Section A-Research paper

UTILIZATION OF CASSAVA PEELS AS BIOETHANOL USING HYDROLYSIS AND FERMENTATION PROCESSES

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Abstract

The increasing use of fossil fuels causes a high demand for fossil energy sources, which in addition to being unfriendly to the environment, causing air pollution, are also non-renewable. Therefore, renewable alternative fuels are needed. One alternative fuel is bioethanol, where biobutanol can be made from cassava peel raw materials using hydrolysis and fermentation methods. Cassava peel contains starch which can be converted into glucose by hydrolysis process using HCl catalyst and then fermented with *Saccharomyces cerevisiae* microorganisms to produce bioethanol. The experimental results show that the bioethanol content that meets the standards as a fuel mixture is the result of hydrolysis with 26% glucose using a 25% HCl catalyst, the bioethanol content before distillation is 17.11% and after distillation 66.13% on the 10th day of fermentation as best result. The results of the GC-MS (*Gas Chromatography – Mass Spectrometry*) test showed that bioethanol has a wavelength that may indicate the presence of OH- functional groups or hydrogen bonds from bioethanol raw materials.

Keywords: bioethanol; fermentation; glucose; hydrolysis; cassava peel; *Saccharomyces cerevisiae*

INTRODUCTION

Currently, vehicle fuels still use conventional fossil energy sources, which in the long run can cause energy sources to run out and become unsustainable. For this reason, efforts have been made, including by making alternative fuels because almost all petroleum-based fuels can be replaced by renewable fuels produced from biomass and are more environmentally friendly, such as bioethanol.

Bioethanol is ethanol or alcohol compounds obtained through the fermentation process of foodstuffs containing starch or sugar with the help of microorganisms, and is one of the leading bioenergy sources that can reduce pollutant emissions. Bioethanol is a clean and renewable biofuel with the main benefit of reducing environmental pollution. Bioethanol can be used as a fuel mixture that is oxygenated with gasoline to produce more complete combustion and reduce polluting emissions. (Hermansyah, 2018).

Bioethanol is ethanol or alcohol compounds obtained through the fermentation process of foodstuffs containing starch or sugar with the help of microorganisms, and is one of the leading bioenergy sources that can reduce pollutant emissions, but has not been widely developed. According to (Erna, 2017), the need for ethanol is increasing both as a solvent, disinfectant, raw material for chemical factories, as well as as an alternative energy substitute for fuel oil (BBM). Ethanol (C_2H_5OH) is a solution obtained from the fermentation process of sugar from carbohydrate sources using the help of microorganisms.

In Indonesia, there are many biological natural resources that can be used as raw materials to produce bioethanol, one of which is cassava peel. This type of plant is a plant that is commonly planted in

almost all of Indonesia, so that this type of plant is a potential plant to be considered as a raw material for making bioethanol (Winarso, 2014).

One of the potential plants that can be used as raw material is to utilize the starch contained in cassava peels and fibrous materials (cellulose) such as organic waste and rice straw have now become an alternative to produce bioethanol. So that this type of plant is a potential plant to be considered as a source of raw material for making bioethanol (Hermansyah, 2018).

Cassava (*Manihot esculenta Crantz*) is a plant that is easily found in various places in Indonesia, one of which is in the Surabaya area. People in the Surabaya area only process the cassava tuber part, while the skin from cassava is disposed of as waste. Therefore, its utilization must be optimized as a source of renewable energy to reduce environmental pollution and prevent the use of fossil fuels from being depleted.

The purpose of this study was to determine the effect of HCl concentration at the hydrolysis stage and fermentation time with the obtained bioethanol content and the best conditions with the selection of HCl catalyst on the obtained ethanol content.

