The Hydrolysis of Cow's Rumen and Rice Straw Mixture using Sulfuric Acid and The Fermentation of Its Hydrolysate in Bioethanol Production

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Abstract. This study aims to investigate the effect of cow's rumen and rice straw ratio on hydrolysis as well as sulfuric acid concentration, temperature, and time were also examined. The fermentation was also carried out, with the main focus on the effect of the yeast concentration. The more straw in the material, the higher the glucose. In the rumen and straw ratio of 40:60 and 80:20%, 0.3 M sulfuric acid, 90 ºC, and 60 minutes, the glucose was 10.60 and 5.87 g/L. Hydrolysis at 90 was better than 80 ºC. In the rumen and straw mixture of 40:60%, 0.6 M sulfuric acid, and 75 minutes, the glucose was 18.47 and 15.11 g/L. The catalyst concentration of 0.6 was also better than 0.3 M, in which the glucose could reach 17.32 and 8.46 g/L at a rumen and straw ratio of 0:100, 90 ºC, and 45 minutes. Glucose in the hydrolysate could be fermented well. The higher the yeast concentration, the higher the ethanol content, respectively 1.22, 1.35, and 1.52% in 72 hours at yeast concentrations of 9, 15, and 20 g/L. Ethanol rose sharply within 24 hours and after 72 hours it continued to rise even though it started to slow down at 60 hours.

1 Introduction

Global energy needs continue to increase due to the high development of human population. Primary energy needs are still fulfilled from fossil fuels such as petroleum, coal, and natural gas [1]. Imprudent exploitation of fossil fuels causes an increase in

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greenhouse gases [2]. Renewable and environmentally friendly energy must be developed as a form of solution, one of which is bioethanol, because it contains 34.7% oxygen [1]; [3]; [4]. Due to the high octane number of bioethanol (105-110); bioethanol provides advantage of a higher heat of vaporization [5].

In Indonesia, most bioethanol is produced from raw materials such as sugar and starch, which are also food ingredients; therefore there are concerns whether it could threaten national food security [6]. The use of agricultural and livestock waste containing cellulose as raw material for bioethanol is starting to be optimized [7]. The potential of biomass waste obtained from agriculture in Indonesia reaches around 146.7 million tons per year or equivalent to about 470 GJ per year [8]. Especially, in Jawa Tengah, the dry rice grain production achieves about 9.62 million ton, therefore, the rice straw obtained will be about 1.5 times of 9.62 million ton (14.43 million ton). Rice straw contains cellulose and hemicellulose by more than 50% [9]. Rice straw has a high content of cellulose (33-47), hemicellulose (19-27), and lignin (23.4% w/w) [10]; [11]). In general, rice straw is only used for animal feed and is burned or left to rot. Rotting biomass waste will produce pollutants and contribute to greenhouse gases. According to the mention above, it is more obvious to use rice straw as a raw material for bioethanol production. Meanwhile, in the agriculture area, many farmers own a cow farm. The cow's rumen as a farm waste is found in the cow's stomach and functions as a place to store and ferment food. In addition to various microbes, namely bacteria, fungi, archaea, and protozoa, it also contains cellulose and hemicellulose. These microbes can degrade the plant cell walls and fibrous substances into absorbable compounds such as proteins and fatty acids. The rumen temperature is 39.6 ^{o}C, which makes it a potential habitat for microorganisms, especially yeast [12]. Rumen is also one of the best habitats for microorganisms to hydrolyze cellulose [13]. Thus, a mixture of rice straw and cow's rumen allows for the bioethanol production with the addition of *Saccharomyces cerevisiae*. The research result can be used to evaluate the bioethanol production in agriculture area.

In general, bioethanol synthesis from biomass consists of two main stages, specifically hydrolysis and fermentation [10]. Hydrolysis is a chemical reaction using water to break down a substance into smaller molecules. Hydrolysis can use concentrated acids, such as sulfuric acids, hydrochloride, nitrate, or phosphate. The acids will act as catalyst by activating the water. At this stage, rice straw must be dried first to get moisture content below 10%. Large amounts of water will dilute the acid thereby weakening the hydrolysis process [14]; [15]. Fermentation is chemical change in an organic substrate that can take place due to catalysts known as enzymes, which are formed due to the presence of certain living microbes [16]. Ethanol fermentation is carried out using yeast (*Saccharomyces cerevisiae*) due to its resistance to low pH and it has high tolerance to ethanol [17]. This study investigates the hydrolysis reaction of the mixture of rice straw and cow's rumen with sulfuric acid and the fermentation of its hydrolysate using *Saccharomyces cerevisiae* in producing bioethanol.

