Bioethanol Production from Tapioca Solid Waste (*Onggok*) in a Batch Reactor

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***Abstract*— Tapioca solid waste (*Onggok*) is a byproduct of processing tapioca flour in the form of dregs and contains a lot of fiber, especially cellulose and hemicellulose that can be developed benefits by treating the waste through an enzymatic process by hydrolysis as Bioethanol. Bioconversion technology is an enzymatic conversion of materials by hydrolysis, which can be used to increase the value of *Onggok*. This research aims to convert starch from *Onggok* in the tapioca flour industry into Bioethanol through enzyme hydrolysis and fermentation processes. This study was conducted to determine the effect of the ethanol content produced from the concentration of 50 g/l, 100 g/l, and 150 g/l *Onggok* concentrations with 10 ml, 15 ml, and 20 ml enzymes. The *Onggok* samples were hydrolyzed using Alpha-amylase enzymes with 10 ml, 15 ml, 20 ml, and 10 ml Glucoamylase enzymes. In the liquefaction process, reducing sugar content was analyzed every 30 min for 2 hours, then in the saccharification process, reducing content was analyzed every 30 min for 3 hours. The acid hydrolysis solution was adjusted to pH 4.5, fermented for three days, and analyzed every 12 hours. From the study results, it was found that the optimal treatment variable was the concentration variable of 150 g/l *Onggok* with a concentration of 20 ml of Alpha-amylase enzyme. The ethanol content obtained from the fermentation process was 3.98% (v/v).**

***Keywords*— Bioethanol, *Onggok*, Alpha-amylase, Glucoamylase, Hydrolysis, Fermentation**

I. INTRODUCTION

Ethanol is called alcohol because it can be obtained by fermentation of grains and industrial wastes [1], [2]. Actually, the fermentation of all materials containing carbohydrates such as grapes, potatoes, agricultural residue as well as rice produces Ethanol [3]–[5]. Ethanol used for beverages and gasohol is still made by fermentation. Ethanol used as a solvent is made by hydration of ethylene, a petrochemical substance obtained from the breakdown reaction of petroleum.

Indonesia is a developing country with increasing demand for oil. For example, there is a decline in Indonesia's daily oil production. So that, Bioethanol as an alternative energy form to be developed further. Plants that have the potential to produce Bioethanol are plants that have high carbohydrates, for example, cassava, sugarcane, corn, and straw. The tapioca industry is one of Indonesia's many agricultural industries (agro industry) [6], [7].

Petok Village, Mojo, Kediri District, East java has a tapioca flour processing industry to support abundant food availability. The industry accommodates raw materials from farmers in the Petok area with a capacity of between 2-and 5 tons per day. This tapioca flour processing industry has side effects on solid and liquid waste [8]. *Onggok* includes organic waste because it is generated as a residue from processing cassava, one of the organic materials. *Onggok* is obtained from grating and pressing, and if not handled carefully, it can cause a great potential to pollute the environment. Most of the tapioca industries are located near densely populated settlements. On the banks of rivers, so dumped piles around industrial sites will be fatal to the environment and living things that inhabit the surrounding area. *Onggok* is a tapioca factory waste. The conversion rate of cassava to *onggok* is between 60-65%.

Bioethanol can be obtained from various sources of raw materials, such as sugar, starch, or cellulose[6], [9]–[12]. This research uses tapioca industrial waste piles from Home Industry in Petok Village, Mojo, Kediri District as raw materials. Ethanol has many benefits; namely, it can be consumed by humans as an ingredient in alcoholic beverages and as a raw material for pharmaceuticals and cosmetics. Ethanol is also used as a flavouring agent, medicine, and antifreeze component.