Cassava

Cassava (*Manihot esculenta Crantz*) is a shrub that belongs to the *Euphorbiaceae* family. Cassava is widely cultivated in tropical areas located at equatorial temperatures between 30^{o}C north and 30^{o}C south. Based on the research of (Artiyani & Soedjono, 2011), cassava peel contains quite high cellulose, around 43.6% and contains 36.5% starch or amylum. Meanwhile, according to Hikmiyati & Yanie's 2009 research (Hikmiyati & Yanie, 2009), cassava peel waste can be used as an alternative energy source in the form of ethanol. The percentage of waste from the outer shell (brown and rough) was 0.5-2% of the total weight of fresh cassava and the waste from the inner shell (reddish white and smooth) was 8-15%. The fiber content in cassava peel is only 10.5% dry weight. Based on Ifada's research, 2019, the percentage of cassava peels is approximately 20% of the tubers so that per kg of cassava tubers produces 0.2 kg of cassava peels (Ifada, 2019).

Starch

Starch is a polysaccharide consisting only of glucose monomers linked together by glycosidic bonds. It consists of two types of glucans namely amylose, a linear glucose polymer having only α-1,4 glycosidic bonds and amylopectin, a branched glucose polymer containing mainly α-1,4 glycosidic in the linear part and some α-1,6 in the branched structure.

Most starches contain about 20-30% amylose and the remainder are amylose amylopectin. Some starches do not contain amylose such as waxy corn starch, waxy rice starch, amylose-free potato, amylose-free cassava. Granular starch is less susceptible to enzymatic hydrolysis. After being heated with excess water, the structure of the starch granules is disturbed, making the glucose polymer more soluble and more susceptible to enzyme attack.

At the same time, the starch slurry becomes more viscous. This process is known as gelatinization and the temperature at which the starch changes its properties is called the gelatinization temperature. (Aurelio & Pardo, 2012)

Delignification

Delignification is the first step to break the bonds between cellulose, hemicellulose and lignin. The delignification process is a process of removing lignin from raw materials so that the result of this process is cellulose with a large enough purity. Temperature, pressure and concentration of cooking solution during the delignification process are factors that will affect the speed of the dissolution reaction of lignin, cellulose and hemicellulose. Cellulose will not be damaged during the lignin dissolution process if the concentration of the cooking solution used is low and the temperature used is appropriate. The use of temperatures above 180^{o}C causes higher cellulose degradation, where at this temperature the lignin has been completely dissolved and the remaining cooking ingredients will degrade the cellulose.

The dissolution of lignin can be divided into three phases. The initial phase of delignification occurs at temperatures below 140^{o}C and is controlled by diffusion. Above 140^{o}C, the delignification rate becomes

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controlled by chemical reactions and continues to accelerate with increasing temperature. The dissolution rate of lignin remains high during this "large delignification" phase until about 90% of the lignin has been removed. The slow final phase is called "remaining delignification" and can be adjusted to several degrees by varying the amount of alkali and cooking temperature.

The process of destroying the structure of the material with lignocellulose content is one of the steps to convert lignocellulose into sugar compounds. The right process can reduce the total cost of converting lignocellulose into alternative fuels. The delignification process is believed to be a potential process as a preliminary process in the raw material preparation stage to increase the surface area and reactivity of the acid catalyst for the hydrolysis process.

In the chemical delignification process, chemicals are used at a certain temperature, pressure, concentration and time. The chemicals used depend on the type of process and the type of raw material. One of the delignifications using acid is hydrochloric acid (HCl) where hydrochloric acid acts as an acid catalyst that can damage the polymer chains of cellulose and lignin. The reaction of breaking the bond of lignocellulose with sulfuric acid can be seen as follows:

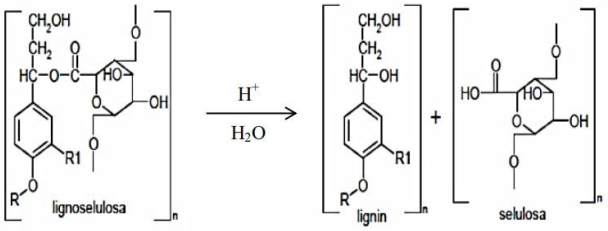

Figure 1. Lignocellulosic Bond Breaking Reaction using Acid Catalyst

The reaction in Figure 1 affects the ester bond between lignin and carbohydrates (cellulose and hemicellulose) and can release cellulose by hydrolysis of the ester bond between lignin and cellulose. Acid catalysts can hydrolyze esters into carboxylic acids and alcohols where the carbonyl oxygen of an ester can be protonated, so that the carbon is positively charged and can be attacked by weak nucleophiles such as water which then produces alcohol and carboxylic acid. This process aims to break lignin bonds, remove lignin and hemicellulose content, damage the crystal structure of cellulose and increase the porosity of the material. Damage to the crystalline structure of cellulose will facilitate the breakdown of cellulose into glucose. In addition, hemicellulose also decomposes into simple sugar compounds: glucose, galactose, mannose, hexose, pentose, xylose and arabinose. Furthermore, these simple sugar compounds will be fermented by microorganisms to produce ethanol. High acid concentration and long-time cause cellulose and hemicellulose to be more easily degraded into glucose and other sugar compounds, so that the contact between cellulose and acid is also greater and the hydrolysis reaction runs more perfectly. However, along with the high concentration and reaction time, the inhibitor produced also increases (Fessenden & Fessenden, 1982).

Hydrolysis

Hydrolysis is the process of breaking down polysaccharides in lignocellulosic biomass, namely cellulose and hemicellulose into sugar monomers that can be done chemically or enzymatically. The high content of polysaccharides makes it a promising raw material for bioethanol. Carbohydrate fractions can be converted into sugars through acid hydrolysis, acid-enzyme hydrolysis, or enzyme hydrolysis. Complete hydrolysis of polysaccharides will produce monosaccharides which are used as substrates in the bioethanol fermentation process. One part of the molecule has hydrogen ions (H^+) and the other part has hydroxyl ions (OH^-).

Generally, this hydrolysis occurs when a salt of a weak acid or weak base (or both) is dissolved in water. However, under normal conditions only a few reactions can occur between water and organic components. The addition of acids, bases, or enzymes is generally carried out to make the hydrolysis reaction occur under conditions that the addition of water does not have a hydrolytic effect. Acids, bases and enzymes in hydrolysis reactions are referred to as catalysts, i.e., substances that can accelerate the reaction. The total reaction mechanism of hydrolysis is as follows:

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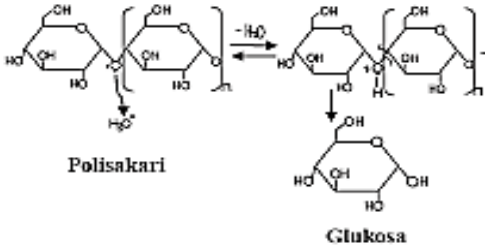

Figure 2. Hydrolysis Reaction Mechanism (Taherzadeh & Karimi, 2007)

Yeast

Regardless of the time and method of fermentation, the yeast responsible for the transformation of sugar or molasses belongs to the genus *Saccharomyces*. *Saccharomyces* is a genus of yeast that is widely used in the industrial fermentation of products that use alcohol as the final product, both for fuel and the production of alcoholic beverages. This microorganism is mostly indicated for this purpose as it collects all the materials needed to carry out the alcohol production process. Ability to quickly convert sugar to ethanol, high tolerance to the product formed, osmotolerance tolerance (tolerance to high temperature variations), and cell activity in acidic environments (Aurelio & Pardo, 2012).

Fermentation

There are several factors that affect the fermentation process, one of which is the temperature of the fermentation. The temperature during the fermentation process will determine the type of dominant microorganism that will grow. Microorganisms have a maximum and optimal temperature for their growth. Fermentation is an oxidation process which includes the overhaul of organic media in anaerobic or facultative anaerobic microorganisms by using organic compounds as the final electron acceptor. Carbohydrate fermentation by yeast is an anaerobic process of producing ethanol and carbon dioxide.

The speed of ethanol fermentation is influenced by several factors such as the composition of the substrate, the rate of nutrient use, the level of inoculation, the physiological state of the yeast, the activity of the enzymes of the EMP pathway, the tolerance of yeast to high sugar and alcohol and conditions during fermentation. White sweet potato is a food plant that has a fairly high glucose content, ranging from 15-20% after hydrolysis. The glucose content allows it to be used as a raw material for making bioethanol through a fermentation process. Anaerobic fermentation process at pH 4-5 using yeast (*Saccharomyces cerevisiae*) as a microorganism that will decompose glucose into ethanol (Moede, 2017).

