Bioethanol production from cassava-based industrial wastes using acid hydrolysis and simple fermentation

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Abstract. Tapioca flour industry produces starchy wastes. Improper handling of this waste can cause environmental problems. The bioconversion of starchy waste to bioethanol has been widely performed. However, the utilization of solid waste from the tapioca flour industry for bioethanol production is still limited. This research is a preliminary investigation to determine the level of ethanol produced from cassava pulp and peel by the fermentation process. Cassava pulp and peel were prepared for the delignification process, hydrolysis process, fermentation process, and finally, the purification process. The fermentation process was carried out by varying fermentation periods, i.e., 2, 4, 6, 8, 10, 12, and 14 days. The highest ethanol concentration at 6.2% was obtained after eight days of fermentation.

1. Introduction
A critical factor in human life that affects all lines of life, ranging from government, economics, education, and society, is the availability of energy. Energy consumption will increase along with the increase in the human population. At present, fossil fuel is still the most important source of energy in Indonesia, and its share has increased from 43% in 2010 to 62% in 2014 [1]. Moreover, transportation played a significant role in energy consumption and pollution subsequently. Caused by the intense growth of greenhouse gas emission, efficient and sustainable improvement of the transportation sector has elevated the concern in many nations [2]. The main problem of fossil fuels as the energy source is the CO₂ emission that can cause environmental pollution. The greenhouse gases can form layers in the atmosphere to retain solar heat and causing the global warming [3]. In addition to causing environmental pollution, fossil fuels also have limited availability, thus causing a world energy crisis. The world energy crisis is a problem that is being faced by many countries, including Indonesia. This crisis occurs due to dependence on the fulfillment of energy from fuels used from fossil fuels. Efforts to utilize alternative energy sources can be used as a solution in reducing use as fossil fuels [4].

Currently, an alternative energy that is being developed is bioethanol. Bioethanol is renewable, low-pollution bioenergy and can be produced from low-cost materials containing sugar and starch such as corn stover, potato peels, wheat peel, sugar cane bagasse, and molasses. The need for ethanol, such as for solvent, disinfectant, chemical factory raw material, has also been increased these days. Ethanol (C₂H₅OH) could be produced from the fermentation process of sugar from carbohydrate sources with the help of microorganisms. On the other hand, the use of agricultural land to produce bioenergy crops will compete with the cultivation of food crops. Bioenergy production from cultivated plants requires higher costs compared to energy production from petroleum. Therefore, an
alternative source of cheap and abundant raw materials is needed [5]. A production based on small holder production may involve a transformation of the existing production system and it is therefore imperative to investigate opportunities and barriers for expansion of cassava production [6].

Indonesia is one of the countries that produce the most abundant cassava in the world. The demand for tapioca flour with cassava as the raw material tends to increase along with the increasing number of food industries that use tapioca as its main ingredient. At present, Indonesia has not been able to fulfill the global market demand for cassava due to an increase every year by 10% or 1.3 million tons per year. Almost 70% of the Indonesian cassava crop product comes from Sumatra, while the rest is produced from Java and Sulawesi. This indicates that Indonesia has vast business potential and tapioca demand [7].

Tapioca flour industries always produce starchy wastes, which can be converted into a value-added product. Improper handling of this solid starchy waste can cause environmental pollutions. Waste control and management efforts are essential to creating a clean, safe, and healthy environment. Along with the increasing amount and quality of wastes, various reasonable efforts to reduce solid waste have been carried out, such as recycling or reusing [8]. Tapioca flour industrial wastes such as cassava peel and ‘onggok’ (solid waste) can be used as an energy source in the form of ethanol. Cassava peel and ‘onggok’ are starch-rich wastes, the potential for bioethanol production. Bioethanol production refers to the four major aspects, i.e., raw materials, conversion technology, hydrolysis processes, and fermentation configurations [9]. In this research, the cassava peel and pulp fermentation process for bioethanol production utilized Saccharomyces cerevisiae [10].