However, attention has turned to ethanol production as a fuel and chemical solvent in recent years. The starch hydrolysis process in this study was carried out by hydrolysis using the enzyme -amylase as a biocatalyst. The hydrolysis reaction is slow, so it is necessary to use a catalyst to speed up the response. The action of enzymes in hydrolyzing starch is to cut 1,4 α-glycoside bonds, but not 1,6 -glycoside bonds [8]. Another thing that needs to be considered in ethanol fermentation and the use of required nutrients is the type of microbes used for ethanol production. According to [8], using hydrolase enzymes, starch, fiber, sucrose, and oligosaccharides can be hydrolyzed into simple sugars that are ready to be fermented. Some of the microbes that can be used in Ethanol production include *Saccharomyces sp*, *Rhizopus sp*, *Mucor sp*, *Aspergillus niger*, *Aspergillus layori*, *Zymomonas mobilis*, and *Kluyveromyces fragilis* [4], [7].

The production of ethanol/bioethanol with plant raw materials containing starch or carbohydrates is carried out by converting carbohydrates into water-soluble sugars (glucose)—conversion of plant raw materials containing starch or carbohydrates into Bioethanol. The three primary process stages in producing Bioethanol are pretreatment, hydrolysis, and fermentation [7]. Glucose can be made from starch, and the manufacturing process can be distinguished based on the auxiliary substances used, namely acid hydrolysis and enzyme hydrolysis [5], [13], [14]. Based on the two types of hydrolysis, enzyme hydrolysis is currently more widely developed, while acid hydrolysis is less developed. The process of making glucose from starch is used by enzyme hydrolysis. Converting

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carbohydrates into water-soluble sugar (glucose) is done by adding water and enzymes. Then, the fermentation or fermentation of Sugar into Ethanol is carried out by adding yeast or yeast [8].

In this study, *onggok* was obtained from home industries in Kediri District. The experiments aimed to evaluate the effects *onggok* concentrations (50, 75, and 150 g/l) for the optimal bioethanol production via enzymatic hydrolysis in a batch fermentation process. The effect of alpha-amylase and glucoamylase activities was also studied.

II. METHOD

A. Materials

Tapioca flour home industries produce solid wastes that are called *Onggok* in Petok Village, Kediri. These materials are used for ethanol production. PT Sorini, Pasuruan, East Java supported an enzyme of Alpha-Amylase and Glucoamylase for experiments for sugar production. Yeast was purchased from a food Store in Surabaya.

B. Method

1. Hydrolysis Process

Onggok is a very moist solid obtained from home industries. Before treatment, this *onggok* was dried under sunshine or oven at a temperature of 105ºC. The dried *onggok* was then used to do the experiment. Hydrolysis was conducted in two steps. First, Liquefaction, the dried *onggok* (50, 100, and 150 g) were put in each a 1000 ml beaker glass and added with l000 ml of distilled water, and 10, 15, and 20 ml of Alpha-amylase enzymes. Then, it was heated at a temperature of 90°C-100°C for two hours. Afterward, this slurry was cooled. Second, Saccharification, this slurry was added with glucoamylase enzyme with the volume of 10 ml and heated at a temperature of 60°C - 66°C for three hours.

2. Fermentation Process

The filtrate from the hydrolysis process was put into a fermenter bottle, and yeast starter, Urea, and NPK were added and then shaken. After that, the fermentation bottle and the gas produced flowed through another bottle filled with water. The fermentation was carried out for 3 d, and the reducing sugar concentration was analyzed every 12 with a fermentation temperature of 30°C, then filtered and took the best filtrate for the distillation process.

3. Analytical Method

The analysis of chemicals in this experiment was reducing sugar concentration and ethanol content. The reducing sugar analysis was conducted using the lane-Eynon method, and the study of ethanol content was using the Gas Chromatography (GC) method.

III. RESULTS AND DISCUSSION

3.1 Result of Sugar Analysis

In the manufacture of Bioethanol, the most crucial element of the raw material for *onggok* is carbohydrate and starch content. The more carbohydrates and starch contained; the more volume of Bioethanol obtained. Starch will be converted into reducing sugar in hydrolysis. After reducing sugar formation, the yeast Saccharomyces cerevisiae will convert it into Ethanol in the fermentation process. Ethanol is the main product of the manufacture of Bioethanol. In order to increase the high concentration of Ethanol, it is necessary to carry out a distillation process. The following data were obtained from the experiments on making Bioethanol that have been carried out.

