MAKING ETHANOL FROM ELEPHANT GRASS *(Pennisetum purpureum schumach)* USING ACID HYDROLYSIS and FERMENTATION METHOD by *Saccharomyces cerevisiae*

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ABSTRACT: Elephant grass *(Pennisetum purpureum schumach)* not fully utilized by the people in North Sumatra. Though Elephant Grass containing lignocellulose namely cellulose, hemicellulose and lignin, which can be utilized as a valuable product that is as bioethanol. This research aims to study the benefits of elephant grass (Pennisetum purpureum schumach) to be made by the hydrolysis and ethanol fermentation to determine the effect of sampling sites on the cellulose content of elephant grass (Pennisetum purpureum schumach) is the area Lubuk Pakam, Tuntungan and Stabat; the influence of an acid catalyst H_2SO_4 and HCl for hydrolysis and fermentation time 2, 4, 6 and 8 days to produce the optimum ethanol. The results showed that the highest cellulose content is sampled area Lubuk Pakam which is further used in the process of hydrolysis. A better catalyst in the process of hydrolysis to produce ethanol that is optimum HCl and optimum fermentation time to produce the highest ethanol content is 6 days with HCl catalyst as much as 27.83%.

1. INTRODUCTION

Indonesia has a climate which facilitates the growth of bulrush, resulting in availability bulrush can be continuously overflow. Bulrush is a plant that is underutilized. This adult elephant grass is only used as fodder, sometimes the grass is also considered as weeds. But the elephant grass has a high cellulose content (40.85%) which can be used as an ingredient producer of ethanol (Sari, 2009).

Naper also known as elephant grass or rumpuuganda. Karakreristik morphology elephant grass was growing tall, dense merumpun, plant height can reach 7 m, thick-trunked dank eras,long leaves and flowering like Popsicles. Bulrush nutrient content consists of: 19.9% dry matter (DM); 10.2% crude protein (CP); 1.6% fat; 34.2% crude fiber; 11.7% ash; and 42.3% extract materials without nitrogen (BETN).

Bulrush has several varieties, including varieties of Africa and Hawaii.

a. African varieties characterized by stems and small leaves, grow upright, flowering and production is lower than Hawaiian varieties.
b. Hawaiian varieties characterized by stems and broad leaves, slightly flared clump growth, high production and flowering (Rukmana, 2005).

Cellulose and Lignin

Cellulose contained in plants as the material forming the cell wall. Cells can be said is wholly cotton cellulose. In our bodies can not digest cellulose because we do not have the enzymes that can break down cellulose. With dilute acid can not be hydrolyzed, but by acid with high concentration can be hydrolyzed to cellobiose and D-glucose. Cellobiose is a disaccharide composed of two glucose molecules glycosidic bond between carbon atoms 1 to 4 carbon atoms (Anna, 2006).

Lignin is one of the main components of plant cells, therefore lignin also has a direct impact on plant characteristics. For example, lignin is very influential in the manufacture of pulp and paper. The content of lignin in ruminant feed is very influential on the ease of feeding it to digest. Food that is low in ligninnya easily digested by an animal.

The special properties of the cellulose chain, not only of β 1,4 glycosidic bond, is also a consequence of the proper stereochemistry of D-glucose on any stereo center. Where D- galactose and D- Alosa bind to the same model, they rightly did not leave a place for the manufacture of polymers with properties such as cellulose. Then we got another view of why D-glucose gets a special position in chemical plants and animals.

Test Benedict

Modification of Fehling reagent is Benedict's reagent, which is a mixture of 17.3 grams of cupric sulfate, 173 grams of sodium sulfate and 100 grams of sodium. carbonate in 100 grams of water. Heating reducing carbohydrate with a reagent Benedict will change color from blue green → yellow → → → reddish and finally brick red precipitate

is formed when the concentration of carbohydrate oxidation cuprous reducing high enough. As with Fehling reagent, in this reaction, reducing carbohydrates will be oxidized to onat acid, whereas Benedict's reagent (as Cu 2+) would be reduced to cuprous oxide. So, in this test a process of oxidation and reduction processes (Sumardjo, 2006).

Yeast

In the process of yeast fermentation ethanol used. This yeast can convert the glucose into alcohol and CO_2. Yeasts are single-celled microorganisms, lacking chlorophyll and belonged eumycetes. Of these groups are known several types, including Anamenesis *Saccharomyces* *Schizosaccharomyces* Pombe and *Saccharomyces cerevisiae*. Each has a
different ability to produce alcohol.