2 Research Methodology

The equipment used in this study were autoclave, watch glass, scales, dropper pipette, Buchner flask, measuring pipette, ball filler, glass stirring rod, beaker glass, measuring cup, Memmert oven, aluminum foil, funnel, vacuum pump, filter paper, test tubes, test tube rack, spectrophotometer, cuvette, Conway cup, fermentation airlock, glass bottle, incubator, hot plate, laboratory spatula, refrigerator, and 16 mesh sieve. The materials used were rumen obtained from the Rumah Pemotongan Hewan Semarang (Semarang Slaughterhouse) and rice straw obtained from agricultural waste in Ambarawa, as well as H2SO4, NaOH, Nelson A and B, Alum, Urea, NPK, Peptone, Yeast, Aquadest, Arsenomolybdate, Vaseline,

which were obtained from Laboratorium Teknologi Pengolahan Biomassa Universitas Negeri Semarang (Biomass Processing Technology Laboratory, Semarang State University).

2.1 Research procedure

Dried rice straw was sifted using a 16 mesh sieve, as was the cow's rumen. Before being used, rice straw and cow's rumen were analyzed for their content of water, ash, fat, crude fiber, protein, and carbohydrate in Laboratorium Bioteknologi - BRIN. The two ingredients were weighed according to their ratio between rumen and straw (as follows 0:100, 20:80, 40:60, 60:40, 80:20, and 100:0%), and the weight of the hydrolyzed ingredient was 25 g.

Hydrolysis reaction was performed using 200 mL of sulfuric acid as catalyst with a concentration of 0.3 and 0.6 M, and using 25 g of raw material in a 250 mL glass beaker which was placed in an autoclave. The autoclave temperature was varied at 80 and 90 ^{o}C and the hydrolysis durations were 30, 45, 60, 75, and 90 minutes. After hydrolysis process was complete, the hydrolysate was filtered and cooled, then stored in the refrigerator. The hydrolysate was analyzed for glucose concentration using the Nelson Somogyi method.

Glucose analysis was commenced by diluting 1 mL of hydrolysate using aquadest up to 100 mL, then adding 1 mL of Nelson's solution (Nelson A:Nelson B at 25:1). The mixture was heated for 30 minutes and then cooled. Subsequently 1 mL of arsenomolybdate was added and shaken; 7 mL of distilled water was added and shaken. The absorption was measured at 540 nm. The measurement results were included in the regression equation that had been obtained, using a standard glucose monohydrate solution, in which x = absorbance and y = glucose concentration (ppm) (Equation (1).

$$y = 0.0079x + 0.1986 \quad (1)$$

The fermentation was executed using 150 mL of hydrolysate obtained from hydrolysis process under the following conditions: rumen and straw ratio of 20:80%, temperature of 80 ^{o}C, catalyst concentration of 0.6 M, and duration of 90 minutes. Before the fermentation, the pH of the hydrolysate was adjusted to 5 by adding NaOH 1 N. The hydrolysate was put into a fermentation bottle and 0.05 g of glucose, 0.03 g of NPK, 0.5 g of peptone, and 0.025 g of urea were added; then stirred them until homogeneous, and afterwards the mixture was sterilized using an autoclave at 121 ^{o}C for 15 minutes. The hydrolysate was cooled to room temperature and after that instant yeast was added, using the following variations 9, 15, and 20 g/L. The bottle was closed with a fermentation airlock and placed in an incubator at 30 ^{o}C. The fermentation was carried out for 72 hours and samples were taken every 12 hours to analyze the ethanol concentration using the Conway method.

Ethanol analysis was commenced by determining the maximum wavelength using a spectrophotometer between 400-550 nm, which was at 447 nm. Regression curves were generated from several standard ethanol solutions at concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0% and the results were presented in Equation (2), in which x = absorbance and y = ethanol concentration (% volume).

$$y = -0.2936x + 0.0159 \quad (2)$$

The ethanol concentration analysis was accomplished by pouring 5 mL of dichromic acid into the center of the cup, followed by adding 5 mL of sample solution and 1 mL of sodium carbonate to the edge of the cup. Subsequently cover the cup coated with vaseline and being heated at 80 ^{o}C for 25 minutes. The solution was cooled to room temperature for

7 minutes. Before checking the absorbance, the remaining dichromic acid solution was diluted until it reached 25 mL in a measuring flask.