The production of Ethanol from cassava is generally carried out by enzymatic hydrolysis because the enzyme is specific and does not produce byproducts that interfere with the growth of microorganisms.

Reducing sugar from cellulose (polysaccharides) generally uses enzymatic hydrolysis or acid hydrolysis. However, there are five types of hydrolysis such as water hydrolysis, acid hydrolysis, alkaline hydrolysis, fusion hydrolysis carried out with or without H_2O at high temperatures, and enzyme hydrolysis (using an enzyme catalyst, thus preventing side reactions). Another advantage of enzymatic hydrolysis is that (1) it can increase the product; (2) it works at neutral pH and low temperature; and (3) it is specific and selective for the substrate [12]. Enzymes are widely used, for example, amylase, glucose-isomerase, papain, bromelain, lipase, and protease.

3.2 The Effect of Alpha-amylase Enzyme on the production of reducing sugar

The hydrolysis process aims to break bonds and remove lignin and hemicellulose content as well as damage the crystal structure of cellulose into simple sugar compounds [8].

Figure 1 shows the relationship between the volume addition of alpha-amylase and reducing sugar production at an *onggok* concentration of 50 g/l. The results depicted that at 50 g/l *onggok* and alpha-amylase volume of 10 ml, the reducing sugar obtained was 16.25 g/l for one hour and 25 g/l for two hours. At the volume of 15 ml of the alpha-amylase, the reducing sugar obtained was 23.21 g/l at one hour and 29.54 g/l at two hours. At 20 ml of the alpha-amylase, it was found that the reducing sugar was 27.1 g/l for 1 hour and 32.25 g/l for two hours.

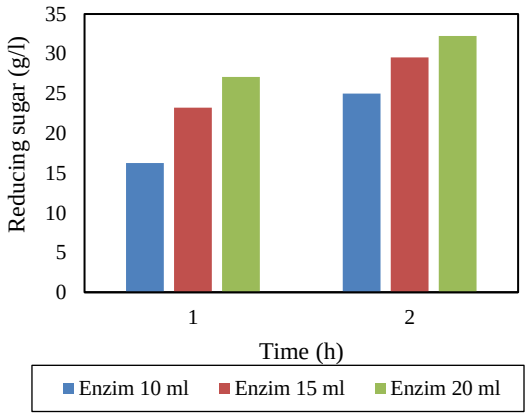

Figure 1. Effect of Alpha-amylase addition in Liquefaction on reducing sugar production with *an onggok* concentration of 50 g/l.

Figure 2 shows the relationship between the concentration of the enzyme of Glucoamylase and the production of reducing sugar at a concentration of 50 g/l *onggok*. The results depicted that at 50 g/l of *onggok* and

Glucoamylase of 10 ml, the reducing sugar obtained was 36.1 g/l for one hour, 40.62 g/l for two hours, 43.33 g/l for three hours, and 46.42 for three hours. At 15 ml of the Glucoamylase, the reducing sugar obtained was 38.23 g/l for one hour, 43.62 g/l for two hours, and 46.42 g/l for two hours. At the volume of 20 ml of the Glucoamylase, it was found that the reducing sugar was 40.62, 46.42, and 50 g/l for one, two, and three hours, respectively.

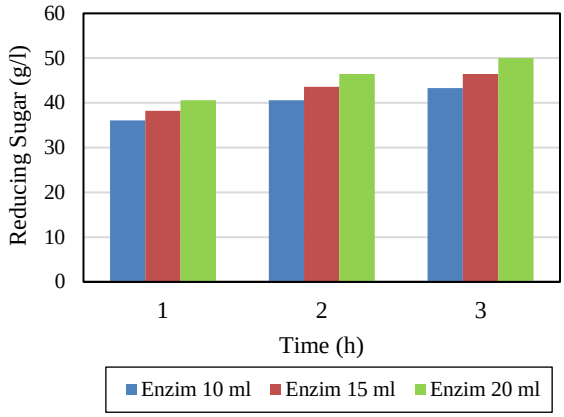

Figure 2. Effect of Enzymatic activity of Glucoamylase in the Saccharification on reducing sugar production with 50 g/l *onggok*.