Saccharomyces is a genus of fungi kingdom that includes many types of yeast. *Saccharomyces* comes from the Latin which means sugar fungus. Many members of this genus are considered very important in the production of food. One of them is the *Saccharomyces cerevisiae* used in making bread and alcohol production industry because it possesses can ferment maltose rapidly and have the ability to metabolize a substrate. *Saccharomyces cerevisiae* can produce ethanol in large quantities and have a high tolerance for alcohol (Anonymous, 2011).

1. The terms required in selecting the yeast for fermentation, are: Fastbreed.
2. Resistant to high alcohol.
3. Resistant to high temperatures.
4. Having stable properties.
5. Fastholding fermented adaptation to media Factors that affect the lives of yeast:

a. Nutrients

In its activities, requires the addition of yeast nutrient for growth and development, for example:

- Element C: No on carbs
- Elements N: with the addition of a fertilizer containing nitrogen, ZA, Urea, Ammonia danPepton
- Elements P: the addition of a phosphate fertilizer NPK, TSP, DSP andothers
- Minerals
- Vitamins

b. Acidity

For alcoholic fermentation, yeast media require acidic conditions, which is between pH 4.8 to 5.0. PH adjustment, the addition of sulfuric acid if the substrates alkalis or sodium bicarbonate if the substrates acid.

c. Temperatures

And the optimum temperature for breeding is 28-30°C at the time of fermentation, an increase in the heat, because the extreme. To prevent fermentation temperature does not rise, need refrigeration so that the temperature is kept constant 28-30°C.

d. Air

Alcoholic fermentation takes place in anaerobic (without air). However, the air required in the process of seeding before fermentation, for breeding yeast cells.

Ethanol

Ethyl alcohol or ethanol, by far the most dikeal. Ethanol is produced biologically by fermentation of sugar or starch. With no oxygen, enzymes in yeast or bacteria culture catalyze the reaction. This process produces energy, wherein the microorganism, in turn, use it for growth and other benefits. Commercially, ethanol is made through addition reaction in which water is combined with ethylene at about 280 ° C and 300 ° C atm. Ethanol has not spelled out the application as solvents for organic chemicals and as a starting compound for the manufacture of dyes, synthetic drugs, cosmetics and explosives. Ethanol is also a part of the alcoholic beverage. Ethanol is the only type of straight-chain alcohols which are not toxic (more precisely slightly toxic); our body produces an enzyme called alcohol dehydrogenase, which helps with the metabolism of ethanol to acetaldehyde (Chang, 2005)

Bioethanol can be produced by fermentation of the three types of carbohydrates:

1. Starchy materials, such as cassava or manioc, sweet potato, corn starch, corn, grain sorghum, wheat, potato, canna, arrowroot, tubers, dahlia and others.
2. Sugary material, in the form of molasses, sugar cane juice, coconut juice, juice of sweet sorghum stalks, palm juice (palm), sap palm, mother-of-pearl, palm and others.

3. The cellulose material, such as logging waste, agricultural waste such as rice straw, bagasse, corncob (cobs) of maize, cassava (tapioca waste), elephant grass, banana stalks, sawdust and others.

Physical and chemical properties Ethanol or bioethanol

1. Chemical properties Heavy analysis

- C (carbon): 52.1
- H (hydrogen): 13.1
- O (oxygen): 34.7
- C/H (carbon/hydrogen): 4.0
- The need air (air kg / kg: 9.0 fuel)

2. Physical properties
   - Molecular weight: 46.07g/mol
   - Density: 0.7894 g/cm3
   - Boiling Point: 78°C
   - Melting point: -112°C
   - Flash point: 17°C
   - Water solubility: yes
   - Refractive Index: 1.3614
   - Viscosity: 1.17

   Source: (Djojonegoro W, 2005)

   Benefits of Ethanol The use of ethanol in everyday life include:

   - As raw materials of alcoholic beverages
   - As the fuel Direct-ethanol fuel cells (DEFC)
   - As rocket fuel
   - As a basic chemical organic compounds
   - As an antiseptic
   - As a solvent for perfumes, paints and drug solution
   - As a solvent organic compounds
   - As a vehicle fuel, good for a mix of premium fuel or as fuel for lighting fuel to create acetic acid
   - Used in the manufacture of some deodorant
   - Used in medicine to treat depression and drug

