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Production of Bioethanol from Solid Wastes of Tapioca Flour Industry Through Enzymation and Fermentation Process

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Abstract

Solid wastes from the tapioca flour industry still contain a lot of starch which is only used as animal feed. Even though the starch content in the solid waste can be converted into bioethanol to increase the benefits of solid waste itself. The purpose of this study was to utilize the potential of solid waste from the tapioca flour industry to produce bioethanol through an enzymatic and fermentation process. The solid waste was firstly dried in an oven at 60^0C for 24 hours, then it was mashed. The fine size of solid waste was then conducted a liquefaction process with the addition of water to a volume of 2 litres, then was added $CaCl_2$ 40 ppm and α-amylase of 2-5% (w/v) and heated at 90^0C-100^0C for 2 hours. The next step was that saccharification process was conducted, which was a solution added with HCl to pH 4, and glucoamylase of 2-5% (w/v) then was heated at a temperature of 60^0C for 4 hours. The result showed that the highest reducing sugar obtained was 100 g/l with the use of solid waste of 75 g/l.

Keywords: *Bioethanol; fermentation; liquefaction; reducing sugar; saccharification.*

Introduction

The increase in fossil fuel prices in 2005 which had touched more than 70 US dollars per barrel was an important momentum for the development of biofuels in the country. This difficult condition has a positive impact on the use of biofuel in Indonesia. Biofuel, which was initially neglected, finally had a better bargaining position. In fact, many believe that biofuel can be used as an alternative to reduce such a large dependence on fossil fuel (Swadaya, 2008). Under these conditions, the government has issued a presidential regulation of the Republic of Indonesia Number 5 in the year of 2006 concerning National Energy policy to develop alternative energy sources as a substitute for fossil fuel.

Vegetable-based fuel, for example, bioethanol, is made from abundant biological resources in Indonesia. Bioethanol is made from sugary or starchy ingredients such as cassava, cane sugar, cane juice, sorghum, sweet potato, etc. (Assegaf, 2009; Soeprijanto et al., 2009). Cassava Plants (*Manihot esculenta*) is one of the commodities that are easy to cultivate and treatment is not difficult. Aside from being a food ingredient, cassava is also used in animal feed. The ingredients contain about 60% water, 25-35% starch, and protein, minerals, fiber, calcium, and phosphate. Cassava is a higher energy source than rice, corn, sweet potatoes and sorghum. Cassava consists of several types, and the types that have the highest starch content are *Adira 1* with a starch content of 45% and *Adira 4* with levels only (18% -22%) (Febrian, 2014). Unused material as a producer of carbohydrate is tapioca solid waste. The Indonesian Technology Research and Research Agency stated that the starch content of tapioca solid waste was approximately 67.8%. In accordance with these data, the tapioca solid waste is very potent as a source of biofuel, namely bioethanol (Marlinda, 2009). Tapioca solid waste which is often called *onggok* is one of the results of the waste produced from the tapioca flour industry. The use of tapioca solid waste so far in addition to being used as animal feed can also be used to make glucose syrup or ethanol fuel. Tapioca solid waste is a carbohydrate in the form of polysaccharide in the form of anhydrous monosaccharide polymer with general formula $(C_6H_{10}O_5)_n$. The main constituent of starch is amylose and amylopectin. Amylose composed of mutual glucose units relating through 1-4 glucoside bonds, while amylopectin is polysaccharides are composed of 1-4α glycosides and has branch chains 1-6α glucosides.

Bioethanol production from plants containing starch or carbohydrates is carried out through the process of converting carbohydrates into glucose (sugar) by several methods including by acid hydrolysis and enzymatically. Hydrolysis is a decomposition reaction between a compound and water to make a compound it breaks or breaks down.

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In the starch hydrolysis reaction with water, water will attack starch on bonds 1-4α glucoside produce dextrin, syrup or glucose depends on the degree breaking of the inner polysaccharide chain pat. But this reaction between water and starch takes place so slowly that the use of a catalyst is needed for enlarging water activity. The catalyst used can be either an acid or an enzyme. The commonly used acid catalysts are hydrochloric acid, nitric acid, and sulfuric acid. In industry, hydrochloric acid is generally used as a catalyst. This selection is based on that salt formation after neutralization results are the salt which is not dangerous for health.

