Ethanol Production from Non Food Tubers of Iles-iles (*Amorphophallus campanulatus*) using Hydrolyzes by Commercial Enzymes (*α* and *β amylase*) and Fermentation by *Saccharomices cereviseae*

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Abstract :

The decrease of oil production caused the increase on the price of fossil fuels. This paper was investigated the possibility of *Amorphophallus campanulatus* or known as "iles-iles" by Javanese people, which is known have a high carbohydrate content, as a raw material to produce bioethanol. The first stage of the process was hydrolyzes the starch, combined by liquefaction and saccharification of the starch from "iles-iles" using *α* and *β amylase*. The process was followed by fermentation of glucose with the help of *S. cerevisiae*. To obtain the maximum ethanol content, several parameter had been studied, such as the type *S. cerevisiae* (pure, dry, wet and instant), the dosage of α-amylase, β-amylase and also DAP dosage as a nutrient support for S. cerevisiae. The result shows that the highest ethanol concentration obtained in fermentation using dry *S. cereviseae* for 72 h with 10.2% (v/v) of ethanol. The highest total sugar content by hydrolysis was achieved by 0.0032 mL α-amylase/g, while β-amylase was 0.0064 mL β-amylase/g (12.5% of glucose). This is show that with increasing of α and β amylase dosages, the total sugar formed was increased. The DAP (Diammonium phosphate) was used as a Nitrogen supply which is needed by *S. cerevisiae* to growth and as a results can increase the level of ethanol produced. The additional of DAP in the fermentation prove that it can enhance 8.45% (v/v) of ethanol. Therefore, it can be concluded that the highest levels of ethanol with conventional methods of "iles-iles" was obtained at 72 h using the dry *S. cerevisiae*, with 0.0032 and 0.0064 mL enzyme/g of α and β amylase, respectively. This result shows that the plant seems to be a potential raw material for bioethanol.

Keywords: bioethanol, DAP , "iles-iles", *S. cerevisiae*, α amylase, β amylase,

1. Introduction

The decrease of oil domestic production caused the increase on the price of fossil fuels **[1]**. These facts catch the interest of many researchers to find the new technology to replace fuel oil with the environmentally-friendly fuel. One way to solve this problem is using alternative energy such as bio-fuels. A bio-fuels must be technically feasible, economically competitive, environmentally acceptable, and readily available **[2]**. One of the examples is bioethanol or C_2H_5OH, which known as a type of bio-fuel that has the potential to replace fossil fuel **[3]**.

Biofuels are made from bio-based materials through thermochemical processes. The most famous and widely used raw material from stracy material to produce bioethanol is cassava and corn, which known as a main food in several parts, especially in Indonesia. However, the use of cassava for bioethanol production is affect to the availability of food because in some area, especially in Indonesia, cassava is used as a main food, as well rice **[4]**.Therefore, locally available and abundant raw materials are still required to produce bioethanol.

Other source, agricultural wastes, such as wood, plants, switch grass, and cotton waste, which contain of cellulose, can be used to produce bioethanol to replace the starch in the fermentation process, in which the consumption of this waste does not interfere with the availability of food **[5]**. However, this method is difficult, high cost and still needs more technological improvements, such as liquefaction and saccharification by special enzymes to hydrolyze cellulose into sugar **[6]**. Wheat straw, for examples, can also be used as the source of bioethanol, but the conventional fermentation *with Saccharomyces cerevisiae* cannot ferment multiple sugar substrates to ethanol because it should involve the recombinant of *E. coli* **[7]**.

Moreover, organic waste can also be used as source of biofuel, but the production of bioethanol from organic waste involves the complicated process, such as the demolition waste, solid liquid separation process, continuous

fermentation and anaerobic conditions **[8]**. Therefore, the alternative process to gain the maximum conversion with the simple treatment is still required. One of the materials which is abundant and have high cellulose content and can be used as a raw material to produce bioethanol is "iles-iles" (*Amorphophallus campanulatus*). Iles-iles contains the highest of starch (77% starch), with another component of fiber (8.5%), crude protein (14%), reduction of sugar (3-5%) ash and vitamins (3.4-5.3%) . Iles-iles have been widely planted as in Central Java and East Java. It is used by the community, especially in rural as a food substitute for rice as its high carbohydrates but its utilization is still relatively small, this is due to its competing with another carbohydrate sources (cassava and corn) and its containing calcium oxalate that cause intense itching. Due to the high content of carbohydrate in the Iles-iles, this experiment will be conducted to study the possibility of Iles-iles as an alternative raw material for the production of bioethanol. The objective of this study is to study the variations in the type *S. cerevisiae* and the composition of α and β amylase on the hydrolysis of the glucose levels to produce bioethanol.

