4 at 120 ºC for 30 minutes. After hydrolysis, the hydrolysis liquid was analyzed for sugar content using a hand-refractometer and continued with ethanol production tests.

Bioethanol production using cell-free

Prepare 5 Erlenmeyer and labelled according to the treatment, namely control (without yeast), yeast concentration of 0.5% (1 g yeast + 200 ml sugarcane waste hydrolysis), 1% yeast concentration (2 g yeast + 200 ml sugarcane waste hydrolysis), 1.5% yeast concentration (3 g yeast + 200 ml sugarcane waste hydrolysis) and 2% yeast concentration (4 g yeast + 200 ml sugarcane waste hydrolysis). The hydrolysis solution was then added with urea powder of 1 g each, and then closed using a swan neck lid. On the neck of the goose is given distilled water in order to minimize microorganisms or air so as not to enter the Erlenmeyer during fermentation. Incubate on an incubator shaker at 150 rpm for 24 hours at 37ºC. After incubation, the parameters of ethanol content, residual sugar and pH were measured[13].

Bioethanol production using immobilized cells

Yeast as much as 100 mL was mixed with 150 mL of 2% alginate. The mixture of *S. cerevisiae* and alginate was then dripped on 0.2 M $CaCl_2$ solution as much as 200 mL ($CaCl_2$ was made by 7.351 g of $CaCl_2$ and then added 200 ml of distilled water). This work was carried out on a hotplate stirrer while rotating to obtain alginate granules and then filtered to separate the granules with $CaCl_2$. Alginate granules containing *S. cerevisiae* cells were put into the sugarcane waste hydrolysis solution according to the treatment, namely control (without immobilized cells), 0.5% immobilized cell concentration (1 g cell + 200 ml bagasse hydrolysis), 1% immobilized cell concentration (2 gr cell + 200 g sugarcane waste), 1.5% immobilized cell concentration (3 g cell + 200 ml sugarcane waste hydrolysis) and 2% immobilized cell concentration (4 g cell + 200 gr sugarcane waste hydrolysis). Furthermore, the hydrolysis solution was added with 0.5% urea, 1 g. Then the sample was incubated for 24 hours on an incubator shaker at 150 rpm at 37ºC. After incubation, the results were checked including ethanol content, residual sugar and pH[14].

Result

Based on the results of the study in accordance with the output and indicators to be achieved in the study that the production of bioethanol with indicators in the form of volume of bioethanol produced per unit of time, indicators in the form of conversion efficiency, which measures how efficient

the process of converting sugarcane waste into bioethanol and achievement indicators in the form of ethanol content, pH, and water content in bioethanol look like in tables 2 and 3. However, before the bioethanol production process is carried out, first the hydrolysis process of sugarcane waste using H_2SO_4 solution with the ratio of sugarcane waste and H2SO4 is 500 grams and 3000 ml (**Table 1**). Based on the research process, different ethanol levels were obtained. Ethanol is the result of the fermentation process of biomass in the form of sugar with the help of *S. cerevisiae* (**Tabel 2**).

Table 1. Measurement results of sugarcane waste sample hydrolysis

Sample type	Hydrolysis volume produced	pH	Sugar content
Sugarcane waste	3.300 ml	5.5-6.0	12%

According to the observations made in the first treatment, the results showed that the pH level for all concentrations was 2.0. For the residual sugar content, the results showed that for concentrations of 1% and 4% were 10% while for concentrations of 2% and 3% were 11%. For the control treatment, the residual sugar content was 12%. The alcohol content obtained for the control, 1%, and 2% concentration is 0%, while for the concentration of 3% and 4% is 1%. The distillation results obtained are control concentration; 45 ml, 1%; 24 ml, 2%; 37 ml, 3%; 44 ml, 4%; 29 ml. (**Figure 1**). Second treatment was found that the pH level for all concentrations was 2.0. For residual sugar content, it was found that for 1%, 3% and 4% concentrations were 11% while for control and 2% concentrations were 12%. Alcohol content for control and 1% concentration was 0% while for 2% and 3% concentration was 1%. For 4% concentration, the alcohol content was found to be 3%. The distillation results that were successfully obtained were for the control treatment; 35 ml, 1%; 32 ml, 2%; 38 ml, 3%; 37 ml, and 4%; 41 ml. The distillation result is the result of distillation once and there is still a possibility of ethanol mixed with water.

Table 2. Sample analysis results after fermentation using cell-free *S. cerevisiae*.

