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Hydrolysis and Fermentation of *durian* Seed Flour: Effect of Cu^{2+} and Fe^{3+} during Fermentation on Bioethanol Concentration

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Abstract:- Bioethanol is an alternative fuel as a mixture of conventional fuel. The production of bioethanol involves the fermentation of biomass with live microorganism cells. One of the raw materials that is lacking in use but has great potential for bioethanol production is durian seed flour. The production process of bioethanol from durian seed flour includes enzymatic hydrolysis using a mixture of alpha-amylase and glucoamylase enzymes at concentrations of 100, 150, and 200 g/L of durian seed flour. Subsequently, the fermentation process is carried out using *Saccharomyces cerevisiae* yeast. This study aimed to investigate the effects of adding metal ions Cu^{2+} and Fe^{3+} during the processes of hydrolysis and fermentation. The research methodology begins with the preparation of durian seed flour. Furthermore, the durian seed flour is hydrolyzed by adding metal ions such as Cu^{2+} and Fe^{3+}, followed by the analysis of glucose concentration. Higher substrate concentrations lead to higher glucose concentrations. Hydrolyzing durian seed flour at a concentration of 200 g/L for 9 hours increases the glucose concentration by 83.72% compared to a concentration of 100 g/L of durian flour. Before the fermentation stage, a *Saccharomyces cerevisiae* starter culture is prepared. Based on the experimental results, the addition of Cu^{2+} and Fe^{3+} metal ions has a positive effect as activators in the enzymatic hydrolysis process. The addition of Fe^{3+} metal ions during fermentation for 72 hours yields the highest bioethanol concentration at 11.83%, whereas the addition of Cu^{2+} results in a bioethanol concentration of 9.4%. This aligns with the theory that the addition of Fe^{3+} enhances yeast cell growth during the fermentation process.

Keywords:- *Bioethanol; Durian Seed Flour; Fermentation; Mineral Ion.*

I. INTRODUCTION

The Indonesian government strongly supports the use of renewable energy sources as an alternative energy solution, such as bioethanol. Bioethanol is a fuel derived from biomass. Durian seeds as waste can be utilized as the raw material for bioethanol production. The process of producing bioethanol from durian seeds involves several stages: the creation of durian seed flour, enzymatic hydrolysis followed by fermentation using yeast. *Saccharomyces cerevisiae* is one of the most commonly used yeasts. The hydrolysis process of durian seed flour employs *Stargen™ 002*, a mixture of alpha-amylase and glucoamylase enzymes, to convert the flour into glucose at a temperature of 30°C [1], which differs from conventional hydrolysis processes, which require temperatures of 90-120°C [2]. Several studies have revealed that divalent mineral ions can influence enzymes by forming electrostatic interactions with charged residues on the enzyme [3]. In a study conducted by [4], it was stated that alkaline earth mineral ions, such as Cu^{2+} and Mg^{2+} can enhance enzyme activity. According to [5], transition metal ions like Ca^{2+}, Mn^{2+} and Zn^{2+} can inhibit the efficiency of enzymatic hydrolysis. Furthermore, other mineral ions like K^{+} and Fe^{3+} can expedite the fermentation process [6]. The presence of Ca^{2+} ions can hinder the fermentation process for ethanol production from molasses using *Saccharomyces cerevisiae*. The fermentation rate decreases as the added Ca^{2+} ions increase [7]. Consequently, the addition of different mineral ions in different substrates can yield varying effects. This research aims to investigate the influence of Cu^{2+} and Fe^{3+} on the hydrolysis and fermentation of durian starch on bioethanol concentration. The results of this study are expected to support the Indonesian government's program in providing bioethanol from non-food, economically less valuable raw materials.

II. MATERIALS AND METHODS

A. Materials

Durian seeds are obtained from the Sidikalang District in the city of Medan. *Saccharomyces cerevisiae*, urea, potassium phosphate (KH_2PO_4), magnesium sulfate ($MgSO_4$), a synergistic mixture of α-amylase and glucoamylase enzymes are acquired from the *Indrasari Chemical Supplies* store in Semarang, Indonesia. Meanwhile, NaCl, $FeCl_3$, $CuCl_2$, H_2SO_4, anhydrous glucose, and sodium alginate are sourced from the *Multi Kimia Raya Chemistry Store* in Semarang, Indonesia..