2. Materials and Methods

2.1. Starch preparation

Iles-iles was used as source of substrate to produce bioethanol. The iles-iles obtained locally were washed thoroughly to remove all dirties particle, chopped, dried (70^{o}C to constant weight) and ground to mesh-size. The solid were repeatedly washed with distilled water, oven dried (60^{o}C to constant weight) and then storage at room temperature for further usage.

2.2. Starch hydrolysis

Iles-iles was treated in a two-step enzymatic hydrolysis consisting of liquefaction by α-amylase and saccharification by β-amylase. The hydrolysis was performed at the starch: water ratio of 1: 4 in the flask agitated at 200 rpm. In the first step, the solution was adding specified dosage α-amylase and heated at 95-100^{o}C and pH 6 for 1 h. The second step, β-amylase was added to the solution and it adjusted to pH 5, heated at 60^{o}C for 4 h. The slurry was filtered and the solid material was washed with deionized water and then fermented. To study the effect of enzymes in the process, the amount of α and β amylase were studied at various dosages, which are 0.0008, 0.0016, 0.0032 and 0.0064 mL α-amylase/g starch and/or β-amylase/g starch.

2.3. Ethanol fermentation

Anaerobic batch fermentation experiments were performed at 30^{o}C for 72 h at pH 5.5 in closed bottles and the flask was agitated at 150 rpm. *S. cerevisiae* was used as fermenting organism. Four types of *S. cerevisiae* which are dry, instant, wet and pure were used at 0.8, 0.8, 10, 10% (w/v), respectively. At appropriate times, sampling samples were taken aseptically for 12, 24, 36 and 72 h to know the effect of contact time in fermentation. The flasks were sampled to determine total reducing sugars and alcohol concentration. To obtain the optimum ethanol yield, the effect of addition DAP with the range of 1-8 g/L at constant of 2 g/L urea to sample were studied.

2.4. Sample analysis

Determination of reducing sugar was performed by the method of spectrophotometry. Samples as much as 0.01 mL (10 microns) in a test tube was added the color glucose reagent 1000 microns/mL, then incubated for 10 min in waterbath at temperature 37 ^{o}C. The glucose was detremined by readings on photometer (Boehringer 1040) with a wavelength of 546 nm. The ethanol content obtained from the fermentation were analyzed using GC- Hewlett Packard Agilent 6890N equipped with a flame ionisation detector.

3. Results and discussion

3.1. Iles-iles composition

The chemical composition of iles-iles consist of ash content, moisture content, cellulose, hemicelluloses, starch, and lignin. The percentage of each composition can be seen in Table 1.

Table 1. Dry - iles-iles starch composition

No.	Parameter	Composition
1.	Starch	71.25
2.	Hemi-cellulose	3.30
3.	Cellulose	8.54
4.	Water	8.50
5.	Raw fiber	5.85

Table 1. shows that iles-iles is consisted of high amount of starch. This characteristic makes iles-iles has the possibility to be a potential raw material for bioethanol. The hydrolysis of starch into glucose from iles-iles was through simply mechanism, in which glucose will be converted into ethanol.

3.2. Effect of α-amylase dosages

Starch was traditionally hydrolyzed by acids, but the specificity of the enzymes, their inherent mild reaction conditions and the absence of secondary reactions have made the amylases to be the catalysts generally used for this process [9]. α-amylase obtained from thermoresistant bacteria like *Bacillus licheniformis* or from engineered strains of *Escherichia coli* or *Bacillus subtilis* is used during the first step of hydrolysis of starch suspensions. For amylases to attack starch, these suspensions should be brought to high temperatures (90-110^{o}C) for the breakdown of starch kernels [10]. The usage of α-amylase in hydrolysis of starch was to crack the α-1,4-glucoside into glucose monomers that are used for fermentation [11].The hydrolysis of starch from iles-iles was done with various doses of α-amylase, which are 0.8, 1.60, 3.20 and 6.40 mL at 95-100^{o}C and pH 6 for 1 h, and followed by sacarification proceed at with 3.2 mL β-amylase at 60-65 °C, for 4 h, pH 5 . Figure 1 shows the effect of time for hydrolysis of starch with various dosage of α-amylase at a constant amount β-amylase in the substrate slurry with ratio Iles-iles:H_2O = 1:4 (w/v). The increase of contact time caused the higher of sugar produced. The highest total sugar content (12%v/v) by hydrolysis was achieved by 3.2 mL of α-amylase (%v/w) . This result indicates that the increase in total sugar content is influenced by the composition of α-amylase. The high amount of α-amylase added, caused the increase of glucose from the cracking of α-1.4 glucosidal. According to Whitaker [12], the addition of enzymes dosage will increase the reaction. However, when the reaction was reached equilibrium, the additional of enzymes was not effective.