Some of the factors that affect the fermentation process include:

1. Microbes
   Fermentation is usually carried out using pure cultures produced in the laboratory. These cultures can be stored dry or frozen. Various kinds of microorganisms can be used for the fermentation process, including yeast. Yeast can be in the form of pure material on an agar medium or in the form of preserved dry yeast.
2. Temperature
   The fermentation temperature will determine the dominant type of microbes during fermentation. Each microorganism has an optimal growth temperature, that is, the temperature that provides the best growth and fastest self-propagation. At a temperature of 30^{o}C has the advantage of forming more alcohol because yeast works optimally at that temperature.
3. Time
   Microbes need time in the fermentation process so that they can convert glucose into ethanol. The required fermentation time varies due to several factors, such as sugar content, number of microbes, nutrients, and others.
4. Nutrition
   All microorganisms require nutrients that will provide:
   1) Energy is usually obtained from substances containing carbon.
   2) Nitrogen is used for protein synthesis. One example of a nitrogen source that can be used is urea.

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3) Minerals used by microorganisms, one of which is phosphoric acid which can be taken from NPK fertilizers.
4) Vitamins, most natural carbon and nitrogen sources already contain all or some of the vitamins needed by microorganisms.

(Juwita & Susilowati, 2011)

Ethanol

Ethanol is an organic compound consisting of carbon, hydrogen, and oxygen so that it can be seen as a derivative of a hydrocarbon compound having a hydroxyl group with the formula C_2H_5OH. Ethanol is a liquid, colorless, specific odor, flammable, volatile, and miscible with water in all ratios

Physical Properties of Ethanol

1) Liquid Form
2) Colorless
3) Odorless
4) Boiling point at 1 atm 78,4^oC
5) Freezing point -112^oC
6) Density 1,59 gr/cm^3

(Perry & Green, 2008)

Chemical Properties of Ethanol

1) Molecular Formula C_2H_5OH
2) Molecular Weight 46,07 gram/mol
3) Low molecular weight so it dissolves in water
4) Obtained from sugar fermentation
   Formation of etanol
   $C_6H_{12}O_6 \longrightarrow CH_3CH_2OH$
5) Ethanol combustion produces CO_2 and H_2O
   Ethanol combustión
   $CH_3CH_2OH + 3O_2 \longrightarrow 2CO_2 + 3H_2O + ATP$

(Fessenden & Fessenden, 1982)

RESEARCH METHODS

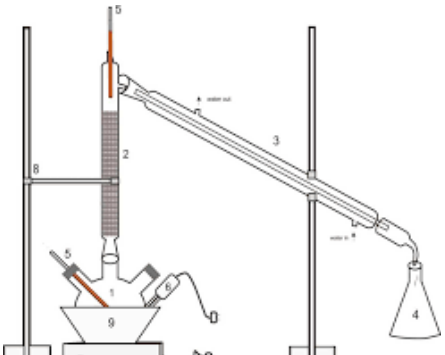
Materials

The materials used in this study were Cassava Peel, *Saccharomyces cerevisiae*, Ammonium Sulfate ($(NH_4)_2SO_4$), Sodium Hydroxide (NaOH), Hydrochloric Acid (HCl), Urea, and Aquadest.

Tools

The tools used in this research are Analytical Balance, Erlenmeyer, Measuring Glass, Volumetric Flask, Dropper, Funnel, Oven, Sieve no. 40, Filter Paper, Stirrer, *Magnetic Stirrer*, Blender, Glass Bottle, Stative and Clamp Holder, Condenser, Hose, Heating Coat, Cork Stopper, Aluminum Foil, Thermometer, Refractometer, Alcoholmeter, and pH meter.