B. Methods

➢ *Preparation of Durian Seed Flour*

Durian seeds are collected, boiled for 20 minutes, peeled, and then thinly sliced. The slices of durian seeds are dried in the sun, subsequently ground into a fine powder using a *Hammermill*, and sifted through a 120-mesh sieve, yielding durian seed flour [1].

➢ *Glucose Analysis*

The hydrolysis filtrate is analyzed for glucose concentration using the dinitrosalicylic acid (DNS) method [8]. The reagent consists of a 1% solution of 3,5-dinitrosalicylic acid, 0.05% sodium sulfite, 20% sodium-potassium tartrate, and 1% sodium hydroxide, added to the sample in a reaction tube at a 3:1 ratio. The mixture is shaken and incubated in boiling water for 8 minutes. Subsequently, the sample is cooled using ice water for 5 minutes, and absorbance is measured at 570 nm using a UV/visible spectrophotometer (*UV-160A, SHIMADZU, Kyoto, Japan*). Pure glucose solutions ranging from 0 to 10 g/L are used as standard solutions, thus the measurement of reducing sugar concentration is expressed in units of g/L.

➢ *Analysis of Bioethanol Content Using UV-Vis Spectrophotometry*

The determination of bioethanol content is performed using a UV-Vis spectrophotometer. The reagent used is dichromic acid. The preparation of the dichromic acid reagent involves weighing 7.5 grams of potassium dichromate, dissolving it in 250 mL of 5 M H_2SO_4 while keeping it cold, and stirring until homogeneous. Next, 5 mL of the sample is mixed with 2 mL of dichromic acid reagent and heated for 5 minutes. Subsequently, the absorbance of the sample is measured at a wavelength of 580 nm using a UV-Vis spectrophotometer. Ethanol solutions with concentrations of 0.1%, 0.2%, 0.3%, 0.4%, and 0.5% are used as standard solutions [1]. The spectrofotometric testing is conducted in the *Integrated Laboratory of Diponegoro University, Semarang*.

➢ *The Effect of Cu^{2+} and Fe^{3+} Addition on the Hydrolysis of Durian Seed Flour on Glucose Concentration*

The hydrolysis of durian seed flour is conducted at concentrations of 100, 150, and 200 g/L as the substrate. $CuCl_2$ and $FeCl_3$ are added to each substrate at a concentration of 40 ppm. Subsequently, the mixture is incubated in a shaker at a speed of 100 rpm for 10 minutes at pH 4. To maintain pH, the slurry is controlled using a 0.01 M sodium phosphate citric acid buffer solution. For the hydrolysis process, the durian seed flour substrate is transferred into test jars and supplemented with 2% (w/w) *Stargen TM 002* at a temperature of 30°C and pH 4, for a duration of 24 hours. Samples are taken at intervals of 3, 6, 9, 12, and 15 hours, followed by centrifugation for 4 minutes at 100 Hz [6]. The centrifuged samples, after separating the filtrate, are then filtered using *Whatman CAT 40 No.1440-125 mm* filter paper to obtain a solid-free filtrate. Subsequently, the filtrate is analyzed to determine the concentration of reducing sugars.

➢ *Preparation of Durian Flour Starter*

The process of creating a starter begins by introducing 15 grams of durian flour into 300 mL of distilled water (aquadest). Subsequently, 35 mL of the durian flour suspension is heated for sterilization. Next, the solution is cooled to a temperature of 30ºC. Add 0.15 grams of KH_2PO_4, 0.15 grams of $MgSO_4$, and 0.15 grams of urea as nutrients. Adjust the solution's pH to 5. Once the pH is adjusted, add 0.15 grams of yeast to the solution and stir until the solution becomes homogeneous. Then, add 0.15 grams of dry yeast on top of the solution. The Erlenmeyer flask is sealed with aluminum foil and perforated appropriately. Place the erlenmeyer flask in an incubator shaker. The preparation of the starter is carried out for 24 hours at an incubator shaker speed of 100 rpm [9].