Bioethanol Production Process The materials that contain monosaccharides ($C_6H_{12}O_6$) as a direct glucose can be fermented into ethanol. However disaccharide starch, or complex carbohydrates must first hydrolyzed into simple components, monosaccharides. Therefore, in order to stage fermentation process may be optimized, the material must undergo preliminary treatment before going into the process of fermentation. Disaccharide such as sugar ($C_{12}H_{22}O_{11}$) should be hydrolyzed to glucose. Polysaccharides such as cellulose must be first converted into glucose. The formation of glucose means the preliminary process has ended and the materials then ready to be fermented. Chemically fermentation process can run long enough, because there is a series of reactions, each of which is influenced by special enzymes.

Hydrolysis

Hydrolysis is a kind of chemical reaction that occurs between water and other compounds. In reaction, the chemical bond will be broken in two molecules causes them to become peceh. Split water molecules to form positively charged hydrogen ions (H +) and hydroxide (OH-) and other molecules split into two simple parts. Principles of cellulose hydrolysis is basically the termination of the polymer chains of cellulose into units of dextrose ($C_6H_{12}O_6$). Termination of the polymer chain can be done by various methods, such as enzymatic, chemical or a combination of both. Enzymatic hydrolysis have fundamental differences compared to hydrolysis chemically and physically in terms of the specificity of the cellulose polymer chain termination. Hydrolysis chemistry and physics would break the chain of the polymer at random, while the enzymatic hydrolysis will break the chains of polymers specifically on certain branching.

Is acid hydrolysis using acid hydrolysis to transform polysaccharides (starch, cellulose) into sugars. In the acid hydrolysis typically used hydrochloric acid (HCl) or sulfuric acid (H_2SO_4) with a certain level. The hydrolysis is usually carried out in a special tank made of stainless steel or copper pipes connected to the heating ducts and pipes airway to regulate the air pressure in the cellulose of grass can be converted to ethanol by acid hydrolysis process with a certain level. Cellulose hydrolysis process has to do with the concentrated acid to produce glucose (Sari, 2009). Hydrolysis of cellulose by acid can be done using dilute strong acid at high temperature and pressure, and can be performed using concentrated acid at high temperature and low pressure. At high temperature hydrolysis process carried out at a temperature range of 160-240 ° C, whereas at low temperature hydrolysis process carried out at a

temperature of 80-140°C. Hydrolysis lignocellulose ingredients will produce simple sugars, such as glucose, xylose, cellobiose and arabinose. Acid normally used for hydrolysis of cellulose is sulfuric acid, phosphoric acid and hydrochloric acid (Octavian, 2013).

Ethanol fermentation had understanding microbial metabolism application to convert raw materials into high-value products, such as organic acids. One of the products produced in the fermentation process is ethanol. Ethanol production can be obtained sugar (sucrose) with anaerobic fermentation (without O_2) by the activity of the yeast *Saccharomyces cerevisiae*.

After hydrolysis stage is the fermentation process to convert glucose (sugar) into ethanol and CO_2. The word comes from the Latin fermentation ferfere which means. Fermentation is a microbiological process that is controlled by man to obtain useful products, where there is the breakdown of carbohydrates and amino acids anaerobically. Decomposition of the complex becomes simple with the help of microorganisms to produce energy. Fermentation is a decomposition activity carbohydrate ingredients. In the decomposition of carbohydrates is done by microbes that can produce enzymes which are reactive substances that control the chemical reactions in the process of fermentation, such as bacteria, yeast and function purification or distillation.

The separation by distillation in principle is based separation methods for their boiling point among the components to be separated. In toeritis when boiling point between components the greater the separation by distillation will take place the better the results obtained are more pure. Distillation is used to attract organic compounds boiling point below 250°C. Distillation compounds with too high boiling point feared would damage the compound to be distilled resulting from oxidation and decomposition. In the distillation of compounds to be taken the desired components boil and the steam is passed through a cooler to thaw. Boiling process is closely related to the presence of surface air. When a liquid is heated, then boiling will occur at the temperature at which the vapor pressure of the liquid to be distilled equal to the vapor pressure at the surface. The air pressure on the surface occurs by the presence of air in the atmosphere. When boiling occurs at 760 mm Hg, the boiling is called the normal boiling and boiling point called the normal boiling point (Sanusi, 2013)

Pycnometer

Pycnometer is an instrument used to measure the value of density or density of the fluid. A wide variety of fluid measured its density, usually when measured in the lab is the density of the OLI and also for cooking oil, Pycnometer is a measure density / relative density / specific gravity. Usually used for liquid and solid samples principle works: by knowing what the mass of the sample to be determined density in the pycnometer volume were completely filled, usually pycnometer volume that is widely used 10ml; 25ml volume and value valid at room temperature shown on the pycnometer(e.g 20°C).2.

