CHAPTER I

INTRODUCTION

1.1. Background

Indonesia is one of the countries in Southeast Asia that is rich in natural resources. Abundant natural resources are the wealth of the country used in various sectors, but continuous exploitation of course causes the depletion of these resources over time. This is what causes the energy crisis. Along with the depletion of reserves of fossil fuel sources (*unrenewable energy*) and increasing world fuel prices and decreasing natural resources, a renewable energy source is necessary to meet these needs. Therefore, alternative fuels from renewable natural resources such as bioethanol are one of the options that are expectable to develop to meet the rising demand for fuel.

The use of bioethanol as a fuel has good prospects because it is multipurpose, including increasing the octane number, increasing combustion efficiency, and functioning as a source of oxygen for cleaner combustion. In addition, nitrogen oxides, and greenhouse gases become pollutants. Bioethanol is also easily biodegradable and safe because it does not pollute water.

Bioethanol (C_2H_5OH) is ethanol produced by fermentation using vegetable raw materials. Bioethanol is deriving from simple sugars, starch, and cellulose. Starch is the form of polysaccharides that can be hydrolyzed into glucose by heating, using a catalyst, and enzymes.

Glucose is then fermented to produce ethanol. Ethanol fermentation is the activity of solving sugars (carbohydrates) into ethanol compounds by releasing CO_2 gas. This fermentation is implementable under anaerobic conditions or in the absence of oxygen. Generally, bioethanol production uses *Saccharomyces cerevisiae* microbes. These microbes can be used for converting sugar to ethanol with good conversion ability, resistance to high levels of ethanol, resistance to low pH and resistance to high temperatures.

One of the biological sources that have great potential as raw materials for bioethanol is bagasse. Bagasse is a by-product of milking sugarcane juice. Bagasse is one of the solid wastes of sugar factories. Bagasse is abundant in Indonesia. Each hectare of sugarcane land can produce 10-15 tons of molasses per hectare or 766-1150 liters of fuel-grade ethanol. In 2013, Indonesia's sugarcane plantation area was 470,000 ha or the maximum potential reached 3.6 million kl of ethanol. In the sugar process in the factory, bagasse is produced by 35-40% of each processed sugar cane, only 5% of the sugar utilized is in the form of *molasses*, *blotong* and water (Silitonga, 2015). Bagasse is a biomass that contains lignocellulose, so it is possible to use it as an alternative energy source such as bioethanol or biogas. The crude fiber component of bagasse consists of 40.59% cellulose, 15.91% hemicellulose, and 17.50% lignin (Gunam, 2011).

So far, the main product produced from sugar cane is sugar, while waste or other by-products do not get much attention. Bagasse is a raw material that has the potential for bioethanol production because bagasse contains sugar and starch. Processing sugarcane bagasse into bioethanol is to make use of useless materials into useful goods and have economic value.

Therefore, by looking at the content of bagasse and its potential to process into bioethanol, it is necessary to conduct research related to the processing of bagasse. The subject focused on the optimum conditions of the inoculum dose and the length of fermentation time used to produce bioethanol.

1.2. Question

Based on the above background, a question can be asked, namely, how are the optimum conditions of the inoculum dose and the length of fermentation time for the produced volume of bioethanol determined?

1.3. Objective

Based on the question, the objective of this study was to determine the optimum condition of the inoculum dose and the length of fermentation time on the produced volume of bioethanol from bagasse.

1.4. Benefit

The results to be obtained in this study are expectable this study would be useful to provide experience and add insight for the author in terms of study on optimization of the inoculum dose and fermentation time

in the manufacture of bioethanol from bagasse and as a reference for other authors related to this study.

CHAPTER II

LITERATURE REVIEW

2.1. Definition of bioethanol

Bioethanol is an alcohol compound with a hydroxyl group (OH), 2 carbon atoms (C), using the chemical formula C2H5OH, made by fermenting sugar of carbohydrate sources (starch) using microorganisms, such as yeast (Hapsari, 2013; Megawati, 2015; Gusmawarni, 2010). Bioethanol can be used as an alternative material because it is environmentally friendly, contains lower CO gas emissions (19-25%), has a high oxygen content (35%) so that it burns more completely, has a higher octane value and can be produced continuously by microorganisms (Retno, 2009). Plants that have the potential to produce bioethanol are plants having high sugar and carbohydrate content such as sugar cane, sap, sorghum, cassava, arrowroot, sweet potato, sago, corn, bananas, straw, corn cobs and wood (Silitonga, 2015; Gusmawarni, 2010).

