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Bioethanol Production from Indonesian Tapioca Waste as Potential Fuel Additive For Low Cost Green Car

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ABSTRACT

People using transportation contribute to a 24% increase demand in energy. Unfortunately, fossil fuel is not renewable and limited in quantity. However, the biomass of tapioca waste is a potential energy source alternative to solve the problem. Today, biosystems for bioethanol production need to be optimized to maximize filtrate qualities and minimize the production cost. First, composition and incubation times need to be optimized. The present research found that to ferment 50 g waste in 200 mL water, we need 2 g of yeast and four days incubation time to obtain 17% ethanol. Without acid hydrolysis, the glucose concentration in the culture for yeast fermentation of tapioca waste was 2616 ppm in the first day and increased significantly in the second day to 2965 ppm. This concentration was measured using the DNS (dinitro salicylic acid) method. Firstly, yeast converted glucose on medium to ethanol. Secondly, the distillation method generated pure ethanol. Thirdly, this ethanol could be mixed with petrol in concentrations, from 10%, 20%, 30%, 40%, and 50%. This variant fuel could be used to test emissions to understand the advantages of tapioca bioethanol compared to fossil fuel. Result of this research should support a government program to enhance low cost green car to achieve lower air pollutant, green energy resolution and cleaner production in the tapioca industry.

Keywords: *Bioethanol, Emission, Formulation, Low Cost Green Car, Tapioca*

1. INTRODUCTION

Indonesia has a variety of potential energy sources, including petroleum, natural gas, coal, and renewable energy resources such as hydro, geothermal, solar, wind, biomass, and wave energy. However, petroleum resources today are limited, as petroleum reserves continue to decline while consumption continues to rise. This is not only in Indonesia; oil consumption by the United States exceeds 800000 barrels per day and has increased 2 times since 2010. Every day there are more than 100,000 barrels of distillate oil exported to Asia by the US including Indonesia (EIA 2017). Increased transportation and limited petroleum resources can not be avoided. Therefore, in overcoming these problems the government needs to make a Low Cost Green Car (LCGC) policy.

A Low Cost Green Car (LCGC) policy is a new policy for four-wheeled vehicles in Indonesia since 2013. This policy is contained in industrial regulations No.33 / M-IND / PER / 7/2013 on the development of production of four-wheeled motor vehicles which are energy efficient and reasonably priced. This policy means a vehicle with a low price and which is environmentally friendly. The policy is for cheap and environmentally friendly cars, which can reduce the consumption of fuel and emissions by private cars in Indonesia (Purba 2015). Therefore, the LCGC also contributes to reducing greenhouse gas emissions. According to Bo *et al*. (2017), the average transportation contribution to greenhouse gas emissions is 24% of total national carbon emissions. Transportation is therefore the main target for conservation and emissions reduction to address climate change.

However, the development of a LCGC as a petrol-car is not currently supported by widespread availability of bioethanol fuels. Non-fossil fuel alternatives such as bioethanol have not yet met the standardization for vehicle engines. Thus, people have not been able to switch from the use of standard petrol and high-octane petrol. Therefore, bioethanol production is required for LCGC fuel vehicles with emissions. The raw materials proposed in this project are tapioca starch waste. This is because the waste of tapioca flour plant is one of the potential materials for ethanol production based on the content of polysaccharides and sugars with ethanol productivity reaching 19.5% of the total mass of the cassava harvest (Wahyuono *et al.* 2015). Therefore the research was conducted to analyze bioethanol production from solid and liquid tapioca waste.

2. METHODOLOGY

Equipment used includes airlock bottles, glassware, distilator (rotary evaporator), analytical balance, pH-meter, vortex, spectrophotometer, pycnometer, emission tester, APD Colony Counter Lite application and hot plates. The materials used include tapioca flour waste, water, commercial *S. cerevisiae*, Na-dichromate, acetate buffer, DNS reagent, sulfuric acid, glucose standard, ethanol standard, HCl, and NaOH.