Series of Equipment



Description:

1. Three neck flasks
2. Distillation column
3. Condenser
4. Erlenmeyer
5. Thermometer
6. Rubber/cork plug
7. Electric stove
8. Stative and clamp holder

9. Heating coat

Figure 4. Series of Distillation Equipment

Run Condition

1. Set Conditions
   The conditions set out in this study are:
   1) The Preliminary Stage: Starch-making from Cassava peel with a size of 40 mesh
   2) Delignification stage using 10% NaOH and 160℃ heating temperature
   3) Hydrolysis stage, heating temperature of solution at 100℃
   4) Fermentation Stage: NaOH concentration of 6 M
   5) Fermentation temperature: 27-30℃, *Saccharomyces cerevisiae*: 14 gram,
   6) Stirring time: 30 minutes
   7) Stirring speed: 250 rpm
2. Condition Variables
   The conditions changed in this study are:
   1) Concentration of HCl at the hydrolysis stage were 7%, 9%, 11%, 13%, 15%, and 25%
   2) Fermentation time of 10, 14, 18, and 22 days

Research Path

The course of the research developed in this study is a modification of the stages of making bioethanol from cassava peel that has been carried out by Erna (Erna, 2017). The stages carried out in this research were:

Preliminary Stage

1. Cassava peel was weighed as much as 1 kg.
2. Soak the cassava peel then cut into small pieces.
3. Dry the cassava peel.
4. Crushed and sieved through a 40-mesh sieve.
5. The cassava peel powder is put in the oven for 2 hours and heated at a temperature of about 105ºC.

Delignification Stage

1. Put 180 grams of sifted cassava peel powder into a pan.
2. Four (4) liters of distilled water and 250 ml of 10% NaOH was added.
3. Heated for 30 minutes at a temperature of 110ºC with a stove and the temperature was monitored using a thermometer.
4. Filter and wash the residue with distilled water until the pH is neutral.
5. Oven for 2 hours at a temperature of 105ºC.
6. Sift through a 40-mesh sieve until a brown color is visible.

Hydrolysis Stage

1. Weigh 15 grams of sieved powder with 6 different treatments.
2. 7% HCl solution was added to the first powder, the second powder was added 9% HCl solution, the third powder was added 11% HCl solution, the fourth powder was added 13% HCl solution, and the fifth powder was added 15% HCl solution, and the rest was added 25% HCl solution, 150 ml each.
3. Heated for 2 hours at a temperature of 100ºC.
4. Glucose levels were analyzed.

Fermentation Stage

1. One hundred and sixty (160) ml of the hydrolyzed filtrate was taken, the filtrate was selected from the analysis of glucose levels with a 25% HCl catalyst.
2. Six (6) M NaOH to pH 4.5 was added.

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3. Seven (7) grams of Ammonium Sulfate and 7 grams of Urea into the hydrolysis solution with a 25% HCl catalyst added.
4. Pasteurization of the filtrate at 80°C for 15 minutes. Cool to room temperature.
5. Added *Saccharomyces cerevisiae* as much as 14 grams.
6. Fermented in glass bottles and covered with aluminum foil and allowed to stand for 10, 14, 18, and 22 days at a temperature of 30°C.
7. The alcohol content was analyzed before distillation.

Separation Stage

1. The fermented products were put into a three-necked flask for distillation.
2. Distillation with a temperature of 78-80°C was done.
3. The alcohol content of bioethanol was measured.

RESULTS AND DISCUSSION

Table 1. Results of Analysis of Cassava Skin Content

Parameters	Yield (%)
Protein	8,13%
Fat	1,27%
Carbohydrate	74,75%
Fiber	15,25%
Water	19%
Starch	75,91%

Source: Nutrition Laboratory, Airlangga University, 2021

Cassava peel is one of the wastes that is not used and is considered as animal feed, but cassava peel can be a product of high economic value, one of which is processed into bioethanol. Every one kilogram of cassava can produce 15-20% cassava peel. Cassava peel has a fairly high starch content, with a high starch content allowing it to be used as an energy source for microorganisms. According to (Guntama, 2019), this cassava peel waste contains quite high carbohydrates of 43.626%. The chemical composition contained in cassava peel per 100 grams consists of 59.40% water and 0.7% protein. This is in accordance with the theory because it takes starch with a high content so that microorganisms get sufficient nutrients during the fermentation process.

