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Low-cost bioethanol production from wild cassava (*Manihot glaziovii*)

Siti Zulaiha
Faculty of Animal Science and Agriculture, Universitas Islam Negeri Sultan Syarif Kasim Riau, Indonesia

Email: siti.zulaiha@uin-suska.ac.id

Abstract: Many countries have concluded that using renewable energy sources is necessary to avoid an energy crisis that would occur as conventional energy supplies run out. The potential availability of biomass in Indonesia enables for the conversion of this biomass into environmentally beneficial alternative energy. Wild cassava is a biomass that can be used as an alternative energy source. The aim of this study was to see if cassava might be used as a simple raw material for manufacturing bioethanol. The bioethanol produced is expected to have the potential to become an environmentally friendly alternative energy. Preparation (drying and gelatinization), hydrolysis (liquification and saccharification), and fermentation are the steps of the bioethanol production process. The findings revealed that cassava can be converted to bioethanol using tempeh yeast (*Rhyzopus oryzae*) and baker's yeast (*Saccharomyses cerevisiae*). *Rhyzhopus oryzae* participates in the saccharification process by taking the place of the costly alpha amylase enzyme. *Saccharomyces cerevisiae*, on the other hand, is involved in the fermentation process. The bioethanol produced can be identified by the presence of a tape smelling odour, and any research findings are consistent with published data.

Key Words: Bioethanol, wild cassava, alternative energy, *Manihot glaziovii*.

1. INTRODUCTION :

Gasoline is an essential liquid, especially in today's world of increased human mobility (Prihandana, 2007). However, as conventional fuel stocks shrink, gasoline's supply will be diminished in the future. There will be an energy crisis as a result of this. Substituting bioenergy, such as bioethanol, for fossil oil is one of the reasonable ways to reduce the energy issue (Prihandana, 2007). Because the octane number of ethanol is quite high, specifically 135, bioethanol can be used to enhance the octane number of gasoline. The higher the octane level, the less likely the gasoline is to burn itself, resulting in a more consistent combustion process and more consistent power. Carbon monoxide (CO) gas emissions will be reduced by a combustion process with more perfect power. Only a 3% bioethanol blend can reduce CO emissions to 1.35% (Anonymous, 2007). Bioethanol is a liquid produced by microorganisms fermenting sugar from a carbohydrate source (starch) (Marques, 2007).

The number of tubers in Pekanbaru city in 2010 was 10,964,000 tons, according to data from the Food Crops Service processed by the Resilience Agency in Riau Province. Based on this number, Pekanbaru's bioethanol production potential is considered to be extremely strategic. On Bangau Sakti street in Pekanbaru city, several tubers, particularly wild cassava, were discovered. According to the preliminary study, nearly all sides of the road are overgrown with wild cassava, which is very valuable, and the local community does not take advantage of the situation. Wild cassava is a tuber that is not present in food and contains the hazardous HCN (cyanide acid). Because wild cassava (*Manihot glaziovii*) contains up to 98.5% carbohydrates, it has the potential to be used as a bioethanol feedstock. Wild cassava may be used to make bioethanol because it is inexpensive and easy to grow.

According to Amalia et al. (2013)'s research, bioethanol can be generated from wild cassava and utilized as a fuel for domestic stoves to speed up the conversion of kerosene to biofuels. Bioethanol can be utilized as a fuel for domestic stoves at a concentration of 94%. The final product had a 94% ethanol level, a 168-hour fermentation time, and a yeast mass of 15 grams.

The saccharification stage is one of several stages in the bioethanol production process from wild cassava. In general, alpha-amylase and gluco-amylase enzymes are utilized at this stage, which are highly expensive and therefore not accessible to the general people. The role of the alpha-amylase enzyme seems to be substituted by the fungus *Rhizopus oryzae*, which can manufacture extra amylase enzymes in an aerobic state (Crueger and Crueger, 1984). This is the groundwork for the author to perform this preliminary research in a straightforward manner, with the research procedure being carried out at a low cost on a household scale.

According to the previous description, the article uses wild cassava as a raw material for ethanol synthesis in order to provide ecofriendly and community-based alternative energy.

2. Materials and methods :

This study is an experiment on generating bioethanol with a specific type of SHF (separate-hydrolysis-fermentation). SHF is an ethanol production method in which the hydrolysis and fermentation stages are carried out independently. Separately from the fermentation process, raw sources containing starch go through a hydrolysis phase (liquification and saccharification). In addition, the technique for producing bioethanol from wild cassava is as follows.

2.1 Preparation of wild cassava

Cassava that grows wild and unutilized is harvested using a purposive sampling strategy that involves identifying young cassava, as seen by the beautiful cream color of the cassava skin. Wild cassava are then covered in plastic and sent to a laboratory for additional processing.

2.2 Gelatinization

To remove the HCN content, wild cassava is cleansed of attaching dirt with clean water, then sliced into thin cubes and dried in the sun for two days. The dried cassava is then mashed until smooth, and the gelatinization procedure is used to turn it into a slurry. To make the hydrolysis process much easier, the gelatinized cassava pulp is separated into solids and liquids.