Sample Preparation

Sampling of tapioca waste was conducted at the Sukaraja Plant, Halang, Bogor. Samples of biomass waste used were solid and liquid waste, with sampling in duplicate. The first batch was made on April 12, 2018, while the second was taken on May 17, 2018.

Preparation of a glucose standard curve was done by the DNS method. Samples of 0.5 mL were mixed with 1.5 mL DNS. Samples were boiled for 10 minutes then diluted 10 times (addition of 9 mL of distilled water in 1 mL sample-reagent), then the absorbance at 540 nm wavelength measured. The standard ethanol curve was prepared by

mixing 8 mL sulfuric acid, 1 mL acetate buffer, 1 mL Na-dichromate and 1 mL sample. This mixture is further vortexed for 1 minute and incubated for 2 hours and its absorbance at a wavelength of 548 nm measured.

Fermentation Stage

The fermentation process began with acid hydrolysis through the addition of HCl. During the fermentation process with *S. cerevisiae* and water, a sugar test was performed by the DNS method and ethanol content tested with Na-dichromate in acidic condition to estimate the fermentation levels in the samples, and to compare solid and liquid waste efficiency.

Distillation Stage

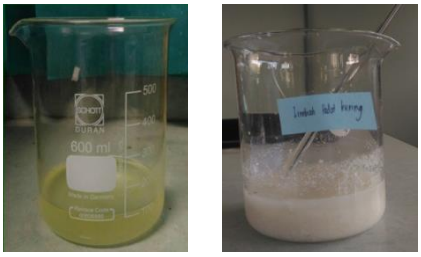
When the distillation begins, the vessel valve was in a closed state. Then the tank was filled with fermentation mixture. The condenser was placed in cold running water and the rotary evaporator was turned on. The distillation is carried out at a temperature of 46-50 ° C to evaporate ethanol under vacuum. After that, specific gravity of the distillate is measured using a pycnometer to estimate purity.

Colony Counting Stage

Calculation the number of cells in a commercial *S. cerevisiae* sample was performed by multilevel dilution followed by the growth of 10^{12} dilution into the PDA (Potato Dextrose Agar). The growth was over 3 days according to the peak graph of ethanol production. Then the number of colony spots formed was calculated using a colony counter.

3. RESULTS

Tapioca waste is the carbohydrate source for yeast in producing bioethanol. Figure 1 and 2 show a sample of solid and liquid waste, and the results of acid hydrolysis as the common first step.



A B

Figure 1 Tapioca liquid waste (A) and solid waste (B)

Figure 2 Acid hydrolysed tapioca solid waste

Table 1 Data absorbance of standar curve [glucose] by DNS method

[Glucose] ppm	Measured $A_{548\ nm}$	Corrected $A_{548\ nm}$
0	0.017	0
200	0.062	0.045
400	0.15	0.133
800	0.32	0.303
1200	0.477	0.46
1600	0.652	0.635
2000	0.782	0.765
5000	1.522	1.505
10000	2.701	2.684