➢ *The Effect of Cu^{2+} and Fe^{3+} Addition on the Fermentation of Durian Seed Flour on Glucose Concentration*

The fermentation process begins with the preparation of an erlenmeyer flask as the fermentation vessel. The product of the hydrolysis process is cooled. Next, add 35 mL of the starter (yeast) to 65 mL of the hydrolysis product in the erlenmeyer flask. Add Cu^{2+} and Fe^{3+} mineral ions at a concentration of 40 ppm, with 2 mL of each. Seal the erlenmeyer flask with aluminum foil. Place the erlenmeyer flask in an incubator shaker. The fermentation process is carried out for 48 hours at an incubator shaker speed of 100 rpm. The fermentation product is then filtered using *Whatman CAT 40 No. 1440-125 mm* filter paper [9].

III. RESULTS AND DISCUSSION

A. Durian Seed Flour Analysis

The analysis of durian flour includes moisture content and ash content. The moisture content and ash content obtained are 6.94% and 5.55%, respectively.

B. The Effect of Durian Seed Flour Concentration on Glucose Concentration

The impact of durian seed flour concentrations of 100, 150, and 200 g/L on the hydrolysis process over 15 hours, as illustrated in Fig.1.

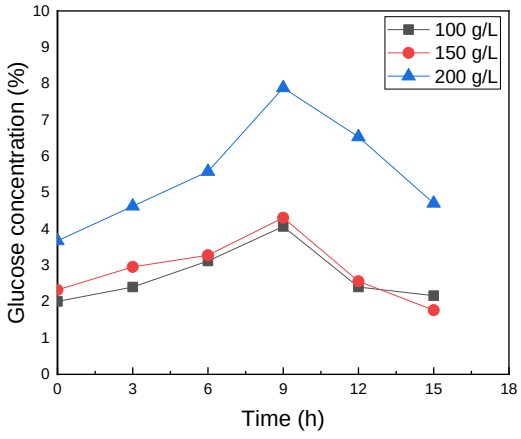

Fig. 1. Effect of durian seed flour concentration on glucose concentration without the addition of metal ions

During the hydrolysis process from 3 to 12 hours, the highest glucose concentration was achieved at the 9-hour mark when using a durian seed flour concentration of 200 g/L. Increasing the concentration of durian seed flour from 100 g/L to 150 g/L did not show a significant difference, but at a concentration of 200 g/L, there was an 83.72% increase in glucose concentration compared to the 100 g/L concentration. This is because as the concentration of flour (as the substrate) increases, more substrate is converted into glucose [10], and the enzyme's active sites are still capable of converting a larger amount of substrate [11].

Reference [12] conducted research on the hydrolysis of sweet cassava starch (100-300 g/L) using a mixture of alpha-amylase and glucoamylase enzymes at a concentration of 1.5% (v/w), and the best glucose concentration obtained was 41.33 g/L at a starch concentration of 200 g/L [6]. This is due to the fact that higher substrate concentrations provide more starch (polysaccharide) available for conversion into reducing sugars [13]. However, when the enzyme reaches saturation, its activity will not increase and may even result in a decrease on glucose concentration. Additionally, there is a possibility of inhibition due to the high substrate concentration [14].

C. The Effect of Cu^{2+} and Fe^{3+} Addition in Durian Seed Flour Suspension During Hydrolysis

The influence of glucose concentration during hydrolysis with the addition of Cu^{2+} and Fe^{3+} is depicted in Fig. 2. The addition of Cu^{2+} during the hydrolysis of durian seed flour for 9 hours did not exhibit a significant impact compared to native durian flour. However, the addition of Fe^{3+} did have an effect, resulting in an increase in glucose concentration from 13.76% (native) to 14.40%. After 9 hours of hydrolysis, the glucose concentration for native durian flour and flour with the addition of Fe^{3+} and Cu^{2+} decreased.

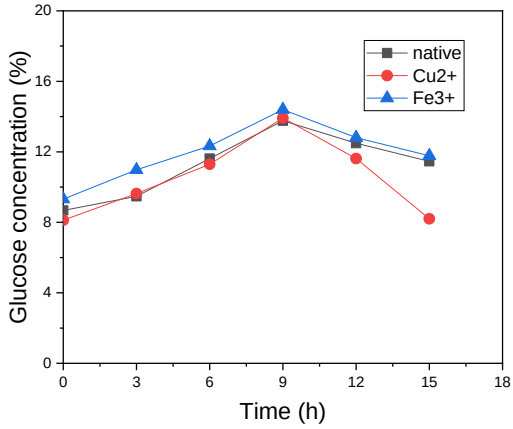

Fig. 2. The effect of adding metal ions on glucose concentration at durian seed flour concentration 200 g/L

