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Enzymatic Hydrolysis of Taro (*Colocasia esculenta*) for the Preparation of Bioethanol

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Abstract

Taro root (*Colocasia esculenta*) was considered as a starchy material for the preparation of bioethanol. Conversion of starch into ethanol was conducted by enzymatic hydrolysis using α-amylase and glucoamylase enzymes, followed by fermentation using baker's yeast *Saccharomyces cerevisiae*. Continuous liquefaction and saccharification were conducted as starch hydrolysis by enzymes. The suitable substrate concentration for liquefaction by using α-amylase was 20 g/L. Time of liquefaction of 2 hr with the enzyme dosage of 7 ml have been resulted for the yield of 1662.5 mg/g of reducing sugar (Dextrose equivalent of 72). For saccharification process 3ml of glucoamylase was satisfactory for the yield of reducing sugar 1900 mg/g (Dextrose equivalent of 82) at the 24 hr of saccharification time. Three days of anaerobic fermentation of starch hydrolysate by yeast dosage of 3.5 g/L produced 12%(v/v) of ethanol.

Key words: Taro root, enzymatic hydrolysis, fermentation

Introduction

Starchy materials have been considered as one of the alternative energy source of liquid fuel such as bioethanol. Wheat, corn, rice, potato and barley are mainly the sources of starchy materials. The hydrolysis of starch is a key step in the processing of starchy material for bioethanol production.

Two major components of starch polymer: amylose, a linear α-D (1-4) glucan and branched amylopectin, a α-D-(1-4) glucan, which has α-D-(1-6) linkages at the branch points. The starch polymer is hydrolyzed to monosaccharide fermentable sugar (glucose) that could subsequently be converted to ethanol by yeast. The hydrolysis is performed by acid or enzymes. The starch liquefying enzymes, α-amylases catalyze the hydrolysis of internal α-D-(1-4) glucosidal linkages in starch in random manner whereas effective glucoamylases, the starch saccharifying enzymes,

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catalyze the hydrolysis of α-D-(1-4) and α-D-(1-6) glucosidal bonds of starch, giving glucose as a final product (Mojovic et al., 2009).

The present study investigated the opportunity for the conversion of starch into ethanol. The level of process variables such as the substrate concentration, dosage of α-amylase and time of liquefaction was studied based on the yield of reducing sugar. The suitable dosage of enzyme dosage and time of saccharification were also studied in starch hydrolysis. The suitable amount of inoculum yeast was also studied for the yield of ethanol strength in fermentation process.

Materials and Methods

Materials

Taro root (Figure 4) harvested from Wakema Township, Ayeyarwady Region was purchased in Thiriminglar Market, Yangon. α-amylase and glucoamylase (Novozymes Co., Ltd, Malaysia) were purchased from MY Associate Co., Ltd (Industrial Ingredients Division), Dagon Centre, Myaynigone, Sanchaung Township, Yangon. Baker's yeast—Saccharomyces cerevisiae-(La—Saf Instant, France) was purchased from Kemiko (Cosmetic and Chemical Dealers), 28th Street, Pabedan Township, Yangon.

Methods

Preparation of Bioethanol by Enzymatic Hydrolysis Followed by Fermentation

Air dried taro powder, 20 g/L was weighed and dissolved in 1L of distilled water. The slurry was gelatinized by heating at 105°C for 5 min. Then, the gelatinized starch was liquefied with 7ml of α-amylase at 95°C for 2 hr by adjusting the pH of 6.5. Thereafter, the liquefied starch was continuously saccharified with 3 ml of glucoamylase at 65°C for 24 hr after adjusting the pH of 6.0. The mash was then cooled to 32°C and inoculated with yeast—*Saccharomyces cerevisiae* under anaerobic condition. Finally, the ethanol was separated from fermented broth by distillation. Liquefaction was carried out with various substrate concentrations of 10 g/L, 15 g/L, 20 g/L, 25g/L and 30 g/L and various dosages of α-amylase of 4 ml, 5 ml, 7 ml, 10 ml and 13 ml with various

liquefaction times of 30 min, 60 min, 120 min, 150 min and 180 min. Saccharification of liquefied starch was continuously conducted by glucoamylase with various dosages of 1 ml, 3ml, 4 ml, 5 ml and 6 ml at various saccharification times of 12 hr, 24 hr, 30 hr, 36 hr and 42 hr. The starch hydrolyzate was fermented with various concentrations of yeast-*Saccharomyces cerevisiae* of 1.5g/L, 2.5 g/L, 3.5 g/L, 4.5 g/L and 5.5 g/L.