3 Results and Discussion

The results of proximate analysis showed that the rice straw contained 10.51 water; 19.42 ash; 0.88 fat; 27.62 crude fiber; 4.31 protein; and 64.88 (%w/w) carbohydrates. Meanwhile, the cow's rumen contained 09.85 water; 10.27 ash; 5.14 fat; 30.35 crude fiber; 7.59 protein; and 67.15 (%w/w) carbohydrates. Rice straw as agricultural waste contained a composition of 37.71 cellulose, 21.99 hemicellulose, and 16.62% lignin [18]; [19]. Meanwhile, cow's rumen also contained a high lignocellulose composition (31.4 cellulose, 20.5 hemicellulose, and 11.6% lignin) [20]. These two materials theoretically could be converted into bioethanol, specifically from rice straw 0.34 liters of ethanol per kg of material and from rumen 0.30 liters of ethanol per kg of material. In the rumen there were also several microorganisms that could be utilized [21]. The overall process optimization would result in maximum conversion of cow's rumen into ethanol. In this study, cow's rumen was used because of its carbohydrate composition.

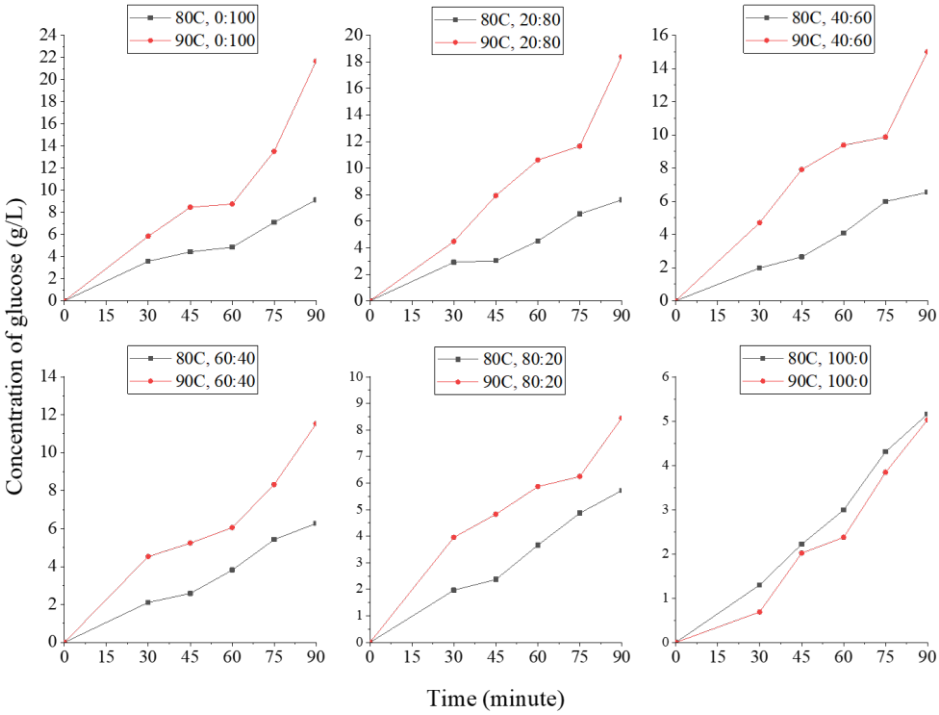

Figure 1. Glucose Concentration Resulting from Hydrolysis of Rumen and Rice Straw Mixture at Catalyst Concentration of 0.3 M

Throughout hydrolysis reaction, the glucose concentration continued to increase. At a temperature of 80 °C, the glucose concentration indicated stability after 90 minutes, but at 90 °C, the concentration continued to increase. The effect of temperature on glucose concentration can be seen in Figures 4.1 and 4.2. Using the same catalyst concentrations and rumen:straw ratio, higher temperature resulted in higher glucose concentration. By using rumen and straw ratio of 0:100%, catalyst concentration of 0.3 M, and temperatures of 80 and 90 °C for 30 minutes, the glucose concentration increased from 3.58 to 5.84 g/L.

Meanwhile, by using rumen and straw ratio of 80:20%, catalyst concentration of 0.6 M, and 45 minutes reaction time, the glucose concentration rose from 7.01 to 9.88 g/L. The average increase of glucose concentration was 4.45 g/L (17.8%) as the temperature increased from 80 to 90 ºC. As the temperature increased, the glucose concentration resulting from hydrolysis would also increase. Increasing temperature could speed up the reaction, so that the glucose concentration produced was also increasing [22]. The hydrolysis of avocado seeds using a sulfuric acid catalyst, the glucose concentration increased by 50% when the temperature was increased from 75 to 95 ºC [23]. The hydrolysis of corn cobs using a sulfuric acid catalyst the glucose concentration increased by 12.24% along with the increasing temperature from 125 to 175 ºC [24]. The glucose concentration resulting from hydrolysis reaction in this study was relatively good. Because 90 ºC shows better result than 80 ºC, therefore for future research, experiment using temperature above 90 ºC has to be conducted to know the optimum hidrolysis temperature.