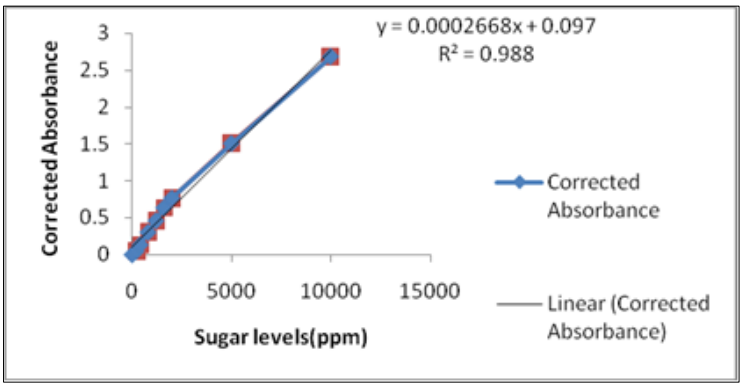


Figure 3 Standard curve of sugar levels by DNS method

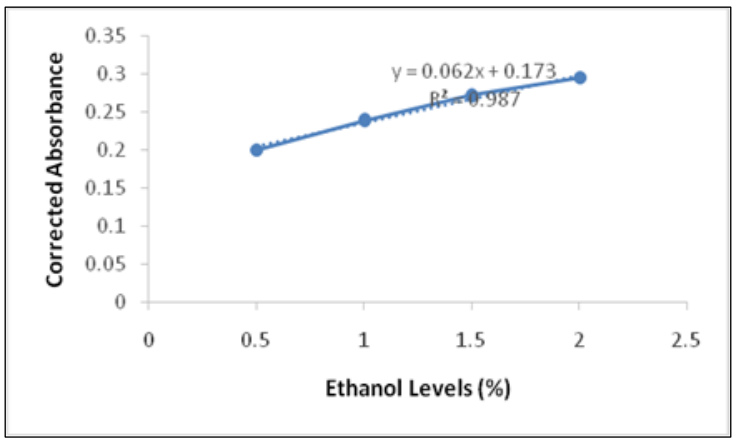


Figure 4 Standard curve of ethanol level

Table 2 Data standard curve of ethanol

Etanol Level (% v/v)	Measurable Absorbance	Corrected Absorbance
0	0.103	0
0.5	0.304	0.201
1	0.343	0.24
1.5	0.375	0.272
2	0.398	0.295

Based on the daily absorbance data from the fermentation sample with the above standard curve, we obtained the data of sugar concentration and ethanol percentage as shown by the following Tables 3, 4, and 5. After obtaining the selected formulation, distillation was carried out with a rotary evaporator under vacuum at a temperature of 46-50^{o}C. The results of the calculation of specific gravity of the pre- and post-distillation materials are shown in Table 6.

Table 3 Ethanol and sugar levels from fermentation A (solid waste)

Day	Ethanol levels (% v/v)	Sugar levels (ppm)
1	6.935	2616.192
2	14.581	2964.768
4	17.065	318.59
8	11	498.5

Table 4 Ethanol and sugar levels from fermentation B (Liquid Waste)

Day	Ethanol levels (% v/v)	Sugar levels (ppm)
0	0	<200
4	16.081	183.658
6	17.565	284.86
10	17	337.33

Table 5 Ethanol and sugar levels from solid waste after acid hydrolysis

Day	Ethanol levels (% v/v)	Sugar levels (ppm)
0	0	<200
2	2.79	<200
6	1.24	<200

Table 6 Density of distillate solid waste tapioca

Sample	Mass (g)	Volume (mL)	Density (g/mL)
Distillate 1	23.9572	25	0.9583
Distillate n	20.4700	25	0.8188
Rest of Distillate	22.8200	25	0.9128

4. DISCUSSION

The LCGC (Low cost green car) is a policy based on the regulation of the Minister of Industry No. 33, 2013 on July 1, 2013. Through the LCGC, the government expects to achieve a 26% emissions reduction in 2020. In addition, the policy of this low-cost car is aimed at reducing the consumption of subsidized fuel. Because the LCGC engine is designed for 92 octane fuel or equivalent to Indonesian premium (grade octane gasoline 92). This octane value is in addition to the emission quality, also closely related to the number of taps produced that affects the level of engine damage. However, although high octane fuel is considered to be more environmentally friendly and free from subsidies, it is too costly for the middle to lower class levels of society. It is necessary to produce high octane fuels at low prices. One way is through the addition and development of ethanol-based fuels (octane 111).

In addition to chemical synthesis, ethanol can also be produced more sustainably by microorganisms hereinafter called Bioethanol. Bioethanol (C_2H_5OH) can be produced from carbohydrate-containing materials by fermentation of glucose using *S. cerevisiae* yeast (Erna *et al* 2016). The results of colony counting of 1 g of the commercial *S. cerevisiae* yeast used in this study showed a value of 9×10^{13} cells.