Determination of Reducing Sugar

Reducing sugar of starch hydrolysate was determined by using Lane and Eynon's method (Pearson, 1976). 10 ml of the mixture containing equal amount of Fehling's solution A (6.928% (w/v) of copper sulphate) and Fehling's B (34.6% (w/v) sodium potassium tartarate in 10% sodium hydroxide) were freshly prepared and it was titrated with starch hydrolysate using methylene blue as indicator.

Starch hydrolysate was filled into a 50 ml burette. 10 ml of the mixture containing equal amount of Fehling's solutions A and B was placed into a 250 ml conical flask, stoppered and mixed thoroughly for 15 sec by swirling and boiled on an electric heater. After the solution had boiled for about 2 min, the sample was added to the boiling solution from the burette and 3 drops of methylene blue was added into it. The titration was continued by adding the sample dropwisely until the blue color disappeared. At the end point, the boiling liquid turned into the brick-red color due to the precipitation of cuprous oxide. This procedure was repeated thrice and the average titre value was calculated. Fermentable sugar (reducing sugar) was determined by using the following equation.

$$\text{Reducing Sugar, mg/g} = \frac{\text{Factor}}{\text{Titre}} \times \frac{100}{10^3}$$

Where factor is the product of titre volume of standard invert sugar solution required to titrate with 10 ml portion of Fehling's solutions and mg of invert sugar in 1 ml of standard invert sugar solution.

Determination of Dextrose Equivalent (DE)

Reducing sugar as dextrose divided by total solid is dextrose equivalent (DE). DE was determined by using the following equation:

$$\text{Dextrose Equivalent, \%} = \frac{\text{Reducing sugar as dextrose}}{\text{Total solid}} \times 100$$

Analysis of Bioethanol

Measurement of pH

pH of bioethanol was measured by a pen type pH meter 009 (I) (China). Firstly, the electrode of pH meter was adjusted to pH 7 by using buffer solution. 30 ml of ethanol sample was added into 150 ml beaker and pH electrode was dipped into it and then measured.

Determination of Alcohol Strength

The alcohol strength of ethanol was measured by distillation method. Ethanol sample 100 g was weighed and filled into a 500 ml round bottom flask. 50 ml of distilled water was added into it. The liquid was distilled until approximately 100 ml of solution was obtained. The solution was then cooled to 15°C and the specific gravity of the solution was measured at 20°C using a specific gravity bottle (capacity of 50 ml). A clean, dried and previously weighed specific gravity bottle (S.G). After that density of the solution was determined from the ratio of the weight of liquid held and the weight of water held in S.G. bottle. Ethanol content by volume from specific gravity at 20°C was read from the table that tabulates the ethanol by volume at 15.56°C from apparent specific gravity at 20°C (Lees, 1975).

Determination of Acidity

Bioethanol, 10 ml was taken into a 250 ml conical flask and the solution was titrated with previously standardized 0.1N sodium hydroxide solution using phenolphthalein as indicator. The acidity was calculated as follows:

$$\text{Acidity (as acetic acid)} = \frac{\text{Titre x 0.006005 x 100}}{\text{Volume of Sample}}$$

0.1N sodium hydroxide = 0.006005 g acetic acid

Determination of Chloride Content

Chloride content was determined by using the MI 414 Chloride Meter (0.0 to 20 mg/L (L) (Martini Instrument, Itali). Calibration of chloride meter was firstly conducted with two reagents solutions, chloride reagent 1(Cl-1) and chloride reagent 2 (Cl-2). 10 ml of distilled water was filled into a cuvet and 0.5 ml of Cl-1 reagent was added into it. The cap was covered and then swirled gently for 30 sec. The cuvet was placed into the holder of chloride meter by positioning the notch on the cap securely into the groove. The value was read on the instrument and then calivrated.Then,the first cuvet was removed. 0.5 ml of Cl-2 reagent was further added to the first cuvet and then swirled gently for 30 sec. Calibration and reading were made again. After that, the cuvet with the sample ethanol was measured and recorded.