The mixture ratio of rumen:straw also affected the hydrolysis. In Figures 4.1 and 4.2, the more rumen was being used, the lower the glucose concentration. At a temperature of 80 ºC, catalyst concentration of 0.6 M, hydrolysis time of 60 minutes, and a rumen and straw ratio of 0:100%, the glucose produced was 16.47 g/L. While at a rumen and straw ratio of 20:80%, the glucose concentration was 14.90 g/L, and at ratio of 40:60; 60:40; 80:20 and 100:0%, the glucose concentrations were 13.66, 11.74, 9.13, and 4.69 g/L respectively. Therefore, the less straw was being used, the lower the glucose concentration. This is because less straw will reduce the amount of holocellulose; which is a source of glucose polymers [9].

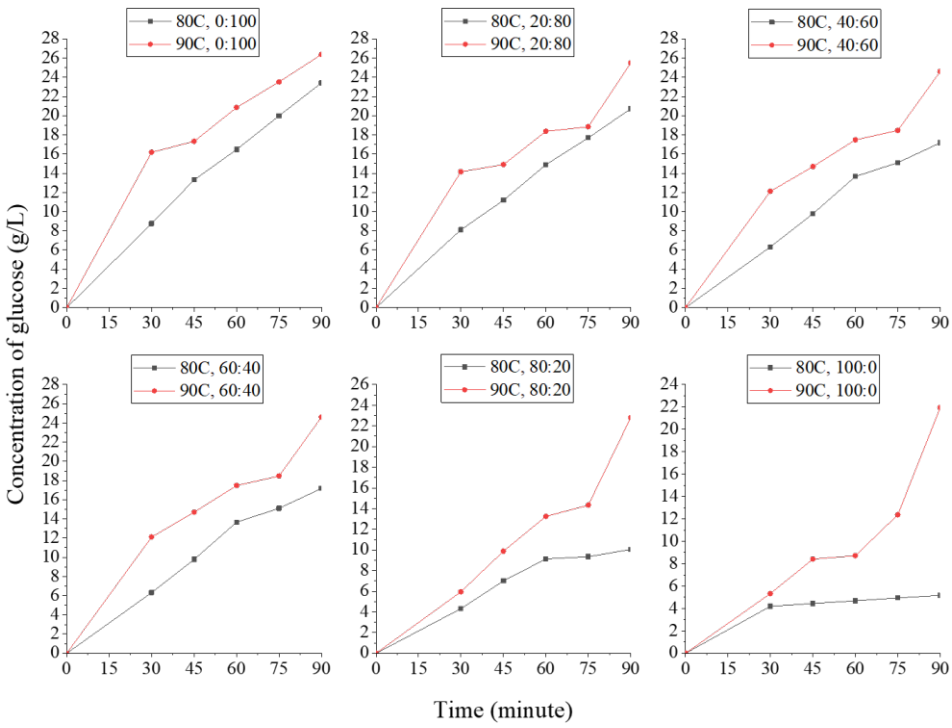

Figure 2. Glucose Concentration Resulting from Hydrolysis of Rumen and Rice Straw Mixture at Catalyst Concentration of 0.6 M

The utilization of rice straw into ethanol has also been studied. Straw was hydrolyzed using an HCl catalyst varied at 7, 14, 21, and 28%, temperature at 100 ºC, and duration of

2.5 hours. The glucose concentration produced was 0.003936 mol/L (0.71 g/L) using a 21% HCl catalyst [25]. In addition, there was also other study that examined the hydrolysis reaction of rice straw mixed with water hyacinth using 1% H_2SO_4 as a catalyst. The resulting glucose concentration was 23.33 mg/L (0.00124 mol/L) at a temperature of 100 ºC for 60 minutes [26]. In this study, the rumen and straw ratio of 0:100% with a catalyst concentration of 0.6 M and a temperature of 90 ºC, the resulting glucose concentration of 23.51 g/L was achieved in 90 minutes. Therefore, the hydrolysis conditions in this study were relatively good and could be improved to obtain more optimal results. There are many studies on cow's rumen that have been conducted. One of them is concerning the bacteria in the cow's rumen that can be used to produce cellulase enzymes, which are used to break down cellulose in producing bioethanol. The hydrolysis of rice straw and sugarcane bagasse (0.05 g each) using cellulase enzyme (0.1 mL) isolated in cow's rumen produced 207.25 and 193.64 mg/mL glucose, respectively for rice straw and sugarcane bagasse [27]. Cow's rumen has fluid containing some bacteria, namely *Butyrivibrio*, *Prevotella*, *Ruminococcus*, unclassified *Lachnospiraceae*, *Ruminococcaceae*, and *Bacteroidales*. These bacteria can degrade lignocellulose into short-chain fatty acids [28].