2. METHODOLOGY

This research is to find out ways of making ethanol from grass, the influence of sampling sites on the cellulose content of the sample, the influence of an acid catalyst to the cellulose content and the effect of fermentation on ethanol is obtained. This research was conducted at the Laboratory of Chemistry Department of Chemistry, Faculty of Mathematics and Natural Sciences, State University of Medan. The method used in this research is the method of acid hydrolysis followed by fermentation.

Variables examined

a) Variable still is the mass of raw material (grass) 28.5 grams, the volume of 1 liter fermentation, hydrolysis time, volume and concentration of catalyst 3 mL.

b) Variable changes consist of:

1. The sampling
2.The type of acid catalyst
3. Time fermentation Equipment and Materials

1. Equipment

a) Hardware Pretreatment and Hydrolysis
   1. Blender
   2. Cuter
   3. Sieves
   4. Glassware
   5. Waterbath
   6. Aluminium foil

b) The soil pH Analysis Tools
   1. pH meter

c) Fermentation Equipment
   1. Fermentor / container fermentation
   2. Hose

d) Purification Equipment
1. The distillation equipment
e) AnalysisTool
1. Pycnomete
2. Materials
- Elephant grass
- Sachharomyces cerevisiae Yeast
- Reagent Benedict
- Aquades
- HCl(p)
- H_2SO_4(p)
- NPK
- Urea
- NaOH
- Iodine

Research Procedure

A. Determination of soil pH using a pH meter
1. Take the dry land that is at the end and the middle of the land who want to test its pH.
2. Mixed with the land acquired into one and dried for several hours to dry.
3. Weigh the soil has dried out of each region as much as 5 grams and each was added to the test tube.
4. Next add 12.5 mL of distilled water into each tube and shaken and allowed to stand a few minutes until the soil settles in the bottom of the tube.
5. Measure the pH of each solution with a pH meter.

B. Analysis of Lignin and Cellulose The analysis procedure cellulose and lignin in the elephant grass is by the method Chesson.

(a) A total of 1 g dried sample was added 150 mL of distilled water, refluxed with a water bath with a temperature of 100°C for 1 hour. The result is filtered, the residue washed with hot water (300 mL). The residue is then dried in an oven until constant then weighed
(b) The residue was added 150 mL 1N H_2SO_4 then refluxed with a water bath for 1 hour at 100 ° C. The results are filtered and washed with distilled water until neutral (300 mL) and then dried.
(c) The dry residue was added 10 mL of 72% H_2SO_4 and soaked at room temperature for 4 hours. Added 150 mL of 1 N H_2SO_4 and refluxed with a water bath for 1 hour in the cooling behind. The residue is filtered and washed with distilled water until neutral (400 mL) and then heated in an oven at 100 ° C and the results are weighed until the weight remains
(d) Further residue diabukan in the furnace at a temperature of 700 ° C and weighed
(e) Calculation of the contents of cellulose and lignin content as follows.

3. RESULTS AND DISCUSSION

soil pH of variation where sampling is Tuntungan, Stabat and Lubuk Pakam has a different pH is Tuntungan and Stabat have a more acidic pH is 4.8 compared Lubuk Pakam with a pH of 5.7. Figure 4.2 Graph Relation between Sampling Points with soil pH Furthermore, from Table 4.2 above shows that the samples came from Lubuk Pakam have a higher cellulose content of 30% while the sample Tuntungan and Stabat have a cellulose content of 20%. So it can be described the effect of pH on the cellulose content of the sample as follows:

Figure 4.3. Graph The relationship between the soil pH to levels Cellulose Sample From Figure 4.2 it can be seen that the samples came from Lubuk Pakammeiliki soil pH is lower (base) compared to samples from Stabat and Tuntungan and in Figure 4.3 can be seen that the pH of the soil affects the cellulose content of samples that area Lubuk Pakam has a cellulose content highest of 30% with a soil pH of 5.7.

