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Thermo-enzymatic Hydrolysis of Cassava Starch by α-amylase and amyloglucosidase

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Abstract**—**One of the greatest challenges for the 21st century society is to meet the growing demand for energy for transportation in a sustainable way. Petroleum based products are in critical state and alternatives for fuel is needed. One of the alternatives is ethanol, which adds octane value to the gasoline blend and provides a cleaner burning. This paper discusses the thermo-enzymatic hydrolysis of *Manihot esculenta* (cassava) starch in order to produce sufficient glucose for ethanol production. The favorable starch concentration for the hydrolysis was found to be 30% with the addition of 0.25% α-amylase for liquefaction and 0.15% amyloglucosidase for saccharification. This combination resulted in producing 204.5 g/l glucose. For fermentation condition, a 30% slurry resulted better glucose consumption by *Saccharomyces cerevisiae* than the 20% slurry. However, the 10% inoculum was considered inadequate for the conversion of glucose to ethanol.

Keywords**:** Starch, *Manihot esculenta*, bioethanol, enzymatic hydrolysis, glucose

I. Introduction

Starch is the most essential component in most of the food preparation. It is also the major source of calories in the tropics. However starch can be manipulated to produce a powerful blend for fuel. The reason for the change to biomass as a source of fuel is due to the fact that petroleum-derived fuel is not sustainable. Based on the data by the Energy Information Administration [1], the petroleum fuel reserves for Malaysia is only 3 billion barrels which is forecast to last another 18 years. In order to compliment the shortfall, research on biomass to produce ethanol is now on the rise.

Cassava (*Manihot esculenta*), the ethanol plant is a perennial woody shrub with up to 32% (fresh) starch content [2]. Its roots are processed into several foods. There are two types of cassava, the sweet and the bitter type. However, the bitter type cassava is not edible and requires extensive processing before consumption due to the high cyanogenic compounds in the roots, named as linamarin and lotaustralin [3]. Bitter cassava has been associated with several health problems which include diabetes mellitus, cancer, iodine deficiency and neurological syndromes [4]. The main reason for this research to utilize these bitter type cassavas is because of not wanting to compete with food supply. If the starch source is to be converted to fuel, it should not suppress the food supply for the nation.

Starch is a storage compound consisting of glucose linked via α-1,4 and α-1,6 glycosidic linkages (amylose and amylopectin) whereas cellulose, another component from biomass is a structural compound composed exclusively of glucose linked via β-1,4, glycosidic bonds. The compactness and complexity of lignocellulose makes it more difficult than starch to enzymatically degrade to fermentable sugars [5]. Hence, the cost of producing a gallon of ethanol from cellulose is higher than production from starch.

Among many microorganisms that have been exploited for ethanol production, *Zymomonas mobilis* and *Saccharomyces cerevisiae* are the most popular. However, *Saccharomyces cerevisiae* still remains as the prime species to be used [6].

Ethanol is a comparative cleaner burning fuel with high octane and fuel-extending properties. The use of petrol blended with 20-24% ethanol is becoming standard practice in Brazil [7]. Therefore, it is highly desirable for a country like Malaysia to use ethanol-petrol blend as transportation fuel to save valuable foreign exchange in importing crude oil for the 18 years to come.

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II. MATERIAL PREPARATION

Starch Preparation: The wildly grown bitter type cassava was obtained from Paya Mat Inson, Pendang, Kedah. The roots were washed and peeled. It was then chopped to small pieces and soaked in water for 5 hours to remove the cyanogenic content. The chips were blended with water and the starch milk was obtained by straining the blends. Fibre was separated from the starchy water and the starch was separated by sedimentation. The starch sediment was dried in the oven until it becomes dry.

Enzymes: Commercial enzymes α-amylase and amyloglucosidase were obtained from Sigma Aldrich. α-amylase was originated by *Bacillus amyloliquefaciens*. One unit dextrinizes 5.26g dry starch per hour. Amyloglucosidase was produced by *Aspergillus niger*. One unit liberates 1 mg of glucose from starch in 3 min.

Inoculum Preparation: The inoculums were prepared using media containing (g/L): glucose, 1; yeast extract, 1; and peptone, 1 at pH 6.5. Volumes of 50 ml media were autoclaved at 121°C for 15 min. After cooling, Baker's yeast, *Saccharomyces cerevisiae* was inoculated and incubated for 24 hours at 150 rpm.

III. PROCEDURE

Thermo-enzymatic Hydrolysis Process: Slurries of tapioca powder at 10, 20 and 30% w/v concentrations in water are prepared. The gelatinization process was done on a heating plate and cooked until it becomes transparent. α-amylase was added into the gelatinized starch at 80°C for the liquefaction to occur. The liquefaction was assumed to be complete by visualizing the starch-iodine reaction. After being liquefied, amyloglucosidase was added for saccharification at 70°C. Glucose concentration was measured using a glucose analyzer (YSI, USA).

Fermentation: Fermentation was performed under aerobic condition. The hydrolyzed starch was added with 10% inoculum and subjected to shaking at 150 rpm. The glucose content was measured using a glucose analyzer every 24 hours. The glucose was assumed to be converted to ethanol as the concentration reduces day by day.