Bioethanol is a very important material because it is a liquid fuel of a renewable source and oxygenates fuel containing 35% oxygen possibly reducing particulates and Nox emissions from combustion products that can be useful as a substitute for fuel. Bioethanol with biological raw materials in addition to being able to reduce the consumption of crude oil can also reduce environmental pollution (Hidayati, 2016). Bioethanol with a content of 95-99% can be usable as a premium substitute (gasoline), while a level of 40% is usable as a substitute for kerosene (Sadimo, 2016).

One of the raw materials for bioethanol that comes from biological sources is bagasse. In the sugar factory process, bagasse produces 35-40% of each processed sugar cane, only 5% of the utilized sugar is in the form of molasses, *blotong* and water (Silitonga, 2015). Each hectare of sugarcane land can produce 10-15 tons of molasses per hectare or 766-1150 liters of fuel-grade ethanol. In 2013, Indonesia's sugarcane plantation area was 470,000 ha or the maximum potential reached 3.6 million kl of ethanol. The production of bioethanol from plants containing starch or carbohydrates is implementable through the process of converting carbohydrates into sugar (glucose). There are two known enzymatic hydrolysis methods, namely SHF and SSF. The SSF method is very important to develop because it can shorten the process of making bioethanol (Hapsari, 2013).

Based on a previous study by Prasetyo utilizing organic waste at the Wonokromo Market of Surabaya using acid hydrolysis and fermentation methods with *Zymomonas mobilis* bacteria proved to contain ethanol. Meanwhile, Muslihah's study resulted in 11.64% optimum ethanol for 6 days from citrus fruit waste, and Faizah's study resulted in optimum ethanol 9.68% for 6 days from tomato waste (Nurhatika, 2013).

2.2. Sugarcane and Morphology

The name of sugar cane is only known in Indonesia. In the international scope, this plant is better known by its scientific name *Saccharum officinarum* L. This plant is a sugar-producing plant. In

addition, its leaves can also be used for animal feed. This plant can only grow in tropical climates (Brilliyana, 2017). In Indonesia, sugar cane producing areas are Java, South Sumatra, West Sumatra, Lampung and the archipelago (Mulyana.W, 2001).

Morphologically, sugarcane plants can be classifiable into several parts, namely stems, leaves, roots and flowers. The stem is the most important part of the plant in sugarcane cultivation. Sugarcane stem growth is the most important stage that greatly determines the amount of sugarcane weight yield. The stem growth is caused by shoot growth and growth at the base of the segment (Brilliyana, 2017). Sugarcane stems are tall, thin, non-branching and growing upright. Poor growth, type of sugarcane, and climatic conditions influence stem height. Stem height of growing well plants can reach 3-5 meters or more. Hard bark is green, yellow, purple, dark red, or a combination thereof. The stem has a layer of wax that is grayish-white. This layer is abundant when the stems are young. The stem is segmented with a segment length of 10-30 cm. The rootstock has shorter internodes. Stem segments can be barrel-shaped, cylindrical, spherical, conical, inverted conical, or convex-concave. Nodes that are the seat of the leaves limit the stem segment. Each leaf axil has round or elliptic eye buds. These buds will grow into seeds (Author Team, 1992; Kristanto, 2011).

Sugarcane leaves are plant organs that play a role in providing food because it is the part where photosynthesis takes place. The more number

of leaves, the more photosynthetic parts increase so that the photosynthate yield will increase. Photosynthate results channel to the vegetative organs of plants to stimulate plant growth (Brilliyana, 2017). Sugarcane leaves are incomplete leaves, because they only consist of midrib and leaf blade, without a petiole. Leaves stem from the stem book with alternating positions. The midrib hugs the stem, the higher it gets, the narrower it becomes. The midrib has hairs and leaf ears. Leaf spines are parallel. The leaf blade is in the form of line 1-2 meters long and 4-7 cm wide with a tapered tip, jagged edges, and a smooth leaf surface (Author Team, 1992).

Sugarcane has fibrous roots that can reach one meter in length and can be classifiable into primary roots and secondary roots. Primary roots are roots that grow from the root buds of stem cuttings. Primary roots are also referred to as cutting roots. The characteristics of primary roots are smooth, branched and these roots do not live long and only function when the plant is still young. While secondary roots or also known as shoot roots are roots that grow from the root in the shoot bud growing from seed cuttings, the shape is larger, softer, and slightly branched. These roots are long-lived and remain as long as the plant is still growing (Author Team, 1992; Kristanto, 2011).

A sugarcane flower is a compound flower composed of panicles formed after vegetative growth with limited growth. Flowers develop in the morning with the flowering period on one panicle varying from five to

twelve days. Sugarcane flowers are perfect flowers. Stalks and pollen hang out after the flowers are mature enough. The pistil is generally purplish. The main axis is branching upwards and gets smaller, thus forming a pyramid. Compound flower length 70-90 cm. Each flower has three petals, one corolla, three stamens and two pistils (Author Team, 1992; James, 2004).

