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THE ROLE OF BIOTECHNOLOGY IN PROCESSING JACKFRUIT SEED WASTE TURNS INTO BIOETHANOL

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Abstract. The jackfruit plant is a type of tropical fruit plant that is multifunctional and can be planted in tropical areas at an altitude of less than 1,000 meters above sea level. Research has been carried out on making bioethanol from waste jackfruit seeds (Artocarpus heterophyllus Lamk). This research aims to find out whether jackfruit seed waste can be used as raw material for making biethanol and to determine the effect of pH on the fermentation process. This research used jackfruit seeds which were hydrolyzed with H2SO, 2.5% for 3 hours at a temperature of 70 °C, then the glucose content was measured and fermented at varying pH = 3, 4, 5, and 6 for 48 hours. The highest bioethanol content of jackfruit seeds was achieved during 4 days of fermentation with an alcohol content of 13.19%.

Key words: *bioethanol, jackfruit seeds, fermentation*

INTRODUCTION

As technology develops and population increases, fuel needs also increase. The fossil fuels that exist today cannot be expected to last for a long time. In 2002, Indonesia had oil reserves of around 5 billion barrels, natural gas around 90 TSCF, and coal around 5 billion tons. If no new reserves are found, it is estimated that petroleum will run out in less than 10 years, natural gas in 30 years, and coal will run out in around 50 years.

Among the alternative fuels currently being developed is bioethanol. Bioethanol is flammable and has a large net heat of combustion, which is approximately 2/3 of the net heat of combustion of gasoline. At a temperature of 25ºC and a pressure of 1 bar, the net heat of combustion of ethanol is 21.03 MJ/liter while gasoline is 30 MJ/liter. Pure ethanol also dissolves completely in gasoline in all proportions and is a high-octane blending component.

Bioethanol is a type of ethanol (alcohol) produced through the fermentation process of organic materials containing sugar or starch by microorganisms, such as yeast or bacteria. Bioethanol is usually produced from biological sources such as plants, grains, or agricultural waste. This differs from conventional ethanol, which is often produced from petroleum.

Jackfruit has a sweet aroma and is liked by many people. Apart from that, the texture of jackfruit flesh is chewier than durian, and the taste of the fruit is sweeter with a touch of savory.

The jackfruit plant (*Artocarpus heterophyllus* Lamk.) is a type of tropical fruit plant that is multifunctional and can be grown in tropical areas at an altitude of less than 1,000 meters above sea level originating from South India. There are two types of jackfruit, namely: *Artocarpus heterophyllus* Lamk or *Artocarpus integer* (Thumb) Merr which is usually called jackfruit and *Artocarpus champeden* (Lour) Stokes or *Artocarpus integrifolia* Lf. which is usually called cempedak. Cempedak has rough hairs on its leaves and has a specific and sharp fragrant aroma, while jackfruit does not. Taxonomic position of jackfruit plants are as follows :

Kingdom	: Plantae
Division	: Spermatophyta
Sub-division	: Angiosperms
Class	: Dicotyledonae
Order	: Morales
Family	: Moraceae
Genus	: Artocarpus
Species	: Artocarpus heterophyllus Lamk.

Jackfruit Seeds

Jackfruit seeds are a local source of starchy material that has the potential to be used as a raw material source for making bioethanol in Indonesia because of its sufficient availability. overflow. Fiber content in seeds high jackfruit, namely around 36.7%. Making bioethanol from jackfruit seeds goes through two stages, namely the acid hydrolysis process which is then followed by the fermentation process. Jackfruit seeds (*A. heterophilus* Lam.) have great potential but not yet exploited. The use of jackfruit seeds in the food sector is very low, only around 10%. This is caused by the lack of public interest in processing jackfruit seeds. The starch content of jackfruit seeds can undergo hydrolysis to become jackfruit seed starch hydrolyzate which has a high sugar content so it can be used as a carbon source in ethanol cultivation. Jackfruit seeds can be used as raw material for making bioethanol. Use of jackfruit seeds as bioethanol has advantages such as jackfruit seeds which are easy to obtain and the price is relatively cheap as well has sufficient starch content so that it can be used as a soluble carbohydrate.

Bioethanol

Bioethanol is ethanol obtained from natural resources that can be renewed as an alternative non-fossil fuel and can generally be used through a fermentation process. Ethanol with the compound name ethyl alcohol with the molecular formula ($C2H5OH$) contains a clear, colorless solution, which is biodegradable, has low toxicity and does not cause major air pollution if it leaks.

