PEMANFAATAN SARI TEBU (*Saccharum officinarum*) DALAM MENGHASILKAN BIOETANOL MELALUI PROSES FERMENTASI

Soeprijanto Soeprijanto [1)]*, Arin Pashadiera Mellina [2)], Raihana Fauzia [3)]

[(1,2,3)]Departemen Teknik Kimia Industri, Fakultas Vokasi Institut Teknologi Sepuluh Nopember, Keputih, Kec. Sukolilo, Kota Surabaya, Jawa Timur 60117, 031-5947274
*Penulis korespondensi: soeprijanto@chem-eng.its.ac.id

Abstrak

Energi Baru Terbarukan (EBT) menjadi solusi untuk mengatasi permasalahan konsumsi energi yang semakin meningkat. Salah satu alternatif sebagai substitusi bahan bakar minyak adalah bioetanol. Sari tebu adalah cairan berwana coklat kehijauan hasil penggilingan batang tebu dan berpotensi menjadi bahan baku pembuatan etanol biofuel. Penelitian ini dilakukan untuk mengetahui pengaruh penambahan variasi nutrient dan yeast Saccharomyches cerevisiae terhadap produksi etanol dari sari tebu melalui proses fermentasi dan untuk mengetahui kadar etanol optimum dalam produksi etanol dengan penambahan variasi nutrient dan yeast Saccharomyches cerevisiae. Prosedur pembuatan bioetanol dengan bahan baku sari tebu dimulai dengan hidrolisis menggunakan HCL 1N, lalu fermentasi gula reduksi menjadi bioetanol dengan menambahkan berbagai variasi nutrient dan yeast Saccharomyches cerevisiae. Selanjutnya untuk pemurnian bioetanol dilakukan proses distilasi pada suhu 78-80°C selama 8 jam. Hasil penelitian ini menunjukkan bahwa dengan adanya variasi penambahan nutrient dan yeast Saccharomyches cerevisiae memiliki dampak yang kurang signifikan terhadap kadar etanol. Sedangkan untuk kadar etanol optimum pada proses fermentasi 3 hari dicapai dengan menggunakan penambahan 0,5% Nutrient + 0,2%Yeast yaitu sebesar 10,46%.

Kata kunci: *bioethanol; proses hidrolisis; Saccharomyces cerevisiae; sari tebu*

UTILIZATION OF SUGARCANE JUICE (*Saccharum officinarum)* IN PRODUCING BIOETHANOL THROUGH FERMENTATION

Abstract

Renewable Energy (RE) is solution to overcome the problem of increasing energy consumption. One of the alternatives to substitute oil fuel is bioethanol. Sugarcane juice is greenish brown liquid that produced by the grinding of sugarcane stems and potentially could be used as raw material for making biofuel ethanol. This study was conducted to determine the influence of additional variations of nutrients and Saccharomyches cerevisiae on ethanol production from sugarcane juice through the fermentation process, and to find out the optimum ethanol content in the ethanol production with additional variations of nutrient and Saccharomyces cerevisiae. The procedure of bioethanol making, using raw materials of sugarcane juice begins with hydrolysis process using HCl 1N, then continued with the fermentation of reduced sugar into bioethanol by adding various variations of nutrient and Saccharomyces cerevisiae. Next, the purification of bioethanol is carried out by distillation process at temperature of 78-80°C for 8 hours. The results of this study indicate that the variation addition of nutrients and yeast Saccharomyces cerevisiae has a less significant impact on ethanol levels. Meanwhile, the optimum ethanol content in the 3-day fermentation process was achieved by using the addition of 0.5% Nutrient + 0.2% Yeast, which was 10.46%.

Keywords*: bioethanol; hydrolysis process; Saccharomyces cerevisiae; sugarcane juice*