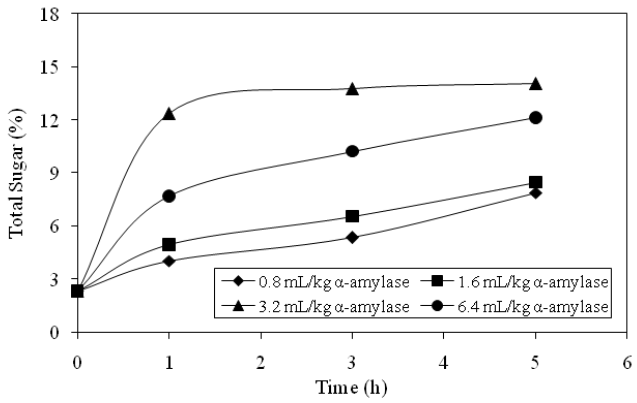

Figure 1. Effect of liquefaction of starch with various dosage of α-amylase at T = 95-100 °C for 1 h and saccharification for 4 h with constant β-amylase

The total sugar produced from saccharified of iles-iles starch were then fermented with *S. cerevisiae* dry at 30^{o}C and pH 4.5 for 72 h. 200 mL of the saccarified starch was added with DAP 0.2 g, 0.4 g urea, 1.6 g of dry *S.cerevisiae*. The correlation between the fermentation time and ethanol and glucose levels to various doses of α-amylase can be seen in Figure 2.

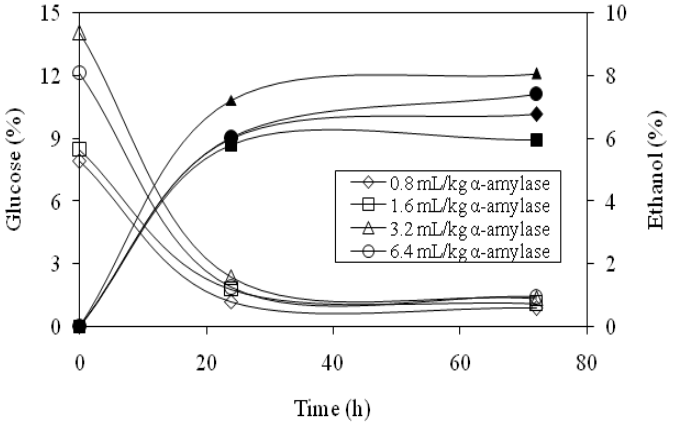

Figure 2. Correlation between the fermentation time with ethanol and glucose levels to various doses of α-amylase (● = Ethanol (% v/v) and ○ = Glucose (% v/v)

The increasing of fermentation time affected the decrease of glucose level produced. This is showed that the glucose level was influenced fermentation time due the capability of *S. cerevisae* to convert sucrose and glucose into ethanol more effectively rather than fructose **[13]**. Glucose from the hydrolysis was converted to ethanol and affects the improvement of decreasing glucose with the increasing of contact time. The highest ethanol concentration of glucose obtained from the hydrolysis with 3.20 mL of α-amylase by 8.06% (v/v). This result was similar to Silverstein et al. **[14]** which reported that with the increasing of α-amylase dosage, the ethanol level was increased

3.3. Effect of β-amylase dosages

There are two processes to hydrolyze iles-iles starch to glucose, which are liquefaction and saccharification using α and β amylase, respectively. According to Balat **[15]**, saccharification is a further hydrolysis of oligosaccharides as result of liquefaction by a single or mixture of enzymes. Due to this result, β-amylase was known plays an important role of hydrolysis. In this experiment, various amount of β-amylase of 0.80, 1.60, 3.20, 6.40 mL at 60-65^{o}C and pH 5 for 4 hours were studied.