Glucose is monosaccharide which is one of the most important carbohydrates used as raw material for making ethanol. Hydrolysis reactions can be shown in Equation 1.

$$(C_6H_{10}O_5)n + nH_2O \rightarrow n(C_6H_{12}O_6) \quad (1)$$

The enzymatic hydrolysis method is more often used because it is more environmentally friendly than an acid catalyst. The glucose obtained is then carried out by fermentation by adding yeast to obtain bioethanol as an energy source (Assegaf, 2009). The technology of starch hydrolysis includes two stages of hydrolysis, i.e, liquefaction, where non-soluble starch is converted into a dissolved polymer and the saccharification where the process is carried out further breaking this fragment into desired starch candy. Liquefaction is carried out with α-amylase which is heat resistant at 95-105^{0}C and pH 5-6.5 and incubated for 2-3 hours. Saccharification is made at 60-65^{0}C and pH 4.5-5 by glucoamylase activity and occurred for 48-72 hours. Two enzymes are widely used to hydrolyze α-1,4 bonds in starch, α-amylase, and β-amylase. α-amylase breaks down starch into glucose and maltose, while β-amylase hydrolyzes unbound in α-1,4 bonds for α-amylase. These two enzymes work imperfectly to break down amylopectin compounds because they can not hydrolyze α-1.6 bonds. To break this bond another enzyme, such as Amilo-1,6-glucosidase is needed.

Ethanol fermentation produces four main products, namely yeast cell division, ethanol, carbon dioxide, and heat. One glucose molecule will produce, stoichiometry, 2 moles of ethanol and 2 moles of carbon dioxide (Equation 2 and 3). With a mass basis, 1 kg of glucose consumed for energy purposes will theoretically produce 0.51 kg of ethanol and 0.49 kg of carbon dioxide. However, in practice, a part of the glucose is consumed to produce new cells (Soeprijanto, 2013).

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

$$C_6H_{22}O_6 \rightarrow C_2H_5OH + 2\ CO_2 \quad (3)$$

This study aims to convert tapioca solid waste to bioethanol from tapioca flour factories in Lumajang, East Java. In this experiment, the limitation of the problem that will be used to obtain bioethanol from tapioca solid waste by hydrolysis of α-amylase enzymes and glucoamylase enzyme then proceed with the fermentation process using *Saccharomyces cerevisiae*.

Experimental Method

Materials. The materials used in this method are solid waste tapioca flour factory, α-amylase enzyme, glucoamylase enzyme, hydrochloric acid, $CaCl_2$, *Saccharomyces cerevisiae*, Urea, and $(NH_4)_2HPO_4$.

***Preparation Step*.** Solid waste which still contains water was dried by drying in the sun. The dry solid tapioca flour mill was then milled with a grinding machine or crushed with a pounder so that it becomes a fine powder.

Liquefaction Process. Weighing 2%, 5%, 7.5% (w/v) of tapioca flour waste then inserting it into a 2000 ml beaker glass, adding $CaCl_2$ 40 ppm (w/v). Measuring pH with a pH meter. Then added HCl to condition the pH of the slurry from 6.5 to 6.6. Add the α-amylase enzyme 2% (w/v) of glucose used. As seen in Figure 1, the slurry was heated for 2 hours at 90^{0}C-100^{0}C.

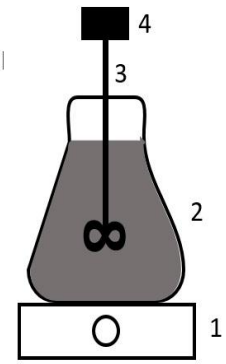

Figure 1. The effect of the concentration of tapioca solid waste using α-amylase and glucoamylase on the production of glucose. *Note:* 1= hot plate; 2 = Slurry of tapioca solid waste; 3 = propeller stirrer; 4 = stirrer motor.

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Saccharification Process. Before the saccharification begins, the final result of the liquefaction stage is cooled at 60^0C. then stir and add HCl to pH 4. Then adding the glucoamylase enzyme is 2% (w/v) of glucose used. Then the slurry was heated at 60^0C for 4 hours – 24 hours, and was shown in Figure 2.