Figure 3 shows the relationship between the concentration of the enzymatic activity of alpha-amylase and the production of reducing sugar at a concentration of 50 gr/l *onggok*. The results depicted that at 100 g/l of *onggok* and alpha-amylase of 10 ml, the reducing sugar obtained was 30.95 g/l for one hour and 32.5 g/l for two hours. At 15 ml of the alpha-amylase, the reducing sugar obtained was 32.5 g/l for one hour and 34.21 g/l for two hours. At 20 ml of the alpha-amylase enzyme, it was found that the reducing sugar was 36.11 g/l for one hour and 38.23 g/l for two hours.

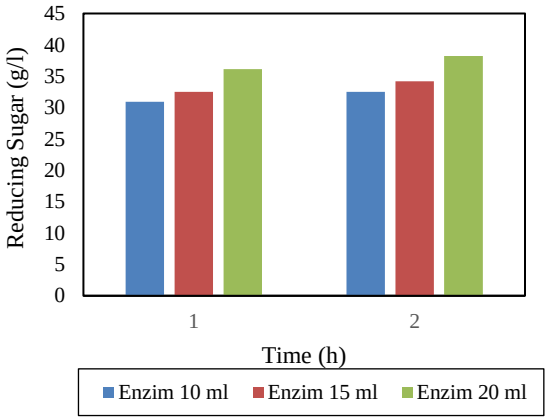

Figure 3. Effect of Alpha-amylase addition in liquefication on reducing sugar production with *an onggok* concentration of 100 g/l.

Figure 4 shows the relationship between the concentration of Glucoamylase and the production of reducing sugar at a concentration of 100 g/l *onggok*. The results depicted that at 100 g/l *onggok* and alpha-amylase enzyme of 10 ml, the reducing sugar obtained was 50 g/l for 1 hour, 54.16 g/l for two hours, and 59.1 g/l for three hours. At 15 ml of the Glucoamylase enzyme, the reducing sugar obtained was 54.16 g/l at 1 hour, 59.1 g/l for two hours, and 65 g/l for three hours. At 20 ml of the Glucoamylase enzyme, it was found that the reducing sugar was 59.1 g/l for 1 hour, 65 g/l for 2 hours, and 72.22 g/l for three hours respectively.

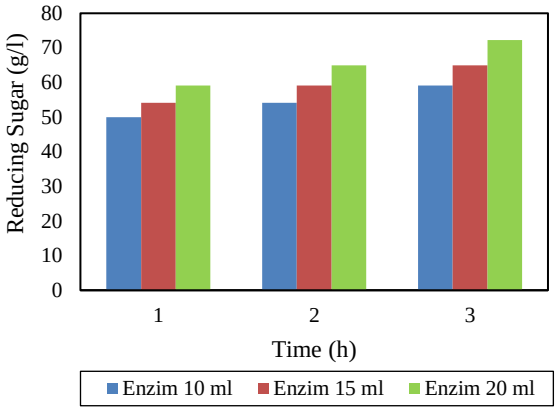

Figure 4. Effect of Glucoamylase Enzyme activity in the Saccharification Process on the production of reducing sugar using 100 g/l *onggok*.

Figure 5 shows the relationship between the enzyme alpha-amylase activity and the production of reducing sugar at a concentration of 150 g/l *onggok*. It can be seen that with the use of the alpha-amylase enzyme 10 ml, the reducing sugar obtained was 40.62 g/l for one hour and 43.33 g/l for two hours. At 15 ml of alpha-amylase, the reducing sugar was 46.42 g/l for one hour and 50 g/l for two hours. At the volume of the alpha-amylase enzyme 20 ml, the reducing sugar obtained was 54.16 g/l for one hour and 59.1 g/l for two hours.