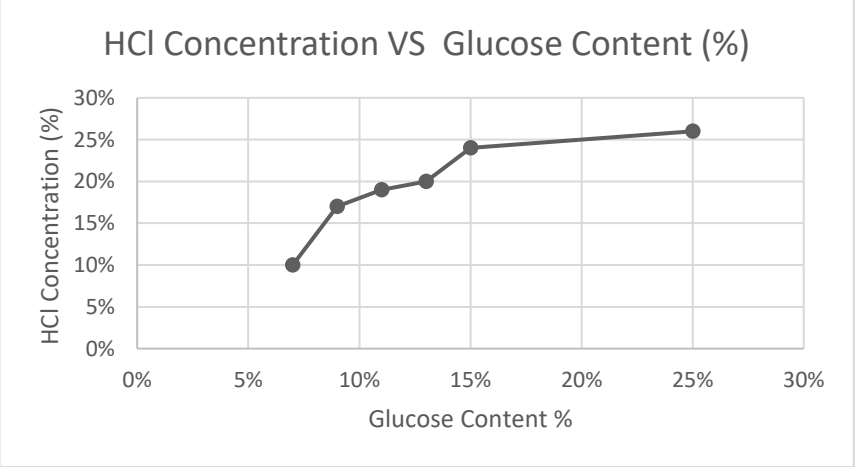

Figure 4. Comparison of HCl Concentration with Glucose Levels in the Hydrolysis Process in Making Bioethanol Waste

The hydrolysis experiment on cassava peels was checked using a refractometer and the results of the hydrolysis process with catalysts of 7% HCl, 9% HCl, 11% HCl, 13% HCl, 15% HCl and 25% HCl obtained solutions with glucose levels of each of 10%, 17%, 19%, 20%, 24%, and 26%. It can be seen in Figure 5 that the greater the concentration of HCl, the greater the glucose level obtained.

This is in accordance with research conducted by Erna (Erna, 2017), with a concentration of 7% H_2SO_4, 5.9% glucose was obtained and 15% H_2SO_4 obtained 7% glucose. Where this can happen because the H+ group of HCl in the hydrolysis process will change the fiber from the cassava peel into a free radical group that will bind to the OH- group of water molecules and produce glucose.