INTRODUCTION

Fossil fuel reserves such as gasoline, diesel, and kerosene become increasingly limited. This is not equal to the increasing energy needs from year to year. According to data from the Directorate General of New Energy and Energy Conservation of the Ministry of Energy and Mineral Resources, Indonesia's dependence on fossil fuels is very high, which consist of petroleum (33%), coal (34%), natural gas (24%), and EBT (7%). Which means that particular energy consumption exceeds the growth of energy consumption in the world. Fuel Oil (BBM) that being used mostly comes from fossil fuels which considered as non-renewable material. In the industrial sector that uses fossil fuels in its operation could cause harmful impacts to the environment due to its emission gases such as CO, CO_2, SO_2, and NOx. To solve this problem, the Government anticipates by issuing Government Regulation No. 79 of 2014 on National Energy Policy (KEN), which its target is to develop Renewable Energy (EBT), whereas the national EBT portion in 2025 reaches at least around 23% and in 2050 around 31%. Biofuels can also reduce greenhouse gas emissions to minimize its impact on global warming (Herliati et al., 2019). Bioethanol is an ethanol product that being produced from biological raw materials and other biomass using biotechnology processes and with a help from certain microorganisms. Bioethanol can be produced from sugarcane juice by converting sucrose, monosaccharides or glucose into ethanol products using help from microorganisms. Bioethanol has good prospect as a renewable energy source to be a substitute for fuel oil e.g. gasohol, which is a mixture of gasoline and ethanol. There are several advantages of bioethanol, such as environmentally friendly, renewable, and the raw materials is very easy to obtain even from organic waste. Ethanol (C_2H_6O) is a colorless, flammable and volatile liquid. Ethanol is widely used as solvents, fuels, and raw material for other useful productions (Danmaliki et al., 2016). Biofuel consumption is predicted to reduce carbon dioxide (CO_2) gas emissions by 2.1 gigatons per year in 2050 if its production were stabilizes (Danmaliki et al., 2016).

One of high-intensity raw materials for the development of bioethanol is sugarcane juice. Sugar cane juice is a liquid that produced from the grinding or breaking of sugarcane stems that have a relatively high sugar content. Fresh sugarcane juice usually has a greenish brown colour with pH number between 5.0-6.0. Sugarcane juice contains high sucrose which can be used as substrate for bioethanol production. In addition to sucrose , sugarcane juice also contains other non-sugar substances. In general, sugarcane juice consists of 73-76% water, 11-16% fiber, and 11-16% of dissolved or suspended solids. Ethanol production from sugarcane plants accounts for about 40% of total bioethanol produced (Li et al., 2012).

Glucose is a monosaccharide which is one of the most important carbohydrates that are being used as raw material for producing ethanol. Hydrolysis reactions are shown in the Equation 1.

$$(C_6H_{10}O_5)n + n\ H_2O \rightarrow n(C_2H_{12}O_6) \qquad (1)$$

In the fermentation of ethanol, there are 4 main products, such as division of yeast cell, C_2H_5OH, CO_2, and heat. Hydrolysis of one mole of glucose will produce stoichiometry, 2 moles of C_2H_5OH, and 2 moles of CO_2 as shown in Equation 2 and 3. Based on a mass, 1 g of $C_6H_{12}O_6$ consumed for energy purposes will theoretically produce 0.51 g of C_2H_5OH and 0.49 g of CO_2. However, in practice, a part of the glucose is consumed to produce new cells (Soeprijanto, 2013: Soeprijanto *et al.*, 2020).

$$C_{12}H_{22}O_{11} + H_2O \rightarrow C_6H_{12}O_6 \qquad (2)$$

$$C_6H_{12}O_6 \rightarrow C_2H_5OH + 2\ CO_2 \qquad (3)$$

Fermentation is the process of converting a substrate into particular product using help from microorganisms. Fermentation is commonly used is batch fermentation. Batch fermentation has advantages including a closed system, no addition of O_2, antifoam, no addition of new media, and acidic or alkaline conditions that can be known by pH control (Abdullah et al., 2015). However, there are some drawbacks of batch fermentation such as limited concentration of ethanol yield, obstacles due to high sugar content, and low productivity. However, batch fermentation is still chosen because it produces higher yields than other methods (Silaban, 2017).

In the production of bioethanol from sugarcane juice using *Saccharomyces cerevisiae* as its yeast. *Saccharomyces cerevisiae* has advantages such as having high fermentation power to glucose, fructose, maltose, and galactose, resistant to other microorganisms, resistant to high content of ethanol, also stable and fast properties for adaptation. Through this fermentation, substrates containing glucose, sucrose, and fructose will be used by yeast in the initial fermentation process. Sucrose by enzyme invertase is hydrolyzed to be outside the cell membrane. While fructose and glucose will enter the cells. The optimum temperature for *Saccharomyces cerevisiae* growth is 25-30ºC and its optimum pH is between 4.5-5.5 (Umaiyah et al., 2013).