Concentration	Parameter		
	pH	Residual sugar	Ethanol
Control	2.0	12%	0%
1%	2.0	10%	0%
2%	2.0	11%	0%
3%	2.0	11%	1%
4%	2.0	10%	1%

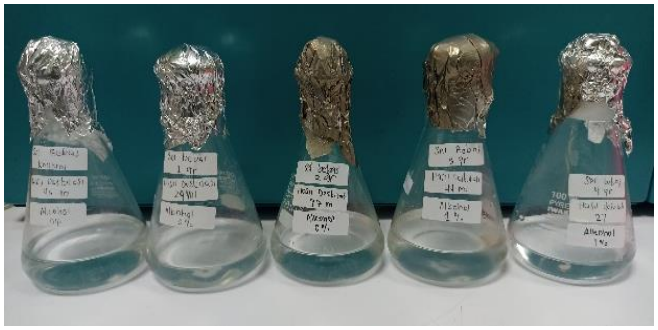

Figure 1. Bioethanol yield after fermentation using cell-free *S. cerevisiae*

Discussion

Based on the distillation results of the samples (**Table 2 and 3**), ethanol was obtained with a very low amount of concentration, where each test sample has a different ethanol scale. The low ethanol production can be influenced by the length of the fermentation process and the solution contained in the sample. This is influenced by the too acidic pH obtained from the hydrolysis of sugarcane waste with the addition of 166 ml of H_2SO_4 diluted in 2.834 ml of aquades, therefore the pH at each concentration inhibits the growth of yeast in producing ethanol. The research activities found good ethanol production results in the utilization of sugarcane waste with a microbial-based bioprocessing approach but at very low levels. It is suspected that the starter used in the fermentation process did not run properly due to the influence of acid in the fermentation medium, so the ethanol production process did not run well. According to Anwar and Subagyo (2020), to produce good ethanol, at least microorganisms such as yeast require media with acidity in the pH range of 4.800-5.0[15]. The same thing was stated by Yuda et al. (2018), optimally that *S. cerevisiae* is able to grow in media with pH conditions ranging from 4.500-5.0[16]. Another theory from Buckle et. al. (1978) states, pH below 3 does not allow microorganisms to metabolize, therefore fermentation can be inhibited if the pH is in that range. So that the amount of ethanol concentration obtained is very low[17].

Table 3. Sample analysis results after fermentation using *S. cerevisiae* immobilized cells.

Concentration	**Parameter**		
	pH	**Residual sugar**	**Ethanol**
Control	2.0	12%	0%
1%	2.0	11%	0%
2%	2.0	12%	1%
3%	2.0	11%	1%
4%	2.0	11%	3%

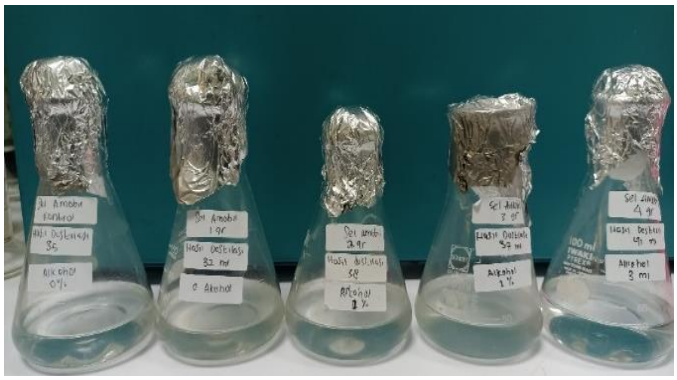

Figure 2. Bioethanol yield after fermentation using immobilized cells of *S. cerevisiae*

In addition, the length of fermentation, the amount of sugar content and the concentration of *S. cerevisiae* inoculum also affect ethanol production. According to Anwar and Subagyo (2020), it requires a fermentation time of 2-3 days to produce ethanol[15]. Agbogbo et al. (2007) stated that the addition of inoculum with a low concentration results in a slow fermentation rate, but can produce higher ethanol because after the cells multiply, the cells will convert sugar into ethanol slowly[18]. Wahyudi (1997) also

stated that a higher concentration of sugar source requires more inoculum to convert sugar into ethanol. Fermentation efficiency can be used as a parameter of the success of a fermentation process[19]. The higher the fermentation efficiency value, the higher the product produced[20].

Conclusions

Based on the results of the study it can be concluded that the production of bioethanol from bagasse biomass waste with changes in fermentation time and hydrolysis time can produce bioethanol with levels that are still low, and sugar levels that decrease. In the ethanol test using free cells, the highest alcohol content was obtained at a concentration of 3% and 4% with the same alcohol content of 1%, with a pH level of 2.0, the amount of residual sugar content of 10% and 11%, with a distillate volume of 44 ml and 29 ml. For the test of ethanol content using immobilized cells, the highest ethanol content was obtained at a concentration of 4%, with a distillate volume of 41 ml, at pH 2.0, with a residual sugar content of 11% and obtained an alcohol content of 3%. So this research shows that sugarcane waste is still less effective to be used as raw material in bioethanol production. However, under these conditions, sugarcane waste used can still provide adequate results in the manufacture of bioethanol.

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Conflicts of Interest

The author declares that there are no conflicts of interest related to this research. All data and information presented in this article are the results of independent research and are not influenced by personal or financial interests.

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