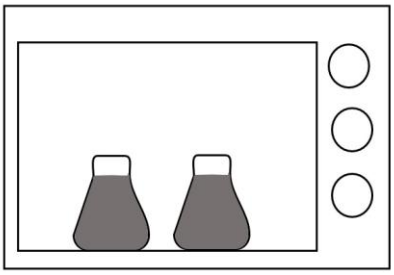

Figure 2. The effect of the concentration of tapioca solid waste using glucoamylase on glucose production.

Fermentation Process. The fermentation process is intended to convert glucose into ethanol/ bioethanol using yeast (*Saccharomyces cerevisiae*) 0.2% and nutrients (Urea 0.5% and $(NH_4)_2HPO_4$ 0.5%). Fermented products are ethanol and carbon dioxide in a mixture of fermented products. Fermentation was conducted for around 3 days. The percent of ethanol/ bioethanol produced from the fermentation process usually only reaches 6-9% (w/v), therefore, to obtain ethanol which has a level of alcohol 95 percent is needed the distillation process.

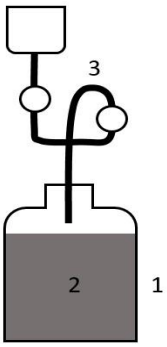

Figure 3. Batch fermentation of ethanol using *Saccharomyces cerevisiae* baker yeast. *Note:* 1 = fermenter; 2 = slurry of tapioca solid waste; 3 = channel of CO_2 gas produced.

Distillation Process. Distillation is carried out to separate ethanol from excess water. This involves steam evaporation. Because the boiling point of ethanol is 80^0C, while the water evaporates at 100^0C, ethanol will evaporate first before water, this vapor is accommodated and re-condensed into a liquid form.

Results and Discussion

Production of Reducing Sugar

The results showed that the higher the concentration of starch used in the fermentation process, the ethanol content produced also increased. In this study, solid tapioca flour was hydrolyzed using the catalyst α-amylase and glucoamylase enzyme, and hydrolysis was carried out through 2 stages, liquefaction at 90^0C, pH 6 for 2 hours and added with $CaCl_2$ (Ca^{2+}= 40 ppm), which served to stabilize enzyme work. At this stage, starch was changed to dextrin. Then the second stage, the saccharification process was carried out at 60^0C, pH 4.5 for 72 hours using an incubator. In this process, the catalyst used is the glucoamylase enzyme which acts to break down dextrin into glucose (reducing sugar).

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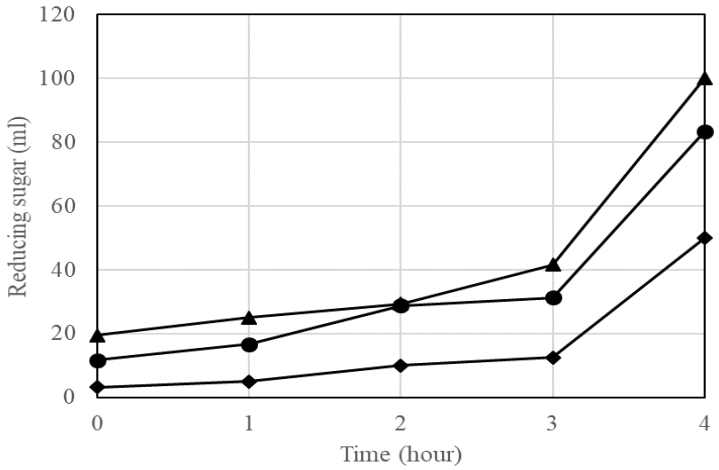

Figure 3. Effect of tapioca flour concentration on reducing sugar production. *Note:* ◆= 2% (20 g/l), ●= 5% (50g/l), ▲= 7.5% (75 g/l).

Bioethanol Production

To obtain high ethanol productivity, the main factor is to optimize the amount of glucose produced in the enzyme hydrolysis. Figure 4 and figure 5 show that ethanol production was influenced by the concentration of glucose and tapioca solid waste added, respectively. The ethanol content obtained increased from 0.2% to 5.41% with increasing amounts of glucose used from 20 g/l to75 g/l. Based on the raw material of tapioca solid waste, the yield of ethanol obtained was 0.39 or 38.72% (v/w).