In the previous study, Utama (2016) stated that the addition of Vitamin B Complex as a nutrient to increase concentration of the ethanol in fermented bioethanol. Fermentation stage used sugarcane juice as much as 200 ml; variations in the addition of Vitamin B Complex 0, 0.1, 0.2, and 0.3% (B/V) of fermented sugarcane juice; and time variations of 6, 7, and 8 days. From the research that has been done, it can be obtained that in fermentation conditions that is the addition of 0.1% Vitamin B Complex with a fermentation time of 7 days to produce bioethanol with the highest concentration of ethanol, which is 30.177%. From these results, it can be known that the addition of B-complex vitamins as fermented nutrients can increase the concentration of ethanol, but the increase in ethanol concentration is inversely proportional to increase in grams of B-complex vitamins that can affect the pH condition of fermentation medium so that there is a decrease in the performance of microbes to produce ethanol. In addition, variations in fermentation time affects the concentration of ethanol that experiences fluctuations. The decrease and increase in ethanol concentration occured because *Saccharomyches cerevisiae* were undergoes a growth phase, whereby the increase in ethanol concentration occurs when the yeast has gone through adaptive phase and entered log phase, while the decrease in ethanol concentration occurs when yeast has entered stationary phase. Amrullah (2021) stated before the fermentation process, evaporation of sugarcane juice that aims to reduce the moisture content in sugarcane juice has to be done, In conjuction with water content in sugarcane juice being reduced, it will increase the sugar content of the sugarcane juice. Fermentation stage were carried out using sugarcane juice volume 300 ml; variations of yeast tape addition 5%, 10%, 15%; and time of 7 days. From the study, the optimum bioethanol content were obtained at yeast concentration of 15% is 6.67. The concentration of yeast does affects the pH of bioethanol, the optimum pH that has been obtained is at the concentration of 5% with a pH number of 3.3. In addition with the increasing amount of yeast that being given leads to decrease in pH value of ethanol products. The concentration of yeast also affects brix, the optimum brix is obtained at the yeast concentration of 15% with brix value of 5%. It can be known that the increase in yeast content can cause decrease in brix content. Nafi'ah (2019) uses hydrolysis by heating the sugarcane juice at 80°C for ±1 hour, then stirring until boiling. Fermentation stage using the volume of sugarcane juice until 5 liters; addition of yeast 1 gr, 2 gr, 3 gr, 4 gr, 5 gr; and time variations of 3, 5, and 7 days. From that study it can be obtained that the highest ethanol content is by giving 5 grams of yeast during 7 days of fermentation is 5000 ppm. Also it can be known that the addition of yeast and the longer of fermentation time does affects how high the content of ethanol that produced. The more yeast that being given could cause the concentration of *Saccharomyches cerevisiae* cells to mobilize more. And longer time it gave means that could gives enzymes more chance to remodel sugar into ethanol.

The purpose of this research is the utilization of sugarcane juice *(Saccharum officinarum L.)* in producing bioethanol to find out the influence of variations of nutrient additions and *Saccharomyches cerevisiae* yeast on the production of ethanol from sugarcane juice through the fermentation process and to find out the optimum ethanol content in ethanol production with the variations of the addition of nutrient and *Saccharomyches cerevisiae* yeast.

RESEARCH METHODS

Materials

The materials used in this method are sugar cane juice obtained from sugarcane juice sellers in the area around ITS campus,. methylene blue, Fehling A, and Fehling B, were used to analyze reducing sugar content in a sample, purchased at a Chemical Store in Surabaya; urea and KH_2PO_4, used as nutrients in fermentation, were supported from Industrial Biotechnology Laboratory, Department of Industrial Chemical Engineering, ITS; baker's yeast and tape yeast were purchased at Food Store.

Experimental Setup

Production procedure of bioethanol with sugarcane juice as raw material in Keputih, Kec. Sukolilo, East Java begins with the acid hydrolysis process with the addition of 1N HCl, after which the glucose is fermented into bioethanol by adding yeast (Saccharomyces cerevisiae). Furthermore, for the purification of bioethanol, a distillation process is carried out. The last stage is product analysis, namely bioethanol.

Hydrolysis Process

Sugarcane juice in hydrolysis with acid hydrolysis using HCl 1N. Inserting sugarane juice 1000 mL, then stir and add HCl 1N to pH 4. Hydrolysis is performed at high temperatures of 100°C by heating on the strirrer hot plate (Figure 1) and the reaction time runs in the range of 30 minutes to 2 hours. The parameters of acid hydrolysis analyzed are reduced sugars formed.