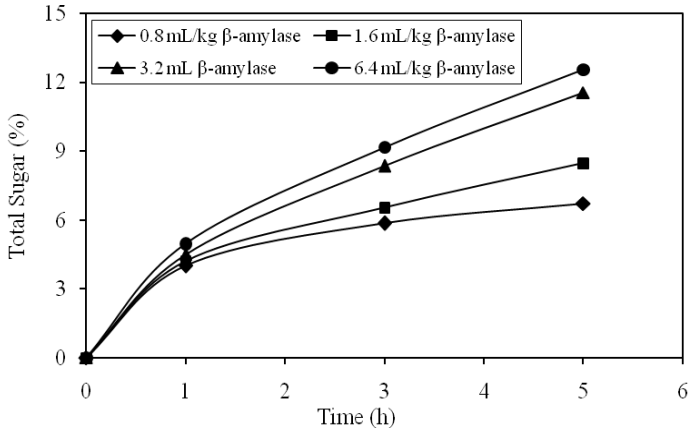

Figure 3. Effect of time for hydrolysis of starch with various dosage of β-amylase

Figure 4 shows the effect of contact time and the amount of β-amylase in saccarification of iles-iles. The Saccharification process was carried out using liquefaction of Iles-iles substrate solution at ratio iles-iles:H_2O = 1:4 (w/v), with conditions of lliquefaction at T = 95-100°C, t = 1 h, pH 6, α-amylase dosages = 1.6 mL/ kg and condition for saccharification at T = 60-65°C, t = 4 h, pH 5. The result shows that the highest of total sugar content formed at a dose of 6.40 ml of β-amylase up to 12.5%. This show that with the increase of β-amylase dosage, the total sugar formed was also increased. It is because the β-amylase or glucoamylase will be hydrolyze amylase and amylopectin into D-glucose by cracking the α-D-(1,4), α-D-(1.6) and α-D-(1,3). In addition, total sugar content was also

influenced by the time of hydrolysis, where with the increase of hydrolysis time, the total sugar content will be increased. However, if the saccharification process was occurring for long time, it caused the polymerization of glucose **[16]**.

After the saccharification process is completed, glucose was converted into ethanol through microbial fermentation using dry *S. Cerevisiae* at 30^{o}C and pH 4.5 for 72 h. The highest ethanol concentration of 8.629% was obtained from the saccharification using 6.40 mL of β-amylase. The increase of amount of glucose produced resulted in increase of ethanol produced, until it reaches equilibrium between substrate and enzyme. Fermentation time also affects the levels of ethanol produced, with the increase of contact time; the ethanol will be increased and resulted in decreasing on glucose amount. This is due to the all *S.ceresiviae* has reacted in converting glucose into ethanol as shown in Figure 5.

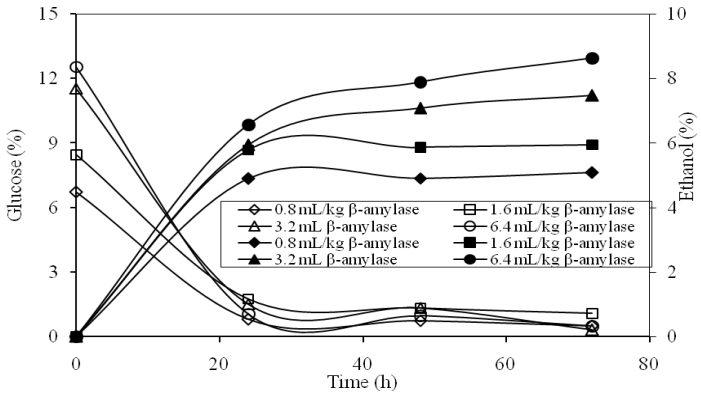

Figure 4. Effect of contact time in fermentation of iles-iles starch for various dosage of β amylase

4. Conclusion

From the research, it could be concluded that iles-iles is an attractive raw material for the production of bioethanol. The highest levels of ethanol from iles-iles achieved at 72 h using the dry *S. Ceresiviae* with 0.0032 mL α-amylase/kg iles-iles, 6.4 mL β-amylase/kg iles-iles and 1 g/L of DAP. In conclusion, iles-iles could be a valuable new source of carbohydrate for bioethanol production. To be exploited, however, additional research is required to resolve current uncertainties and to gain the maximum benefit from the process.

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