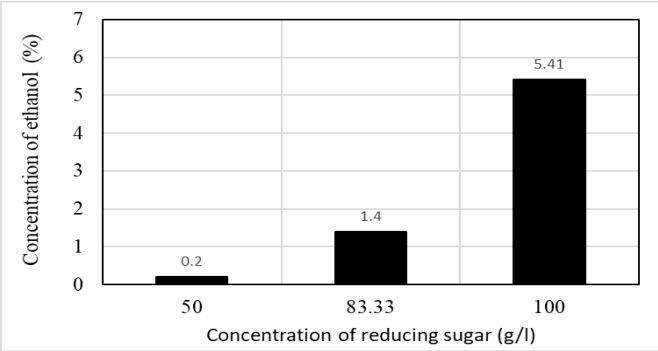

Figure 4. The relationship between the concentration of reducing sugar and ethanol after the fermentation.

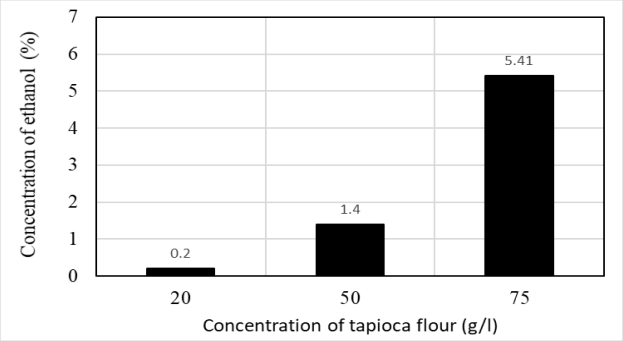

Figure 5. The relationship between concentration of starch and ethanol after the fermentation.

Conclusions

The highest reducing sugar production obtained from saccharification was 100 g/l using a tapioca solid waste concentration of 7.5% (w/v). The highest ethanol production was obtained to be 5.41% (v/v) using sugar concentration of 100 g/l.

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Lembar Tanya Jawab

Moderator : Sri Sukadarti (UPN "Veteran" Yogyakarta)
Notulen : Fauzan Irfandy (UPN "Veteran" Yogyakarta)

1. Penanya : Haries Handoyo (Universitas Gajah Mada)
   Pertanyaan : Pada hasil penelitian tercantum bahwa konsentrasi maksimal yang dihasilkan adalah 5%. Konsentrasi pada hasil proses apakah yang dimaksud dan mengapa tidak dapat dicapai angka lebih dari itu?
   Jawaban : Konsentrasi etanol 5,41% didapatkan dari hasil fermentasi dalam waktu 3 hari (menurut referensi terdahulu).

2. Penanya : Sri Sukadarti (UPN "Veteran" Yogyakarta)
   Pertanyaan : Waktu fermentasi yang dilakukan adalah 3 hari. Apakah sudah dilakukan variasi waktu penelitian?
   Jawaban : Belum dilakukan variasi waktu sehingga belum diketahui kapan terjadinya fase-fase mikroba seperti *lag phase*, *log phase*, *stationary phase*, dan *death phase*.

3. Penanya : Hesty Rimadianny (Badan Pengawas Tenaga Nuklir)
   Pertanyaan : Bagaimana tinjauan ekonomi dari penelitian ini seperti keuntungan dan perbandingan penggunaan bahanbaku dari ampas tapioka (singkong) ini dengan bahan lainnya?
   Jawaban : Penelitian ini masih tahap awal yaitu peninjauan dari proses fermentasinya dan belum dilakukan peninjauan secara ekonomi.

4. Saran : Soeprijanto (Institut Teknologi Surabaya)
   Konsentrasi hasil etanol dari proses enzimasi ini tergantung pada konsentrasi gula. Konsentrasi gula pada penelitian ini adalah 100 g/L. Estimasi etanol yang dihasilkan tidak lebih dari 10%. Selain itu proses enzimatik memang cukup berat dan membutuhkan waktu yang lama untuk mengubah pati menjadi gula, tidak seperti hidrolisa asam yang dapat menghasilkan konsentrasi etanol yang cukup tinggi dalam waktu singkat.

   Haries Handoyo, S.ST. (Universitas Gajah Mada)
   Dalam proses enzimatik memiliki semacam *lethal dose* dimana produksi etanol itu sendiri dapat membunuh mikroba *Saccharomyces cerevisiae*. Maka penambahan gula dan jumlah bakteri harus diperhatikan.