Cassava is a plant in the Euphorbiaceae family and is classified as a tropical plant. The general public has used cassava tubers for tapioca starch production

and as a substitute for staple foods. The processing process produces solid waste/cassava "onggok" and liquid waste from sedimentation. Both of these wastes are reported to contain close to 25% of the cellulose potential for biomass sources for bioethanol production. Constraints in the process of making bioethanol refers to the four major aspects of raw materials, conversion technology, hydrolysis process, and fermentation configuration (Sarkar et al., 2011). Figure 1 shows a sample image of liquid waste, and the result of acid hydrolysis.

Hydrolysis is the process of breaking chemical bonds using water. The hydrolysis process can be carried out chemically by the addition of a catalyst or enzymatically. Acid hydrolysis is intended to catalyze the breaking of cellulose bonds into sugars. However, in this study, it was found that acid hydrolysis did not adequately affect the sugar concentration in the sample as the results were much lower than the direct fermentation. In addition, neutralization with NaOH is considered less environmentally friendly. This hydrolysis was carried out by heating the HCl and the material with a ratio of 3: 1 at 100^0C for 10 minutes and then measuring sugar. DNS test results showed sugar levels were below 200 ppm and lower pH reached was 0.8. Whereas the amount of fermented sugar without hydrolysis is much better as shown in the next section. The amount of sugar is important to measure because it is the material hydrolysed to produce ethanol through fermentation. The data in Figure 3 shows the standard curve of sugar and ethanol to be used as the basis for calculating sugar from fermentation.

Sample A is the fermentation of solid waste and water with *S. cerevisiae*. Measurements of ethanol and sugar levels in sample A were performed on days 1, 2, 4, and 8. The ethanol content of sample A increased from day 1 to day 4 and decreased on the 8th day. The highest ethanol content was found on day 4, indicating the optimal ethanol content of sample A was found at day 4. Until this 4th day also more than 2300 ppm sugar is converted for 4 days fermentation. This is the optimum phase of harvesting, where most of the sugar from the sample is enzymatically converted by the yeast. This result almost matches the study of *Hanum et al*. (2013) which produced 18.99% ethanol in 48 hours. *S. cerevisiae* growth curve in accordance with this study, includes the phase of adaptation, log, lag or stationer and death. The peak or end of the log phase is the proper time for harvesting. In this phase the density of the solution will decrease due to the increase of ethanol concentration by yeast (Khodijah and Abtokhi 2015). After the supply of sugar as a carbon source declined, some of ethanol will be converted to acetic acid by yeast, therefore after the peak phase, there is a significant decrease in ethanol content. In this research, the solid waste fermentation was done on the 4th day.

Based on Table 4, it can be understood that the ethanol content of liquid waste fermentation of tapioca maximum production obtained is 17.57% on the 6th day. With the same amount of products, liquid waste requires longer fermentation time. On the other hand, the measured sugar content of the liquid waste tends to be lower, not more than 300 ppm. Therefore, solid waste fermentation is considered better in producing bioethanol. As in Table 5, it can be seen that solid waste fermentation with acid hydrolysis actually produces sugar lower below 200 ppm. In addition, the resulting ethanol was also low, around 2.79% on the 2nd day of incubation. It can thus be concluded that a more effective and efficient formulation of bioethanol production is a 1: 3 solid waste inwater in with 2 g of *S. cerevisiae*.

If the distillation is optimal, 17 mL of ethanol from 1 liter of fermentation fluid

can be obtained. Through mathematical calculations, it takes 3 kg of waste to produce 1L of bioethanol for 4 days. Surely this is encouraging because it requires a little material with a low economic value to be converted into energy sources with high economic value. The waste base is another advantage of bioethanol production to support cleaner production in the industry. In addition to economics, bioethanol is considered to be more mechanically efficient because it has a high octane value of 111. This exceeds the quality of Indonesian low and high octane petrol as some of the 88 and 92 octane gasoline grade.

The potential result obtained is the value of sugar concentration in 1 day of fermentation without acid hydrolysis is as high as 2600 ppm. This makes for more environmentally friendly energy production without the need for acid hydrolysis that can pollute the environment or require extra production costs. This clean and economical production is supported by ethanol concentrations of up to 17%. This research needs to be continued with a larger scale fermentation to cover fuel demand for a Low Cost Green Car.

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