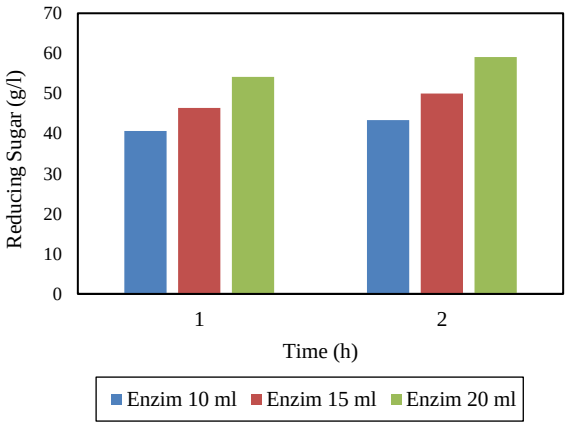

Figure 5. Effect of Alpha-amylase enzyme concentration in Liquefaction on the production of reducing sugar at 150 g/l *onggok*

Figure 6 shows the relationship between the addition of Glucoamylase and the production of reducing sugar at a concentration of 150 g/l of *onggok*. The results indicated that with the use of 10 ml of alpha-amylase and 10 ml of Glucoamylase, the reducing sugar obtained was 65 g/l for one hour, 72.22 g/l for two hours, and 81.25 g/l for 3 hours. At 15 ml alpha-amylase and 10 ml glucoamylase, the reducing sugar was 72.22 gr/l for one hour, 81.25 g/l for two hours, and 92.85 g/l for three hours. At the alpha-amylase of 20 ml and the Glucoamylase of 10 ml, the reducing sugar obtained was 92.28 g/l for one hour, 108.33 g/l for two hours, and 130 g/l for three hours.

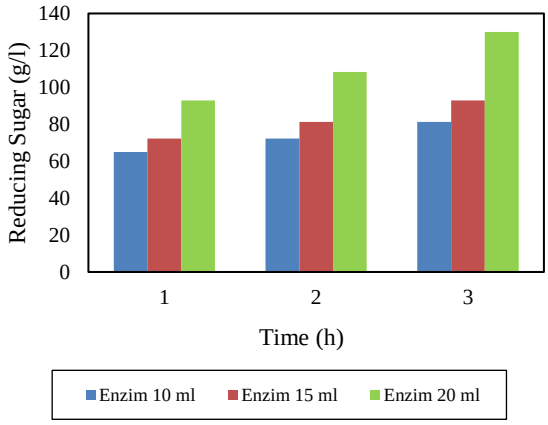

Figure 6. Effect of Glucoamylase addition in the Saccharification process on the production of Reducing sugar at 150 g/l of *onggok*.

The hydrolysis process with enzymes gives a reasonably high reducing sugar level of 54.16-130 g/l under process conditions with an alpha-amylase enzyme concentration of 20 ml, while at a concentration of 10 ml alpha-amylase enzyme, the reducing sugar levels obtained are only 40.62-81.25 gr/l. The results showed that the greater the activity of the alpha-amylase enzyme used, the greater the level of reducing sugar produced, and the higher the concentration of hemp, the greater the glucose produced. The highest value of reducing sugar levels was 130 g/l with a volume of 20 ml of the alpha-amylase. In the hydrolysis process, the amount of 150 g/l of *onggok* with a volume of 10 ml of alpha-amylase produces reducing sugar levels that are much smaller than the other concentrations. In the hydrolysis process, most of what is converted to reducing sugar are only the starch fraction, while very little hemicellulose occurs, and cellulose is impossible.

The level of reducing sugar produced during the hydrolysis process is strongly influenced by the enzyme's concentration and the cassava's concentration. The higher the enzyme concentration and the concentration of *onggok* used, the higher the reducing sugar level obtained. The results obtained showed the effect of alpha-amylase enzyme concentration on the hydrolysis process on increasing reducing sugar levels.

In all this liquefaction process, the enzymatic catalyst used is the alpha-amylase enzyme which acts to break down the starch into dextrin (reducing sugar). The increase in the number of enzymes used would increase the degradation of the starch as the activity of the enzyme increased. Similar results were also found to be in the Saccharification process using the enzyme of Glucoamylase. The increase in the enzyme used would increase in reducing sugar.