Results and Discussion

Bioethanol was prepared from taro by enzymatic hydrolysis using α-amylase and glucoamylase followed by fermentation. The process steps involved in the conversion of starch into ethanol were gelatinization, liquefaction, saccharification and fermentation. The suitable substrate concentration was determined at constant pH, temperature, time and enzyme dosage based on the yield of reducing sugar.

As shown in Figure 1(a), 1(b) and 1(c), the maximum yield of reducing sugar, 1662.5 mg/g (value of DE = 72%) has been resulted at the substrate concentration of 20 g/L, 7 ml dose of α-amylase and 120 min of liquefaction time. The yield of reducing sugar increased together with increase in substrate concentration. However, beyond the optimum point, the yield of reducing sugar decreased. That may be due to decrease in activity of α-amylase to be able to breakdown the whole chain of starch.

Figure 2(a) and 2(b) show the optimum dosage of glucoamylase and time of saccharification for the maximum yield of reducing sugar. At the optimum enzyme dosage of 3 ml and 24hr of saccharification time, Dextrose Equivalent (DE) was 82% as the yield of reducing sugar was being 1900 mg/g. According to Smile (1971), Dextrose Equivalent (DE) represents the percentage hydrolysis of the glycosidic linkages present. Pure glucose has a DE of 100, pure maltose has a DE of about 50 and starch has a DE of effectively zero. During starch hydrolysis, DE indicates the extent

to which the starch has been cleaved. DE value was round about 72% when maltodextrin may be obtained from partially liquefied starch by using α-amylase. After the starch was partially liquefied, maltodextrin may continuously be hydrolyzed to glucose by glucoamylase so that DE value increased to 82%. This result was in accordance with Omojasola (2008), who stated that increase in glucose production depends on the specific binding of the enzyme with substrate. However, beyond the optimum dosage, decrease in reducing sugar occurred. That was probably due to accumulation of an insoluble starch fraction that was not digested by glucoamylase.

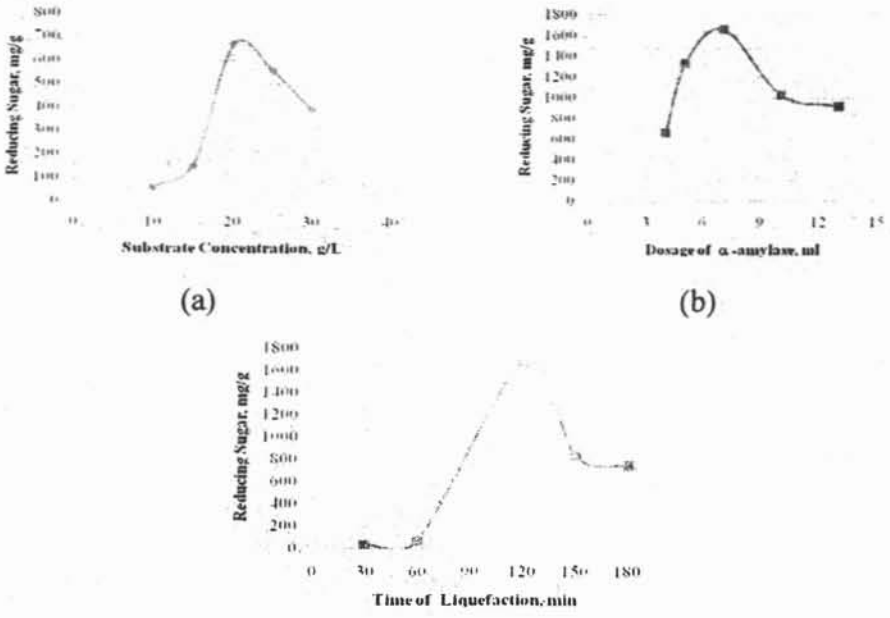

Figure (1) Yield of Reducing Sugar (a) at Various of Substrate Concentration (b) at Diffeent Dosages of α-amylase and (c) at Different Liquefaction Time

In this research work, baker's yeast branded as la-Saf instant was used for fermentation of the starch hydrolysate. Three day period of fermentation was carried out under anaerobic condition. The maximum alcohol strength of 12% by volume obtained at the dosage of 3.5 g/L as shown in Figure 3. Mojovic et al., (2009) pointed out that the strength of ethanol is limited by its toxicity to yeast and the most ethanol tolerant strain

of yeast can survive up to 15% ethanol by volume. The growth of the strain used for this study may be weak in specified ethanol concentration. Moreover, three day period of longer fermentation time offered no efficient production of ethanol.