In contrast to the research conducted by [6] on the hydrolysis of sweet cassava starch for 12 hours with the addition of Ca^{2+}, which resulted in a reducing sugar concentration of 32.82 g/L, indicating a 32.1% increase compared to native cassava starch [6]. Reference [15] stated that the addition of Ca^{2+} leads to a higher increase in reducing sugar concentration compared to the addition of Na^{+} ions because Ca^{2+} can activate enzymes by binding to water and protein residues. Research on enzymatic hydrolysis with the addition of monovalent, divalent, and trivalent metal ions showed that the addition of Ca^{2+} resulted in the highest enzyme activity among other metal ions. This is generally because amylases are known as metalloenzymes and contain at least one Ca^{2+} ion as an integral component to enhance enzyme activity [16].

D. The Effect of Metal Ions Addition on the Fermentation Process and Bioethanol Concentration

The product resulting from the hydrolysis process of durian seed flour is then fermented for 72 hours, both without the addition of metal ions and with the addition of Cu^{2+} and Fe^{3+}. The fermentation results in bioethanol production, as shown in Fig. 3. The addition of Fe^{3+} and Cu^{2+} yielded bioethanol concentrations of 11.3% and 9.4%, respectively, whereas without the addition of metal ions, the bioethanol concentration was 9.2%. The addition of Fe^{3+} increased the bioethanol concentration by 22.83% compared to the metal-free (native) condition, while the addition of Cu^{2+} did not have a significant effect.

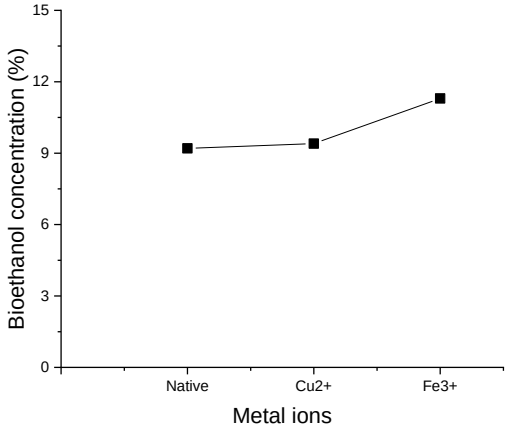

Fig. 3. The effect of adding metal ions on bioethanol concentration

Reference [17] conducted fermentation for 7 days using *Saccharomyces cerevisiae* while adding magnesium ions (Mg^{2+}) at concentrations of 0, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 g/L. The maximum bioethanol concentration obtained was 17 g/L with the addition of Mg^{2+} at a concentration of 1 g/L. Furthermore, [18] added potassium ions (K^{+}), magnesium ions (Mg^{2+}), and copper ions (Cu^{2+}) to the bioethanol fermentation medium with *Saccharomyces cerevisiae*. The highest bioethanol yield, 96.38 g/L, was achieved in the fermentation medium with the addition of potassium ions (K^{+}).

Research by [19] demonstrated that the addition of Fe^{3+} increased the bioethanol concentration by 18% compared to conditions without the addition of metal ions. Fe^{3+} serves as an essential nutrient for yeast cells because it plays a role in various oxidation-reduction reactions within the cell. Reference [20] explained that the effect of adding

Cu^{2+} to fermentation outcomes depends on the source and concentration of copper used. At low concentrations, Cu^{2+} does not impact the growth of Saccharomyces cerevisiae cells but can hinder fermentation performance.

IV. CONCLUSIONS

The addition of Cu^{2+} and Fe^{3+} has yielded positive results as activators in the enzymatic hydrolysis process. The addition of Fe^{3+} resulted in a glucose concentration of 14.4%, while Cu^{2+} produced a glucose concentration of 13.92%. In the fermentation process, the addition of Fe^{3+} yielded a bioethanol concentration of 11.3%, while Cu^{2+} resulted in a bioethanol concentration of 9.4%. Meanwhile, the bioethanol concentration produced from the hydrolysis of durian seed flour without the addition of metal ions was 9.2%. The addition of Fe^{3+} increased the bioethanol concentration by 22.83% compared to the metal-free (native) condition, while the addition of Cu^{2+} did not have a significant effect.

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