When compared to the other variable parameters used in this study, the reducing sugar obtained 130 g/l at 20 ml alpha-amylase and using *onggok* 150 g/l was higher than that by [15], [16]. They found to be the reducing sugar content of 10 g/l using 5% HCl solvent, electromagnetic field induction of 7.18 x10^{-4} Tesla, at a temperature of 100°C for 60 minutes [15]. While reducing sugar produced under this condition was 58.32 g/l on the starch concentration of 200 g/l [16].

3.3 Relationship Between Reducing Sugar and Ethanol content

Figure 7 shows the relationship between the concentration of reducing sugar and the ethanol content at a concentration of 50 g/l *onggok*.

The results indicated that ethanol production was influenced by reducing sugar concentration. The ethanol concentration produced increased from 0.88, 0.9, 0.91, 0.96, 0.97, and 0.98%, respectively, by decreasing the amount of reducing sugar used from 19.70, 14.44, 10.65, 9.28, 7.38, and 6.50 g/l at a volume of 10 ml of alpha-amylase for 12, 24, 36, 48, 60, and 72 hours, respectively. At a volume of 15 ml alpha-amylase enzyme, the Ethanol obtained increased from 0.99, 1.05, 1.26, 1.4, 1.55, and 1.63% by decreasing the amount of reducing sugar from 28.26, 20.96, 14.43, 10.58, 8.78 and 7.64 g/l, for 12, 24, 36, 48, 60, and 72 hours, respectively. At the volume of the alpha-amylase enzyme 20 ml, the Ethanol obtained 1, 1.07, 1.3, 1.55, 1.64, and 1.77%, by decreasing the amount of reducing sugar 34.21, 21.66, 15.12, 11.01, 9.15 and 7.83 g/l, for 12, 24, 36, 48, 60, and 72 hours, respectively.

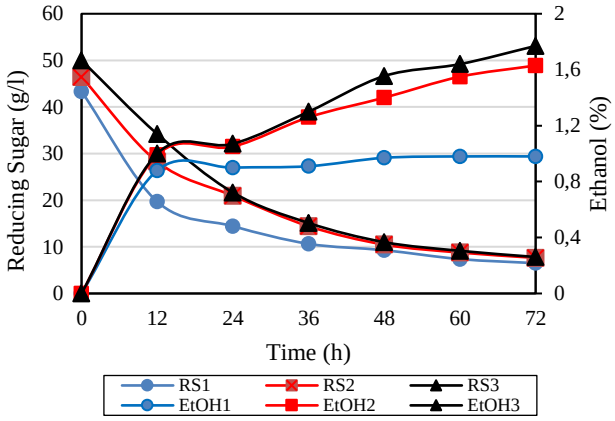

Figure 7. The Relationship Between Reducing Sugar and ethanol content (% v/v) at 50 g/l of *onggok*.

Figure 8 shows the relationship between the concentration of the alpha-amylase enzyme and the ethanol content at a concentration of 100 g/l *onggok*. The results showed that at a volume of 15 ml alpha-amylase enzyme, the ethanol production increased from 2.21, 2.4, 2.59, 2.72, 2.82, and 2.95% by decreasing the reducing sugar used from 43.33, 24.07, 15.85, 11.62, 9.55 and 8.13 g/l for 12, 24, 36, 48, 60, and 72 hours. At the volume of the alpha-amylase enzyme 20 ml, the Ethanol obtained increased from 2.72, 2.97, 3.19, 3.3, 3.39, and 3.59% by decreasing the reducing sugar used from 46.42, 25, 16.66, 11.81, 9.73 and 8.22 g/l for 12, 24, 36, 48, 60, and 72 hours, respectively.

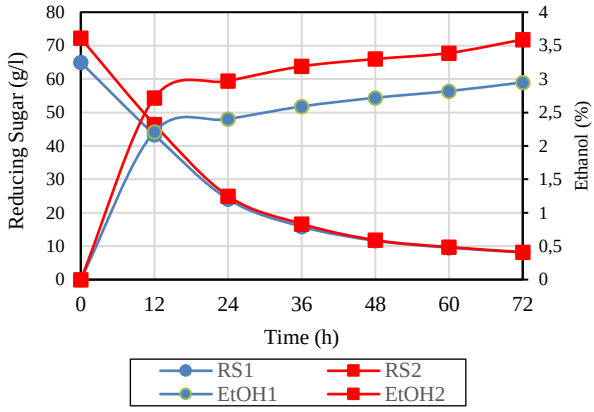

Figure 8. The Relationship Between Reducing Sugar and ethanol content (% v/v) at 100 g/l *onggok*.