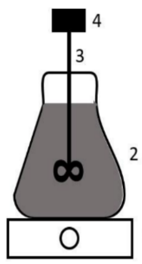

Figure 1. Hydrolysis process of sugarcane juice using acid

Note:
1 = *Hot Plate*
2 = Sugarcane juice
3 = *Propeller Stirrer*
4 = *Stirrer Motor.*

Fermentation Process

The fermentation process is intended to convert glucose into ethanol. Fermentation is carried out in batches (Figure 2) in an anaerobic state at a room temperature of 25-30°C by adding nutrients and yeast *Saccharomyches cerevisiae*. Fermentation was conducted for around 3 days. Fermented products in the form of ethanol and carbon dioxide in the mixture of fermented products. Bioethanol levels resulting from the fermentation process, usually only reaches 6-9% (w/v), so to obtain bioethanol with alcohol 94% needed other processes (purification), namely distillation process.

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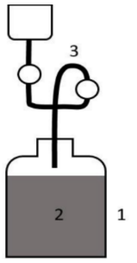

Figure 2. Batch fermentation of ethanol using additional variations of nutrient and yeast

Note:
1 = Fermenter
2 = Sugarcane juice
3 = *Channel of CO_2 gas produced.*

Distillation Process

Distillation was done to separate the content of ethanol with excess water. This process involves evaporation of steam. When distillation takes place, ethanol that has a boiling point of 80°C will evaporate first compared to water that has a boiling point of 100°C. The distillation process is carried out for 8 hours. The distillation results will be analyzed ethanol.

RESULTS AND DISCUSSION

Analysis Results of Reduced Sugar Content After the Hydrolysis Process

Acid hydrolysis technology can be carried out using concentrated acid at low temperatures and the hydrolysis process can be carried out using diluted acid at high temperatures (Fengel and Wegener, 1989). Hydrolysis with highly concentrated acids at low temperatures makes the decomposition of sugar polymers slow. The amount of sugar that has been obtained from decomposition is 90%. Hydrolysis using diluted acid, diluted hydrochloric acid is carried out at high temperature and high pressure. At high temperatures, the decomposition process runs rapidly for polysaccharide compounds, and also rapidly decompose polysaccharide compounds, and also rapidly decompose the monosaccharide compounds that has been released.

Diluted acid processes are carried out at high temperature of 100°C and high pressures of 15 psi to 75 psi, and have reaction times in range between 30 minutes to 2 hours with batch processes (Badger et al., 2002; Kim et al., 2002).

Hydrolysis process with the help of HCl aims to hydrolyze starch, cellulose, and hemicellulose which contained in the material into reduced sugars. Raw material, which is sugarcane juice is hydrolyzed using HCl with a concentration of 1N HCl.

After acid hydrolysis, the reduced sugar contents of sugarcane juice increased from 65.78 g/L to 73.53 g/L. This matched with the literature which stated that the addition of acid will increase the reduced sugar contents and greater the concentration of added acid is, it will increase the reduced sugar contents (Erwinda, 2014).

Analysis Results of Reduced Sugar and Ethanol Content in the Fermentation Process

The fermentation process with the help of the yeast Saccharomyses Cerivisiae aims to convert the reduced sugar that formed in the hydrolysis process into ethanol with a days of fermentation time.

After the fermentation process for 3 days, the results that has been obtained from the initial reduced sugar contents are as follows:

Table 1. Results of Reducing Sugar and Ethanol Content After the Fermentation Process

A	B	C	D
0.5%*Nutrient* +0.2%*Yeast*	73.53	7.57	10.46
1%*Nutrient* +0.4%*Yeast*		6.94	10.39
1.5%*Nutrient* +0.6%*Yeast*		6.25	10.2
2%*Nutrient* +0.8%*Yeast*		5.95	10.15
2.5%*Nutrient* +1%*Yeast*		5.55	10.02

Note:
A = Addition Variable
B = Initial Reduced Sugar Content (gr/L)
C = Final Reduced Sugar Content (gr/L)
D = Ethanol Content (%)

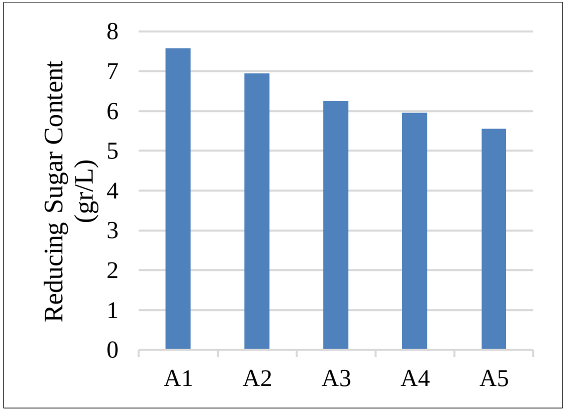

Figure 3. Results of Analysis of Reducing Sugar Content After the Fermentation Process