2.3. Bagasse and its contents

Bagasse is a waste agent usually disposed of in open dumping without further processing so that it will cause environmental disturbances and unpleasant odors (Nugroho, 2008). Preliminary study results show that bagasse is the best raw material for making bioethanol compared to rice straw, corn straw, and wood sawdust (Gunam, 2011).

Bagasse is a solid residue in the sugarcane processing, which so far has not been widely used as a product having added value. Bagasse classified as biomass is very likely to be usable as source of energy, fodder, or lignocellulosic-based products such as paper, biogas, bioethanol and others (Silitonga, 2015). Dry bagasse content is 10% of milled sugarcane, cellulose content/glucan 50%, hemicellulose 25%, and lignin 25% (Utomo, 2014).

2.4. Saccharomyces cerevisiae

Saccharomyces cerevisiae is true yeast belonging to eukaryotes that morphologically only forms *blastospores* in oval, cylindrical, oval or ovoid shapes. *Saccharomyces cerevisiae* reproduce dividing through

"budding cells". Reproduction may be influenced by environmental conditions and the number of nutrients available for cell growth. *Macroscopic appearance* has round colonies, light yellow color, shiny surface, smooth, soft texture, and round cells with 1-8 ascospores. *Saccharomyces cerevisiae* is an anaerobic microorganism. *Saccharomyces cerevisiae* grows very well at a temperature of 20-30 ^{o}C and pH between 4.5 and 5.5 (Silitonga, 2015). The taxonomy of the yeast *Saccharomyces cerevisiae* is classifiable into kingdom *Fungi*, phylum *Ascomycota*, class *Saccharomycetes*, order *Saccharomycetales*, family *Saccharomycetaceae*, genus *Saccharomyces*, and species *Saccharomyces cerevisiae*.

Saccharomyces cereviseae functions to make bread and beer, because *Saccharomyces cereviseae* is fermentative (performs fermentation, which breaks down glucose into carbon dioxide and alcohol). The acquisition of bioethanol content also increased along with the increase in glucose yield. Ethanol fermentation by *S. cereviseae* produces less than 50% ethanol.

2.5. Fermentation

Fermentation comes from the Latin "*ferfere*" which means to boil. Initially the term *fermentation* was usable to denote the process of converting glucose into ethanol that worked anaerobically. However, then the term fermentation evolved again into a whole overhaul of organic compounds carried out by microorganisms (Jannah, 2010). According to

microbiology, industrial fermentation is definable more broadly as a process to convert raw materials into a product by a mass of microbial cells (Hargono, 2015).

According to study by Sadimo (2016), alcoholic fermentation is the process of breaking down carbohydrates into ethanol and CO_2 produced by the activity of type of microbe called yeast in anaerobic conditions. Changes may occur if these microbes come into contact with food suitable for growth. The fermentation process usually produces carbon dioxide gas. Fermentation results are influenced by many factors, such as food or substrate, types of microbes and ambient conditions.

Factors affecting the success of alcoholic fermentation are carbon sources. According to Santosa (2013), growth, yeast requires energy from carbon. Sugar is the preferred substrate; therefore, sugar concentration greatly affects the quantity of alcohol produced.

The degree of acidity, in general the pH for fruit fermentation or yeast cell formation, requires optimum acidity between 3.5-5.0. Beyond that, microbial growth will be disrupted. Adjustment of the pH, NaOH can be usable to increase and nitric acid to lower the pH. Before fermenting, pasteurized fruit juice is added with SO_2. It is to prevent unwanted bacteria and yeast. The source of SO_2 is $NaHSO_3$, potassium or sodium bisulfite.

Nutrition, according to Soebijanto (1986) in Santosa (2013), in the fermentation process, microorganisms really need good nutrition in order to obtain good fermentation results. The right nutrition to supply

microorganisms is nitrogen (N) that can be obtainable from the addition of NH_3, salt ammonium, peptone, amino acids, and urea. The nitrogen required is 400-1000 gr/1000 L of liquid. The phosphate needed is 400 gr/1000L of liquid. Another nutrient is ammonium sulfate with a content of 70-400 gr/1000 L of liquid.

Temperature, a good temperature for bacterial growth is between 20 ^oC – 30 ^oC. The lower the fermentation temperature, the higher the ethanol produced, because, at low temperatures, the fermentation will be more complete and the loss of ethanol carried away by CO_2 gas will be less.