2. Methods
2.1. Materials
Cassava peel and ‘onggok’ or solid wastes were obtained from a local tapioca industry in Semarang, Indonesia. Saccharomyces cerevisiae strain was provided by the Laboratory of Integrated Science, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Indonesia. All chemicals with analytical grade were obtained from Merck, Germany, such as HCl, H2SO4, (NH4)2SO4, and NaOH.

2.2. Pre-treatment of cassava peel and ‘onggok’
Cassava peel was soaked for three days in water and then was cut into smaller pieces. Then, cassava peel and ‘onggok’ were air-dried at room temperature for five days. Cassava peel and ‘onggok’ peel were then ground separately using mortar and pestle until a fine powder was obtained. Subsequently, the cassava peel powder and ‘onggok’ powder were sieved using a 40-mesh sieve. After that, the powders were oven-dried at ± 105 °C for 2 h.

2.3. Delignification of cassava peel and ‘onggok’ powder
Delignification was done by adding 300 mL distilled water and 35 mL 10% (w/v) NaOH to the mixture of 12.5 g cassava peel powder and 12.5 g ‘onggok’ powder in a 500 mL Erlenmeyer flasks. The mixtures were then heated and stirred using a magnetic stirrer for 30 min at 160 °C. Subsequently, the solution was filtered using Whatman No. 1 filter paper. The filtered residue was washed with distilled water until a neutral pH was obtained. Then, it was oven-dried at 105 °C for 2 h. After drying, the delignified powders were then ground using mortar and pestle until a fine powder was obtained. Then, it was sieved using a 40-mesh sieve.

2.4. Hydrolysis of delignified cassava peel and ‘onggok’ powder
The hydrolysis process was performed by varying HCl concentration at 15% (v/v) and 7% (v/v), as well as H2SO4 concentration at 15% and 7%. As much as 25 g cassava peel powder and ‘onggok’ powder were put into beaker glass. Then, the 30 mL acid solutions were added. Then, the mixtures were heated at 100 °C for 2 h. After heating, the mixtures were then filtered using Whatman No. 1 filter paper. The absorbance of filtrate obtained was measured by using a UV-Vis Spectrophotometer at 630 nm.
2.5. Fermentation process

The starter culture of *Saccharomyces cerevisiae* was prepared by adding 10 g of starter powder to 50 mL of 10% (w/v) sucrose. It was then incubated at room temperature for 24 h to obtain $1 \times 10^7$ cells/mL. The fermentation process was carried out by taking the filtrate from the hydrolysis results. The filtrates pH was adjusted to 4.5 by adding 6M NaOH solution. Then, it was added with 0.5% (w/v) (NH₄)₂SO₄ and then was pasteurized at 80 °C for 15 min. After that, it was added with 10% (v/v) of starter culture yeast to obtain $1 \times 10^6$ cells/mL. Then, the fermentation process was carried out by allowing the solutions to stand at room temperature 30 ± 2 °C for 2, 4, 6, 8, 10, 12, and 14 days.

3. Results and Discussion

The cassava-based industrial wastes such as cassava peel and ‘onggok’ or solid waste are the potential to be converted to bioethanol with the simple step of pretreatment and fermentation. In this research, cassava peel and ‘onggok’ were obtained from the local tapioca industry. Onggok is a waste that has a hard texture depending on the results of extortion. This starchy waste is obtained after grating and pressing the cassava (*Manihot esculenta* Crantz and *Manihot utilissima*). The cassava peel waste is obtained from the cassava cleaning processes. Based on the observation in this research, one kilogram of cassava could produce 15-20% cassava peel.