Note:
A1 = addition of 0.5%*Nutrient*+0.2%*Yeast*
A2 = addition of 1%*Nutrient*+0.4%*Yeast*
A3 = addition of 1.5%*Nutrient*+0.6%*Yeast*
A4 = addition of 2%*Nutrient*+0.8%*Yeast*
A5 = addition of 2.5%*Nutrient*+1%*Yeast*

Reviewed from the results of the study in **Table 1**, the results of reduced sugar contents after the fermentation process decreased from the initial reduced sugar contents before fermentation, namely 73.53 gr/L to 7.57; 6.94; 6.25; 5.95; and 5.55 g/L. In this study, the reduced sugar contents that being obtained after the addition of 0.5% Nutrient +0.2% Yeast to 2.5% Nutrient +1% Yeast in the fermentation process only has a slight difference, which means that the variation of the addition of Nutrient and Yeast with the same ratio is not too influential in this study. The lowest reduced sugar content was obtained at the addition variable of 2.5%Nutrient+1%Yeast, which was 5.55 gr/L. Meanwhile, the highest reduced sugar content was in the addition variable of 0.5% Nutrient +0.2% Yeast, which was 7.57 gr/L.

The decrease in reduced sugar contents before and after the fermentation process proved that due to the sugar that contained in the fermentation medium was continuously utilized by *Saccharomyces cerevisiae* cells for cell growth and ethanol formation. Higher the amount of reduced sugar that being used by Saccharomyces cerevisiae cells, higher the ethanol concentration that being produced and conversely less reduced sugar that being used, lower the ethanol concentration produced. This match with the statement of Wignyanto et al. (2001) which states that the more reduced sugars that can be utilized by *Saccharomyces cerevisiae* cells, the higher the concentration of ethanol produced by Saccharomyces cerevisiae cells.

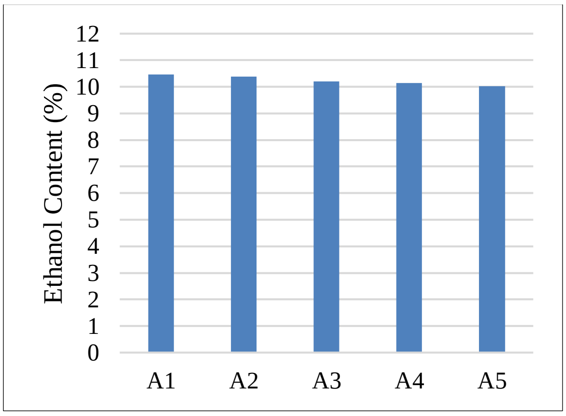

Figure 4. Results of Analysis of Ethanol Content After the Fermentation Process.

Note:
A1 = addition of 0.5%*Nutrient*+0.2%*Yeast*
A2 = addition of 1%*Nutrient*+0.4%*Yeast*
A3 = addition of 1.5%*Nutrient*+0.6%*Yeast*
A4 = addition of 2%*Nutrient*+0.8%*Yeast*
A5 = addition of 2.5%*Nutrient*+1%*Yeast*

According to the results of the study in **Table 1.** Results of Ethanol Content After Fermentation were obtained at 10.46%; 10.39%; 10.2%; 10.15%; and 10.02%. In this study, the ethanol content obtained after the variation of the addition of 0.5% Nutrient + 0.2% Yeast to 2.5% Nutrient + 1% Yeast in the fermentation process was not much different because the reducing sugar content obtained after the fermentation process was only slightly different. In this study, the highest ethanol content was found in the variation variable addition of 0.5% Nutrient +0.2% Yeast with a concentration of 1N HCl of 10.46%. Meanwhile, the lowest ethanol content was found in the addition variable of 2.5% Nutrient +1% Yeast of 10.02%. Greater the reduced sugar contents will results in greater the ethanol produced. According to Khodijah (2015), this is due to the drastic growth of *Saccharomyces cerevisiae* and the supply of nutrients that supports the growth of *Saccharomyces cerevisiae* as the addition of the starter is greater, so that the bacteria that convert the reduced sugar into ethanol is getting bigger, and this process will stop if the alcohol contents cannot be tolerated by microbes.

CONCLUSION

From the process of bioethanol making from sugarcane juice, the it can be concluded that: the addition of nutrients and yeast has less significant impact on ethanol content; the optimum ethanol content in the 3 day fermentation process was achieved by using the addition of 0.5% Nutrient +0.2% Yeast, which its result was 10.46%.

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