2.3 Hydrolysis

Liquification and saccharification are the two steps of the hydrolysis process. During the liquification step, the sample is heated till it thickens, whereas during the saccharification stage, the slurry is cooled to 50°C and 1 tablespoon of Rhyzopus oryzae is added. The slurry is then placed in a container. After cooling for 7 hours, the cassava porridge forms two layers, one with sugar deposits on the bottom and the other with water on top.

2.4 Fermentation

2 layers generated in the previous stage are stirred repeatedly, and 1 tablespoon of Saccharomyces cerevisiae (baker's yeast) is added. The container is then wrapped in plastic covers to create anaerobic conditions. Meanwhile, cut the top of the porous plastic into little pieces with a needle to get CO_2 out of the system.

2.5 Distillation and examination with a pycnometer

The distillation process uses heat to extract ethanol from the fermenting solution. The heating temperature in this distillation process is kept at 78^{o}C, the boiling point of ethanol, such that the ethanol evaporates first and the evaporation flows into the condensed pipe, returning to liquid ethanol. A pycnometer is used to measure the ethanol content of the distillate collected during the distillation process. This method, on the other hand, can be carried out in the advanced research stage.

2.6 Statistical analysis

All experiments were performed in triplicate. The data obtained were analyzed by a qualitative descriptive method.

3. Results and Discussion :

Wild cassava (Figure 1) is a plant whose tuber produces a harmful substance known as HCN. The drying process is one method for removing HCN levels. In addition to lowering HCN levels, wild cassava must be dried to reduce the quantity of water it contains. Following a two-day drying period, the weight of wild cassava reduced by up to 74% in this study. The following table shows the characteristics of cassava before and after drying.

Table 1 represents the wild cassava before it is processed. The drying stage has white features and contains sap that can cause skin irritation. The color of the wild cassava changes to a yellowish tone after drying. This variation is attributable to a less-than-ideal drying procedure. After the drying process, the sample was then heated at a temperature of 100^{o}C for 30 minutes until it became a thickened slurry (gelatinization) and proceeded to the hydrolysis stage.

Hydrolysis is a reaction in which reactants and water combine to break or breakdown a molecule. Water attacks starch at 1-4 alpha glucoside bonds in the hydrolysis reaction, producing dextrin, syrup, or glucose depending on the degree of breakdown of the polysaccharide chains in starch. Because the reaction between water and starch is so sluggish, a catalyst is required to boost the water's activity. An acid or an enzyme can be used as a catalyst. Liquification and saccharification are two steps in the hydrolysis process.

Table 1. Cassava characteristics before and after drying

Characteristics	Before drying	After drying
Color	White	Yellowish
Sap	Contains a lot of sap	Sap begins to decrease

Figure 1. Wild cassava

However, the most common issue is the high cost of enzymes, making them difficult to obtain. To get over this, the authors propose replacing the enzyme with tape yeast (*Rhyzopus oryzae*). *Aspergillus* and *Rhyzopus* fungi are two microorganisms capable of generating amylase enzymes. This fungus has also been widely employed as a starch hydrolyzer and a producer of amylase enzymes in the bioethanol production process. *Aspergillus oryzae, Aspergillus niger*, and *Rhizopus oryzae* are some of the most commonly utilized fungi.

The hydrolysis stage of the liquification process generates a thick gum slurry, whereas the 7-hour saccharification phase produces two layers, one of water and the other of sugar deposits. Fermentation, which is the act of breaking down organic compounds into simple compounds with the help of microorganisms to produce energy, is the next step in the process of creating bioethanol from wild cassava. Ethyl alcohol (ethanol) and CO_2 can be produced by *S. Cerevisiae* fermentation. The odor produced when the fermentation process was completed, especially the presence of a tape-like scent, was employed in this investigation to evaluate the existence of alcohol content in the sample.

The fermentation procedure in this investigation lasted 7 days. According to prior study, the best time to start the fermentation process is after 7 days, when a lot of glucose is converted to ethanol. The fermentation process, however, cannot be carried out for more than 9 days because the amount of ethanol generated would decrease. This is because the main producers of alcohol are yeast, especially *S. cerevisiae*.

The fermentation process was carried out in this investigation under anaerobic conditions. After 7 days, three layers with a tape-like scent formed (from top to bottom in sequence of ethanol, water, and protein). The author only went as far as fermentation in this investigation. The authors will conduct additional study for the distillation stage and measuring the ethanol concentration produced with a pycnometer.

4. CONCLUSIONS :

Preparation (sample collection, drying, and gelatinization), hydrolysis (liquification and saccharification), and fermentation stages are all straightforward steps in the process of producing bioethanol from wild cassava. The use of enzymes in the saccharification stage to obtain ethanol can be replaced by tempeh fungi (*Rhyzopus oryzae*). This is because under aerobic conditions, *Rhyzopus oryzae* can produce additional amylase enzymes. On a small scale, easy bioethanol production is possible, notably through a 7-day fermentation process. Following the fermentation process, a tape-like odour may be detected, indicating the existence of bioethanol.

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