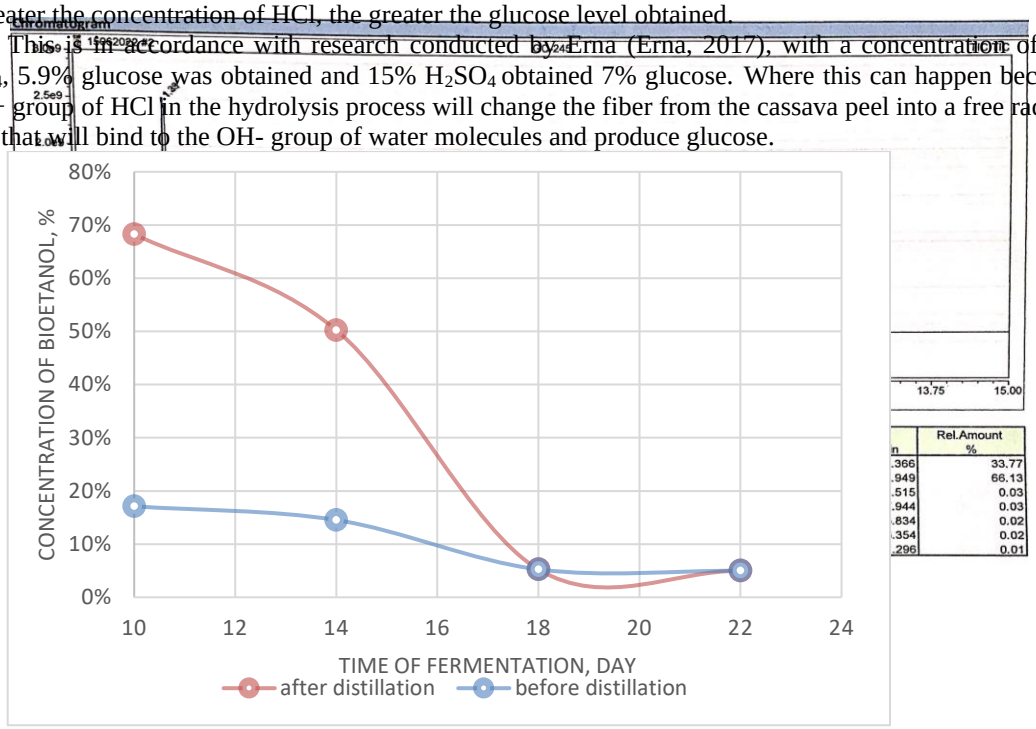

Figure 5. Comparison of Bioethanol Levels Before and After Distillation

Based on Figure 5, the highest bioethanol content through fermentation using 26% glucose before distillation was 17.11% with a fermentation time of 10 days and the lowest bioethanol content before distillation was 4.99% with fermentation time for 22 days. Furthermore, the highest bioethanol content after distillation was 66.13% with a fermentation time of 10 days and the lowest bioethanol content after distillation was 5% with a fermentation time of 22 days.

According to (Azizah, 2012), the length of fermentation is influenced by factors that directly or indirectly affect the fermentation process. For example, the microbe used in this study was *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* can convert sugar into ethanol because of the invertase and zimase enzymes where the invertase enzyme converts sugar into a simple form and the zimase enzyme converts simple sugars into ethanol. Fermentation time also matters because the microbes used can run out of nutrients and stop converting. This causes a decrease in alcohol content on day 14 to day 22 where alcohol content can be reduced if the fermentation time is too long, because the alcohol has been converted into other compounds. Furthermore, separation by distillation is certainly needed to separate bioethanol from impurities that reduce the purity of the alcohol content obtained (Yuliarto, 2012). This is in accordance with the theory where the higher the glucose level, the higher the alcohol content obtained. Based on the research by Sari (Sari, 2012), the fermentation process with 12% glucose content produces 9% ethanol before the distillation process, after the distillation process can produce 60% ethanol content. In this study with a glucose level of 26%, the bioethanol content was 17.11% before distillation and 66.13% after the distillation process.

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Figure 6. Results of GC-MS Analysis on Bioethanol with 25% HCl Catalyst

In the use of 25% HCl catalyst based on the chromatographic graph, it can be seen at 1.40 minutes that ethanol compounds with a concentration of 66.13% were detected, which means the ethanol content without impurities. According to Fitri (Fitri, 2007), the peak branching with different levels is caused by the occurrence of the mobile phase in the detector carrying the separated components. The workings of the detector is by converting the carrier gas signal and the components contained in it into an electronic signal that is useful for qualitative and quantitative analysis of components that are separated between the stationary phase and the mobile phase. The chromatogram which is the result of the physical separation of the components is presented by the detector as a broad series of peaks with respect to time. The results of this study obtained bioethanol content of 66.13%.

CONCLUSION

Based on the research that has been done, it can be concluded as follows:

1. The effect of HCl concentration on the hydrolysis stage is to determine the glucose levels obtained. Where the glucose used in the fermentation process affects the levels of bioethanol obtained. In this study, the highest glucose level was 26% based on test results using a refractometer with a 25% HCl catalyst. Fermentation time also affects the growth of *Saccharomyces cerevisiae* where these microorganisms cannot survive long at unstable temperatures and inadequate nutrients. The faster microbial growth causes high levels of bioethanol.
2. From this study the use of 25% HCl catalyst obtained 26% glucose levels and ethanol levels before and after distillation were 17.11% and 66.13%, respectively, with a fermentation time of day 10.

SUGGESTION

The bioethanol produced in our research is still renewable with different and varied variables. Therefore, further research is needed to obtain better bioethanol levels by looking at the results of our study as a reference.

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