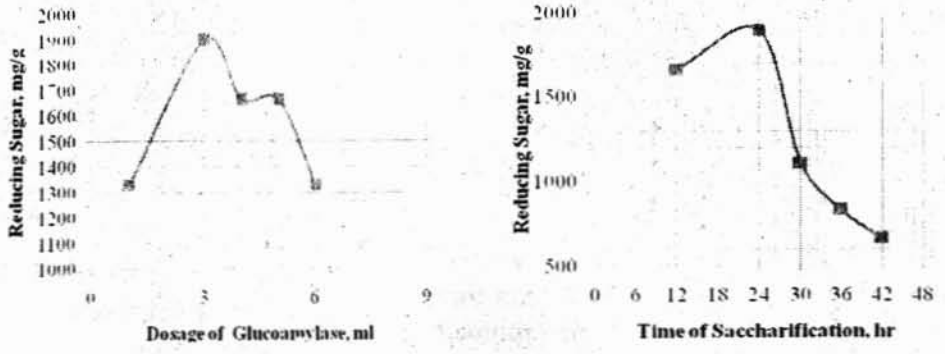

Figure (2) Yield of Reducing Sugar During Saccharification (a) at Different Dosages of glucoamylase and (b) at Different Saccharification Time

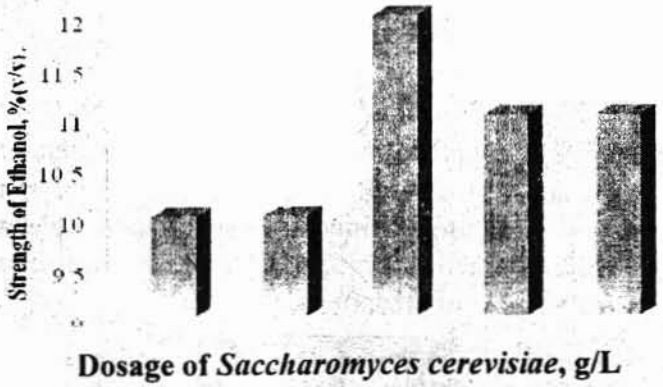

Figure (3) Strength of Ethanol at Various Dosages of *Saccharomyces Cerevisiae*

Table 1 represents the qualitative analysis of the resulting bioethanol. Based on the specifications of the Renewable Fuel Association of United States of America, minimum strength of ethanol is 92% by volume and it should be visibly free of suspended matter. Although acidity and inorganic chloride content of the bioethanol produced followed the specifications stated, its pH and strength were very low in comparison to that for useful bioethanol.

Table 1 Analysis of Bioethanol

Properties	Experimental Value	Literature Value*
Acidity, %	0.0034	0.007
pH	4	6.5
Chloride Content, mg/L	1.1	10(+8)
Alcohol Strength %	12	92
Physical appearance	Visible suspended matter but free of precipitated contaminants, clear and bright	Visible free of suspended matter but free of precipitated contaminants, clear and bright

*Renewable Fuels Association, Industry Guideline, Specification and Procedure

Conclusion

Bioethanol was prepared from taro root by enzymatic hydrolysis. Liquefaction by α-amylase cleaved the carbon linkages of starch resulting DE of 72% and glucoamylase continuously broke down the chain obtaining DE of 82% in saccharification process. The yeast strain chosen for this study gave 12% by volume of ethanol only.

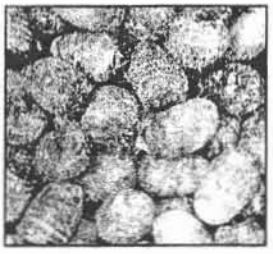

Figure (4) Taro root

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