Hydrolysis

The enzymatic hydrolysis process is widely used because it will reduce the environmental impact compared to the addition using an acid catalyst to the fermentation process which can produce bioethanol . The conversion process that occurs between carbohydrates into simple sugars is called hydrolysis. Hydrolysis is a reaction that occurs between a substance and water ($H2O$) so that the compound will decompose. The hydrolysis reaction process takes a long time so it is necessary to add a catalyst to speed up the reaction in the form of a hydrochloric acid (HCl) or sulfuric acid ($H2SO4$) catalyst.

Fermentation

Fermentation, namely the process of chemical changes in an organic substrate due to the activity of enzymes produced by microorganisms. However, the microorganisms used are not pathogenic so they are safe for the body. Furthermore, the fermentation process produces alcohol, acid and gas compounds. The purpose of the fermentation process is to preserve food. This can occur due to the change in carbohydrates into organic acids so that food can last longer. It is safe for humans to consume fermented food because the fermentation process uses pathogenic microorganisms, therefore consuming fermented food is safe for the body's health. The fermentation process can produce gas, acid and alcohol.

Instant yeast

Instant yeast is a leavening agent used in making cakes and bread. Instant yeast has a faster fermentation process compared to regular yeast, thereby speeding up the production time of bread and cakes . Instant yeast generally consists of active yeast that is dried and ground into powder. The difference between instant yeast and regular yeast lies in the fermentation process. Ordinary yeast takes longer to ferment, so it takes longer to make bread and cakes. Instant yeast can also improve the taste and quality of the final product. Due to the faster fermentation process, instant yeast produces greater amounts of $CO2$ gas in a shorter time.

Yeast Tape

Tape yeast is a mixed population consisting of species of the genera Aspergilius, Saccharomyces, Candida, Hansenulla, and Acetobacter bacteria. Tape yeast is used to make fermented products such as sticky rice tape and cassava tape. Ragi tape comes from rice flour mixed with other ingredients so that it can help in the fermentation process. In this yeast there are microorganisms that can convert carbohydrates (starch) into simple sugars (glucose) which are then converted again into. Fermented carbohydrates (starch) produce large amounts of lactic acid which will lower the pH value, giving rise to a sour taste in the yogurt. Apart from that, tape yeast contains microorganisms which, under anaerobic conditions, produce amylase and amyloglucosidase enzymes, two enzymes that are responsible for breaking down carbohydrates into glucose and maltose.

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MATERIALS AND METHODS

Making Jackfruit Seed Flour: In the process of making jackfruit seed flour, the raw material in the form of 5.6 kg of jackfruit seeds is washed thoroughly to remove dirt attached to the seeds. The clean jackfruit seeds are then bruised and dried for 4 days using sunlight. The dried jackfruit seeds are then weighed to determine the water content in the jackfruit. It was found that the weight of the jackfruit seeds after the drying process was 2.4 kg. The dried jackfruit seeds were then separated from the outer skin that was attached to the seeds. The seeds that have been cleaned from the skin are then peeled to get jackfruit seed flour. The 2.2 kg of jackfruit seed flour obtained was cloudy white in color.

Hydrolysis: Hydrolysis is a process between reactants and water so that a compound breaks down, in this case the compound in question is a polysaccharide (starch) which breaks down into a monosaccharide (glucose). The hydrolysis process using an acid catalyst requires high temperature operating conditions. The hydrolysis process takes place at a temperature of 100°C using a magnetic stirrer with a ratio between the raw material in the form of jackfruit seed flour and the catalyst solution is 1:10. The catalyst used in the hydrolysis process is H2SO 4 0.3 M 600 mL and uses 60 grams of jackfruit seed flour. The use of H2SO4 as a catalyst is the best acid catalyst to break down starch chain bonds into glucose and can dissolve in all concentrations of water. The purpose of adding a catalyst is to speed up the reaction process. The reaction of water with starch is very slow, so it is necessary to add more H2SO4 than water to help the reactivity of water in breaking down compounds so that they decompose more quickly. The results obtained from the hydrolysis process undergo several physical changes, namely the color from light brown to dark brown reddish. These changes are because the compounds in jackfruit seed flour have broken down and become homogeneous. After the hydrolysis process is complete, the solution is cooled to room temperature and then filtered using calico cloth to separate the filtrate and residue. The filtrate obtained was 580 mL and was brownish red in color.