Figure 9 shows the relationship between the concentration of the alpha-amylase enzyme and the ethanol content at a concentration of 150 g/l *onggok*. The results showed that at a volume of 15 ml alpha-amylase enzyme, the Ethanol obtained increased from 2.39, 2.49, 2.54, 2.74, 2.9, and 3% by decreasing the reducing sugar used from 59.12, 27.12, 18.57, 12.74, 10.15, and 8.66 by for 12, 24, 36, 48, 60, and 72 hours. At a volume of alpha-amylase enzyme 20 ml, the Ethanol obtained from 3, 3.22, 3.38, 3.6, 3.8, and 3.98% by decreasing the reducing sugar from 72.22, 28.26, 19.11, 13.26, 10.48, and 8.9 g/l for 12, 24, 36, 48, 60, and 72 hours, respectively.

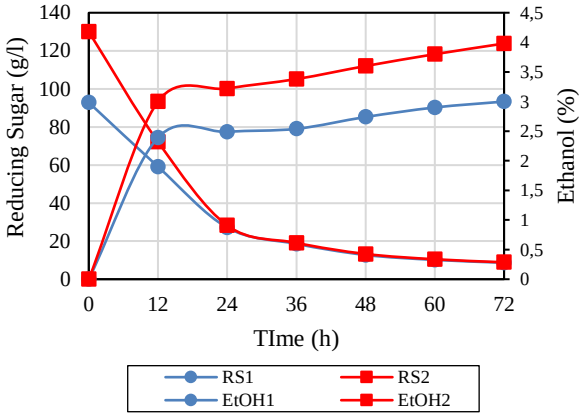

Figure 9. The Relationship Between Reducing Sugar and ethanol content (% v/v) at 150 g/l *onggok*.

Based on the Figure above, it can be concluded that the higher the concentration of the alpha-amylase enzyme used, the higher the Ethanol produced, and the higher the concentration of *onggok*, the higher the Ethanol produced. This is because the reaction rate increases due to the increasing concentration of the enzyme used. So, the higher the alpha-amylase enzyme used, the faster the hydrolysis reaction and the starch molecule that decomposes into reducing sugar increases. When the reducing sugar level increases, the Ethanol produced will also increase. The highest Ethanol produced was 3.98% with a 20 ml alpha-amylase enzyme.

Based on the Figure of the results of the ethanol fermentation process with concentrations of 50, 100, and 150 g/l, the highest Ethanol was obtained at a concentration of 150 g/l *onggok* with a volume of 20 ml alpha-amylase enzyme, which was 3.98% (v/v). The increase in Ethanol was influenced by the length of time of fermentation. The longer the time and the concentration of enzymes used in the fermentation process, the more opportunities for microbes to break down the substrate and vice versa [12].

However, the ethanol content (3.98%) obtained in this study was lower than that of the other authors [2], [17], [18]. Mulyadi et al. [2] depicted that ethanol content obtained was approx. 6.2% based on *menir* (groats) and *onggok*. According to [17], they got 9.3 (v/v) and 8.3% (v/v) of Ethanol from sugarcane molasses using *Z. mobilis* and *S. cerevisiae*, respectively. Ashok et al. [18] obtained an ethanol concentration of 7.95% (v/v) from sweet potatoes using *S. cerevisiae* MTCC-170.

IV. CONCLUSION

It concluded that the more in the addition of alpha-amylase and Glucoamylase would increase the reducing sugar production. With the use of *onggok* at the concentration of 150 g/l and a volume of 20 ml alpha-amylase enzyme, the reducing sugar obtained was 130 g/l. Also, the Ethanol obtained was 3.98%. To improve reducing sugar, it requires some dose of enzyme and *onggok*. The practical product of reducing sugar is increased because it can increase the frequency of collision between *onggok* and enzymes.

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