The components contained in the elephant grass, namely cellulose, hemicellulose and lignin produced from photosynthesis. Factors affecting the growth of a plant is grown is the appropriate soil pH so that plants grow and grow with optimum to obtain food through photosynthesis. So from the three areas where sampling has different cellulose content because the soil pH samples where the growth is also different.

variations in the fermentation time 2, 4, 6 and 8 days and hydrolysis using H_2SO_4 catalyst having the optimum concentration of ethanol that is 6 days of fermentation with an average grade of 20.71%. It can be described as in the graph below: Figure 4.4. Relationship Between Time Graph VS Fermentation Ethanol Content (%).

Further Data Table 4.4 with a variation of fermentation time 2, 4, 6 and 8 days and hydrolysis using HCl catalyst having the optimum concentration of ethanol that is 6 days of fermentation with the average level of 27.83%. It can be described as in the graph below.

Once known bulrush cellulose content using samples Chesson Lubuk Pakam region has the highest cellulose content is 30% with a soil pH of 5.7. Then, the next process is the hydrolysis of elephant grass samples from Lubuk Pakam to convert cellulose into glucose each using catalyst HCl and H_2SO_4 and fermentation time variation 2,4,6 and 8 days. From Figure 4.3 and 4.4 shows that the optimum fermentation time to produce a high ethanol content ie 6 days of fermentation, using either a catalyst HCl and H_2SO_4. This is because the optimum phase of *Saccharomyces cerevisiae*under 6 days an adjustment or growth of *Saccharomyces cerevisiae* and sixth day after death phase occurs with cessation of growth and breeding of *Saccharomyces cerevisiae*. So that at the time of 8 days decreased levels of ethanol as *Saccharomyces cerevisiae* has stopped working and breeding. Fermentation time variations of 2, 4, 6 and 8 as well as variations acid catalysts are HCl and H_2SO_4 obtained higher levels of ethanol that is using HCl catalyst. Can be seen in the graph below:

Figure 4.6 Graph of the relationship between the fermentation time vs. ethanol content (%)

In the process of hydrolysis of cellulose to glucose chain termination used acid catalysts are HCl and H_2SO_4 respectively with dense concentrations. The catalyst serves to accelerate the cellulose chain termination. From Figure 4.5 it can be seen that the higher ethanol content ie using HCl as a catalyst effect on the strength of the acid solubility. Where HCl perfect ionized in water with high levels of pekatnya only 38% and the pK a = -6.0 H_2SO_4 while experiencing two times the separation in a solution but the second is very weak sehinggan concentrated H_2SO_4 concentration is as high as 96% and the price pKa1 = -3, 0 and pKa2 = 1.99. The lower the pK a greater acid strength and vice versa pK a lower-strength acid. So from differences in the strength of the acid H_2SO_4 and HCl to produce different levels of ethanol that is more optimum HCl to produce ethanol that is 27.83%.

4. CONCLUSION

From the results of research and discussion can be concluded as follows:

1. Elephant grass can produce ethanol because it contains cellulose. Ways of making ethanol from grass that first the cellulose is hydrolyzed to glucose. Then fermented using bacteria *Saccharomyces cerevisiae* and fermented in distilled at 80 ° C.
2. The sampling influences the bulrush cellulose content by measuring the pH of the soil of each region is Lubuk Pakam with a pH of 5.7 and cellulose content of 30%; Tuntungan and Stabat pH and the levels of the same cellulose is pH 4.8 and 20% cellulose content. So it can be concluded that the location where sampling has an influence on the growth of elephant grass so it has a different content of cellulose, lignin and hemicellulose.
3. Time of fermentation affect the levels of ethanol produced, namely fermentation time of 2 days have levels of around 10-12%; 4-day fermentation period had higher levels of about 13-15%; 6 days of fermentation time has an optimum level of about 20-30%; 8-day fermentation period has decreased by about 7-11%. So we can conclude the optimum time of fermentation ethanol in the elephant grass *Saccharomyces cerevisiae* using bacteria to produce ethanol fermentation highest of six days.
4. Hydrolysis with H_2SO_4 catalyst has lower levels of ethanol compared with HCl catalyst for perfect ionized HCl in water and HCl lower pK so that the strength of the acid is greater.

While H_2SO_4 has a pK higher and lower acid strength so as HCl produce higher ethanol content.

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