Starter Making: The process of making a starter functions to activate bacteria before the fermentation process is carried out. The starter making process is carried out by taking 9% of the total volume of hydrolysis results, namely 54 ml and then adding NaOH. The function of adding NaOH is to increase the pH, when the solution initially has a pH of 1, then add NaOH until it reaches a pH of 4.5. The pH adjustment process functions to optimize the activation process because bacteria do not survive in solutions that are too acidic. After reaching a pH of 4.5, nutrients are added in the form of NPK and urea as much as 3 grams/liter. The addition of nutrients functions to provide nitrogen and potassium elements in the solution. After adding the nutrients, 9 grams of bread yeast was added to the solution, the mixture was then stirred and left to rest for 30 minutes.

Fermentation Process: Fermentation is the process of converting reducing sugars into bioethanol using the help of microorganisms. Generally, bioethanol production uses the fungus Saccharomyces cerevisiae. This fungus can be used to convert sugar into ethanol with good conversion capabilities (I. Nyoman W. 2011). Before carrying out the fermentation stage, first make an inoculum which aims to shorten the lag (adjustment) phase, namely by adapting the cells to the fermentation medium. The solution used to make the inoculum is 100 ml of distilled water to which 5 g of glucose, 0.5 g of yeast extract, 0.5 g of KH2PO4, 0.15 g of NH4Cl, 0.07 g of MgSO4, and 0.17 g KCl which is then mixed until homogeneous and covered tightly with sterile cotton/cotton cloth. The inoculum medium was sterilized in an autoclave at a temperature of 121ºC for 15 minutes, after which the inoculation medium was cooled to room temperature. After the temperature of the inoculation medium reaches room temperature, Saccharomyces cerevisiae is put in 2-3 oases, then inoculated in a rotary shaker at 180 rpm for 24 hours (Amalia, 2014) (Siburian et al., 2015).

In this stage, variations in fermentation time were used, namely 1, 3, 5.7, 9 and 11 days to determine the bioethanol content obtained from the fermentation process. The fermentation process begins by mixing 600 ml of the liquid phase resulting from hydrolysis containing sugar with a pH of 4 with 2-3 oases of Saccharomyces cerevisiae fungus and adding 12 ml of inoculum. The solution that has been mixed homogeneously is ready to ferment for 1, 3, 5, 7, 9, and 11 days and at each time variation, samples are taken, then extracted (distilled) and stored in the freezer. After waiting until the maximum time for the variation, the fermented solution is ready to be distilled. (Alegre, et al. 2003; Karimi, et al. 2006; Rouhollah, et al. 2007).

RESULTS AND DISCUSSION

Bioethanol from jackfruit seeds: The raw materials for making bioethanol are sugary, starchy and fibrous materials. This research tries to make bioethanol from jackfruit seeds which are a starchy material. The hydrolysis process is carried out for each H2SO4 acid concentration variable (0.1, 0.5 and 1 M). The acid hydrolysis process is carried out to convert 36.7% of the cellulose in ripe jackfruit seeds in the form of starch into glucose. The highest glucose content resulting from hydrolysis is achieved when the concentration of the H2SO4 solution is 0.5 M. In the hydrolysis process the H+ group from H2SO4 will change the starch groups from jackfruit seeds into free radical groups. The free radical group will then bond with the OH - group from water and react at a temperature of 100ºC for 1 hour which will produce glucose. When the concentration of the H2SO4 solution is 0.1 M, the need for H+ from H2SO4 is not sufficient so that not many free radical groups are formed from jackfruit seed starch and the glucose produced is not optimal.