In the hydrolysis of mixture of rumen and straw, at all mixture ratios and temperatures, the glucose concentration at a catalyst concentration of 0.6 M was higher than at 0.3 M (see Figures 4.1. and 4.2.). The higher the catalyst concentration, the higher the glucose hydrolysis results [29]. The glucose concentration increased because the concentration of hydrochloric acid as a catalyst in the hydrolysis of cassava shells also increased [30]. In this study, at rumen and straw ratio of 40:60% using temperature of 80 ºC and duration of 75 minutes, the glucose concentrations obtained were 9.87 and 18.47 g/L respectively at a catalyst concentration of 0.3 and 0.6 M. Meanwhile, at a temperature of 90 ºC, using rumen and straw ratio of 0:100% and duration of 60 minutes, the glucose concentrations obtained were 4.85 and 8.74 g/L.

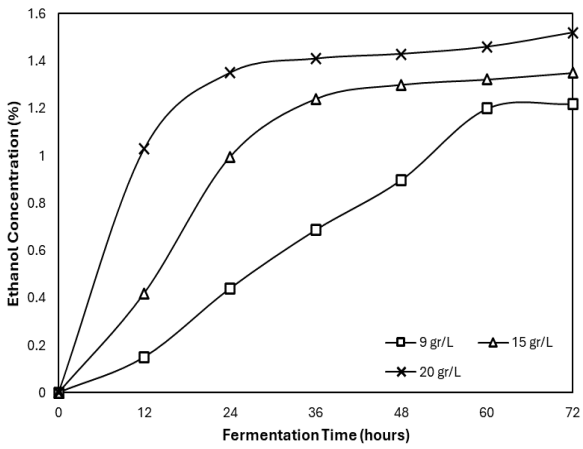

Figure 3. Relationship between Ethanol Concentration and Fermentation Time During the Fermentation of Hydrolysate Resulting from Hydrolysis of Cow's Rumen and Rice Straw Mixture

During fermentation, the number of *Saccharomyces cerevisiae* became an important factor influencing the fermentation [31]. Figure 4.3. depicted the results of hydrolysate fermentation from the hydrolysis of cow's rumen and rice straw mixture using instant yeast at concentrations of 9, 15 and 20 g/L. The ethanol production curve was relatively good at 15 and 20 g/L. Rapid production occurred from the start of fermentation until 24 hours, then slowed down and then stabilized after 48 hours. This was in line with study by Prawati and Trisakti [32], describing that ethanol resulting from fermentation increased on the first

day, continued to increase before finally decreased. The condition when the ethanol concentration was stable or did not increase anymore was due to the number of living cells was decreasing, thus the activity of yeast to convert glucose into ethanol was also decreasing [33]. The yeast concentration of 9 g/L was too low for the hydrolysate fermentation; accordingly the ethanol produced was the lowest and the slowest. The higher the amount of yeast being applied used in the fermentation process, the more optimal the ethanol content would be. In addition, the optimum mass of *Saccharomyces cerevisiae* was 1.5%-2% [34]. In this study, the amount of yeast that were used were 9 (0.9), 15 (1.5), and 20 g/L (2.0%). The glucose concentration in the hydrolysate was 22.82 g/L. Theoretically, if being fermented it would produce ethanol as much as 11.41 g/L (yield 51%). The maximum ethanol obtained was 1.52% (yield 30.24%) using 20 g/L yeast and duration of 72 hours.
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4 Conclusion

The cow's rumen and rice straw mixture is extremely promising to be converted into bioethanol. The mixture is hydrolyzed into glucose using diluted sulfuric acid and subsequently being fermented into ethanol using yeast. The more straw being applied, the more glucose is produced, and accordingly the amount of ethanol is also higher. The hydrolysis process at a catalyst concentration of 0.6 M produced higher glucose than 0.3 M. Similarly, the hydrolysis reaction at temperature of 90 oC produced higher glucose than at 80 oC. In the fermentation process, higher concentration of yeast being applied produced more ethanol. Moreover, 72 hours of fermentation using yeast concentration of 20 g/L generated ethanol concentration as much as 1.52% (yield 80%).

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