IV. RESULTS AND DISCUSSION

Starch concentration of 10 – 30% was prepared and each of the concentrations was added with 5 different concentrations of enzymes. The effect of hydrolysis was investigated by comparing all the 15 conditions as shown in Figure 1.

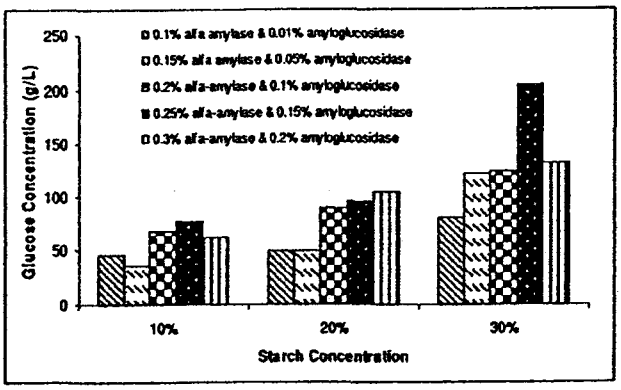

Figure 1 Effect of enzymes concentration on different starch concentration.

All results presented in the figures are taken as average from triplicate trials.

For the 10% starch slurry, the lowest glucose liberated was achieved by adding 0.15% α-amylase and 0.05% amyloglucosidase. The next enzyme concentration was 0.25% α-amylase and 0.15% amyloglucosidase where the glucose produced was 76.7 g/l. However, after the enzymes concentrations were increased to 0.3% α-amylase and 0.2% amyloglucosidase, the glucose production dropped to 62.5 g/l. This showed that for a 10% slurry, the optimum enzyme combination is 0.25% α-amylase and 0.15% amyloglucosidase.

Both the concentrations of enzymes 0.1% α-amylase, 0.01% amyloglucosidase and 0.15% α-amylase, 0.05% amyloglucosidase gave the same performance in producing glucose, 49 g/L in the 20% starch slurry. However, there was a sudden increment after the 0.2% α-amylase and 0.1% amyloglucosidase were added. The glucose obtained was about 41 g/L higher than the previous enzyme combination. By increasing the enzymes concentrations to 0.25% α-amylase and 0.15% amyloglucosidase, the glucose concentration only increased slightly to 95.4 g/l.

By implementing 30% starch, the glucose concentration seems to be higher. Just by adding 0.1% α-amylase and 0.01% amyloglucosidase, the saccharification yielded a higher result compared to the 10 and 20% slurry, where the glucose was 80.6 g/l. Conversely, between 0.15% α-amylase, 0.05% amyloglucosidase and 0.2% α-amylase, 0.1% amyloglucosidase, similar results were obtained. Both conditions generated 122 g/l glucose. After adding 0.25% α-amylase and 0.15% amyloglucosidase, the glucose concentration was 204.5 g/l. This concentration seems to be the best condition since the addition of higher enzymes concentrations resulted in lower glucose. The result was almost similar to the work by [8] where the optimum slurry concentration was 25%.

For the study on glucose consumption by *Saccharomyces cerevisiae*, two concentrations of slurries, 20 and 30% were used. Both concentrations were hydrolyzed using 0.1% α-amylase and 0.01% amyloglucosidase. The fermentation was carried out for 5 consecutive days.

Based on Figure 2, a 20% slurry had an initial glucose concentration of 210 g/l.

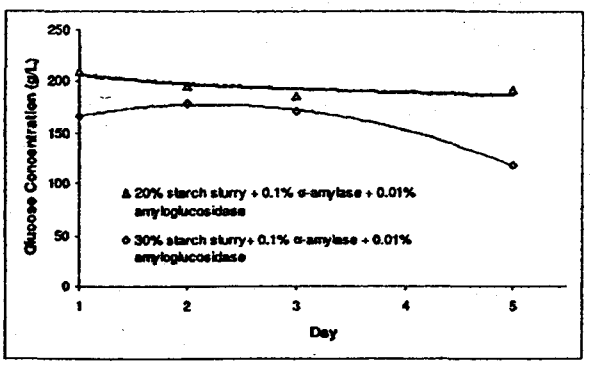

Figure 2 Reduction of glucose concentrations during fermentation with 10% inoculum.

The rate of glucose consumption appeared to be slow and somehow became almost constant after 5 days.

Compared to the 30% slurry, an increment of glucose was detected on the second day and then started to decrease the following days. This may be caused by the enzymes is still active in converting starch to glucose resulting in higher glucose. After the second day, the glucose was consumed by *Saccharomyces cerevisiae* and converted to ethanol. Until the fifth day, the concentration of glucose was detected at 118 g/l. This condition is rather slow in terms of ethanol production capacity. Therefore, the 10% inoculum may not be sufficient to convert the glucose to ethanol effectively. Another reason for the slow conversion may be caused by the high glucose concentration in the fermentation environment which can toxicate the yeast.

V. CONCLUSIONS

The highest glucose production, 204.5 g/l was obtained by implementing 30% starch slurry and by adding 0.25% α-amylase (for liquefaction) and 0.15% amyloglucosidase (for saccharification). During fermentation, the 30% slurry showed a decrease on glucose concentration due the conversion of glucose to ethanol. However, the rate was slow and 10% inoculum was considered insufficient for the bioconversion. The research will be continued in investigating other possible parameters that could result in higher glucose production thus producing higher ethanol.

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