Increasing the concentration of the H2SO4 solution will form more free radical groups, but increasing the concentration of the H2SO4 solution causes less water in the composition of the hydrolysis solution. So the need for OH - as a free radical scavenger is reduced and less glucose is produced. Thus, the most optimum acid concentration during the hydrolysis reaction to hydrolyze starch from jackfruit seeds into glucose is 1 M. The higher the glucose concentration, the greater the bioethanol that will be formed because the material that will be fermented into bioethanol is glucose. So the concentration of H2SO4 solution of 1 M is what is used to hydrolyze jackfruit seeds before carrying out the next stage, namely fermentation with a variable length of fermentation time. The results of the study showed that the highest glucose levels obtained were at a concentration of H2SO4 0.1 M of 4.17%, H2SO4 0.5 M of 5.35% and a concentration of H2SO4 1 M of 7. 06% for a temperature of 60ºC in 30 minutes. For a temperature of 60ºC for 30 minutes, the glucose level was 4.61%, for 60 minutes 11.67 and for 90 minutes 4.72%. The hydrolysis temperature of 800ºC for 1 hour was 56.71%, the hydrolysis temperature of 100ºC was 13.44% and the hydrolysis temperature of 120ºC was 3.99% (can be seen in table 3). So the best hydrolysis time to use is at a temperature of 100ºC for 60 minutes with a concentration of H2SO4 1 M. The reagent used is Luff Schoorl solution which is a mixture of cupric sulfate, sodium carbonate and citric acid.

The monosaccharides formed were added to 25 ml of Luff Schoorl solution and then boiled for 10 minutes. After that it is cooled and KI is added. Cupric oxide functions as an oxidizer, in the presence of reducing sugar the cupric oxide turns into cuprous oxide. Cuprous oxide in this reaction will liberate iodine from the KI salt, where the amount of iodine released is equivalent to cuprous oxide. In the research carried out, biethanol was made by fermenting jackfruit seed powder (*Artocarpus heterophyllus*) at pH 5 with variations in fermentation time of 1 day, 2 days, 3 days, 4 days, 5 days, 6 days and 7 days. In this fermentation, the yeast type Saccharomyces cerevisiae is used which is fermentative, that is, it can ferment sugar into bioethanol in anaerobic conditions (without air), whereas if there is air, this yeast will be oxidative, that is, it oxidizes sugar into carbon dioxide and water. Jackfruit seeds (*Artocarpus heterophyllus*) which have been hydrolyzed and the glucose content calculated, the pH is adjusted to pH 5 then 10% Sacharomyces cerevisiae (fermipan) is added and fermented for 1 day, 2 days, 3 days, 4 days, 5 days, 6 days and 7 days at room temperature. Fermentation takes place anaerobically, where the Erlenmeyer (fermenter) fermentation site is closed with a rubber stopper with a hole, then a hose is placed in the hole and the other end of the hose is dipped in water so that air from outside cannot enter and the gas produced can be released through the hose.

During fermentation, gas bubbles occur in the water which come out through a hose whose end is connected to the fermenter bottle, this shows that a fermentation process has occurred which is characterized by the formation of carbon dioxide gas. The fermented sample is distilled using a simple distillation apparatus, where this process is intended to separate the mixture of water and alcohol based on the real difference in boiling point. The research results showed that using the refractive index method produced refractive index values on day 1, day 2, day 3, day 4, day 5, day 6 and day 7 with a refractive index value of 1.3382 respectively; 1.3386; 1.3391; 1.3312; 1.3409; 1.3406 and 1.3397. This is because the microorganisms (yeasts) are not yet active (initial phase) so their growth rate is low which causes relatively little bioethanol product to be produced.

The highest bioethanol content was achieved at 4 days of fermentation with an alcohol content of 13.19% . Can be seen that at 4 days of fermentation the highest bioethanol content was obtained. This is because in these conditions the microorganisms work perfectly where their growth rate reaches a maximum so that the bioethanol produced is higher.

Meanwhile, during subsequent fermentation, the product and levels of bioethanol produced decrease, which is caused by the activity of microorganisms starting to decrease, meaning that the microorganism cells that produce enzymes as biocatalysts for converting substrates into bioethanol have begun to decrease or experience a death phase. Analysis of samples with the highest refractive index results was continued using gas chromatography. A sample of 0.2 µl was injected into the injector of the gas chromatograph at an injector temperature of 100°C with a column temperature of 70°C , where the detector response was depicted in a recorder based on the potential difference in the form of a voltage versus time plot, thus producing a chromatogram with retention time and peak area. bioethanol. From these data, the bioethanol content can be calculated by comparing the peak area of bioethanol in the sample with the peak area of standard alcohol (ethanol 99.98%).

CONCLUSION

Making bioethanol from jackfruit seeds is carried out with the help of microorganism Saccharomyces cerevisiae under conditions. The highest bioethanol content was achieved in 4 days with an alcohol content of 13.19%, is because in these conditions microorganisms work perfectly where the growth rate reaches maximum, so the bioethanol produced is higher.

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