The purpose of pretreatment and delignification processes can help the lignocellulose breakdown simple sugars. The oligo- and monosaccharides could be released and help to ease the fermentation process by the yeast [11]. According to the previous research, 10% NaOH is the optimum concentration for pretreating the cassava peel and ‘onggok’ with the lignin content at 2.035% and sugar level at 4.279% after pretreatment [12]. In this research, the hydrolysis process was carried out four times by adding the various concentration of 15% and 7% H₂SO₄; 15% and 7% HCl. Hydrolysis was performed, and the results of the hydrolysis process could be observed by color-changing after the treatment as presented in Table 1.

| Acids  | Concentration (% v/v) | Colour   |
|--------|------------------------|----------|
|        | 15                     | Dark brown |
|        | 7                      | Light brown |
| H₂SO₄  | 15                     | Dark brown |
|        | 7                      | Light brown |
| HCl    | 15                     | Dark brown |
|        | 7                      | Light brown |

The hydrolysis process using the acids can cause the color-changing since the lignin, hemicellulose, and cellulose have been converted to glucose. Dark brown color indicates that the optimum conversion was obtained. On the other hand, the presence of high acid concentrations dramatically affects the strength of acid hydrolysis which causes continued degradation between hemicellulose and cellulose to glucose. In this research, 15% concentration of acids was optimum for the hydrolysis process [13]. The glucose concentration of each filtrate was analyzed, and the results are shown in Table 2.

| Acids  | Concentration (% v/v) | Glucose Concentration (%|)
|--------|------------------------|-------------------------|
|        | 15                     | 4.8 ± 0.021             |
|        | 7                      | 6.0 ± 0.017             |
| H₂SO₄  | 15                     | 8.7 ± 0.034             |
|        | 7                      | 0.6 ± 0.042             |
| HCl    | 15                     |                         |
|        | 7                      |                         |
In the hydrolysis process, the highest glucose levels were obtained at 15% HCl. H\(^+\) in HCl forms the free radicals from cassava peel and ‘onggok’ powder and reacts with the OH\(^-\) group from water to produce glucose [3], whereas 7% HCl did not produce sufficient free radicals; therefore the glucose concentration is low. Both 15% and 7% H\(_2\)SO\(_4\) gave low acid levels; therefore, the water content decreases and results in low glucose levels. The fermentation process is the most critical stage in ethanol production. Fermentation is a biochemical process in which microbes play a role in fermentation by producing enzymes that can convert substrates into bioethanol. The fermentation process involves *Saccharomyces cerevisiae*. The fermentation medium is a crucial factor in the bioconversion of the substrate into the product. In this research, cassava peel and ‘onggok’ powder were used as the medium to support yeast growth and bioconversion of the starchy medium to bioethanol. Cassava peel and ‘onggok’ powder are the carbon source that can be utilized by the yeast.

In alcohol fermentation, the fermentation medium must contain carbon sources that can be utilized, such as fructose, galactose, and sucrose. *Saccharomyces cerevisiae* can produce zymase and invertase, which convert glucose to bioethanol. Glucose fermentation goes faster, and spontaneously occurs since it is a nature of alcohol fermentation. The fermentation of 25 g cassava-based industrial waste to alcohol produced bioethanol in the concentration ranging from 3.1-6.2%, as presented in Figure 1.

![Figure 1. Bioethanol concentration (%) and glucose concentration (%) during the fermentation process from 0-14 days](image)

Ethanol concentration tends to increase started from day 2 to day 8. It reached the optimum concentration on day eight at 6.2%. Figure 1 shows the trend of the glucose that was utilized by the yeast strain to support its growth and to develop an ethanol product. After day 10, the trend of ethanol production was decreased since glucose was used up by the yeast. It could probably because ethanol was converted to other compounds such as carboxylic acids or esters. It indicates that high levels of ethanol are related to more sugar used by the yeast strain. Therefore, the sugar concentration was decreased, and the lower concentration of ethanol was obtained. The higher the concentration of substrates or reducing sugars that are broken down by microorganism cells into ethanol, the higher the ethanol produced [11]. It is in line with the research, which showed that ethanol production by *Saccharomyces cerevisiae* from cassava at 15.82% was achieved within six days with glucose levels at 3.198% [14]. Maximum ethanol produced after 72h period of fermentation by S. cerevisiae resulted in an ethanol yield of 0.44 with a fermentation efficiency of 85.4 % [15].

4. Conclusion

Bioconversion of 25 g cassava peel and ‘onggok’ powder using the fermentation method by *Saccharomyces cerevisiae* produced the highest ethanol at 6.2% with 6.8% glucose levels within eight days of fermentation.
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