Fermentation time, the usual fermentation time is 3-14 days. According to Judoamidjojo (1992), the time needed for fermentation is 7 days. If the time is too short, *Saccharomyces cereviseae* is still in its infancy so that the alcohol produced is in small quantities. On the other hand, and if it is too long, *Saccharomyces cereviseae* will die so that the alcohol produced is not optimal.

Sugar content, according to Sardjoko (1991) in Santosa (2013), sugar content will greatly affect the fermentation process, the optimum sugar content given for fermentation is 25% for starters; sugar content used is 16%.

Volume starter, according to Nurhatika (2013), starter is a certain microbial culture grown in a substrate or medium for certain process purposes. The volume of a good starter for fermentation is 1/10 part of the

volume of the substrate. In the fermentation process, glucose of fermentation converts into ethanol (Santosa, 2013).

2.6. Normal Distillation

Normal (simple) distillation is used to separate compounds that can evaporate below 130 ^{o}C. In normal distillation, boiling will occur when the vapor pressure of the heated liquid is equal to the air pressure on the surface of the liquid. The surface of the liquid to distill must be lower so that the heating evenly distributes so that evaporation will be complete (Sitorus, 2013).

CHAPTER III

METHOD

3.1. Time and Location

This study was located in the Biochemistry Laboratory of the University of North Sumatra, from February to March 2019.

3.2. Tool and Material

The tools used in this study were blender, analytical balance, *Erlenmeyer*, measuring flask, measuring cup, glass jar, stirring rod, filter paper, sieve, distillation device and other instruments.

The materials used in this study were 100 grams of bagasse, 6 liters of distilled water, yeast, and urea.

3.3. Sampling Location

Sampling was at the place of retailers who usually sold sugarcane ice as a soft drink.

3.4. Method

This study used an *experimental* method to determine the produced volume of bioethanol based on the difference in dosage and the length of fermentation time used.

3.5. Procedure

3.5.1. Preparation for Materials

The bagasse was sorted (separated from the skin and the bagasse or pulp), then the bagasse was placed on the sun to dry (±3 days). After drying, the bagasse was chopped and ground using a blender to produce

flour. This process was carried out to condition *lignocellulosic* materials in terms of structure and size by breaking down and removing the content of lignin and hemicellulose, destroying the crystal structure of cellulose and increasing the porosity of the material. Damage to the crystalline structure of cellulose would facilitate the breakdown of cellulose into glucose. In addition, hemicellulose would also break down or solve into simple sugars (glucose, galactose, hexose, and pentose) (Nurhatika, 2013).

3.5.2. Fermentation Process

The fermentation process was carried out by adding a number of inoculum, namely, *Saccharomyces cereviseae* into the fermentation medium as much as 0%, 12%, 14% and 16%. Fermentation was carried out at room temperature. The fermentation time varied at 24, 48, 72, 96 and 120 hours to observe the effect of fermentation time on the bioethanol produced by the addition of 0.05% urea of the mass to ferment (Rahmadani, 2017).

3.5.3. Distillation Process

The results of the fermentation were then purified through a distillation process at a temperature of 75^{o}C. The remainder of the distillation was filtered and the solid (*vinasse*) was taken. Meanwhile, the distillate was accommodated and the volume of the resulting distillate was measured (Silitonga, 2015).

3.6. Analysis of data

The data obtained were then analyzed by using a randomized block design (RBD). Factor A is the level of inoculum dose used (0%, 12%, 14%, 16%) and factor B is the length of fermentation time used (24, 48, 72, 96, 120 hours).

CHAPTER V

CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The optimum condition of the inoculum dose was 14% with a long fermentation time of 72 hours to produce 1.7 ml of bioethanol. This means that the length of fermentation time and the dose of inoculum used affected the volume of the produced bioethanol.

5.2. Recommendation

Based on the done study, the author suggests that it is necessary to conduct continuous research on bagasse in more treatment levels and doses used. The government needs to apply several policies that can encourage the use of bagasse as raw material for bioethanol, such as intensifying research and development for better mastering of the technology of converting lignocellulosic biomass into bioethanol.

PROOFREADING

1.	In world	:	world
2.	price	:	prices
3.	raising	:	rising
4.	and	:	,and
5.	can	:	That can
6.	usable	:	used
7.	material	:	materials
8.	Fuel grade	:	Fuel -grade
9.	factory	:	The factory
10.	environmental	:	environmentally
11.	reducing	:	reduce
12.	previous	:	A previous
13.	Grayish white	:	Grayish-white
14.	l convex concave	:	convex-concave
15.	sugarcane	:	A sugarcane
16.	***cereviseae***	:	***cerevisiae***
17.	amount	:	number
18.	located	:	Was located
19.	Has no effect on	:	Does not effect
20.	basing	:	based
21.	few	:	fewer
22.	suggest		suggests