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Observation on the Yield of Reducing Sugar from Rice for the Preparation of Bioethanol by Acid Hydrolysis

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Abstract

Rice is considered as a source of starch enriched material for the preparation of bioethanol. Conversion of rice into reducing sugar (i.e. fermentable sugar) prior to continuous fermentation process was carried out by acid hydrolysis. Starch hydrolysis such as liquefaction and saccharification of gelatinized rice, was conducted by using hydrochloric acid (HCl) and sulphuric acid (H_2SO_4) with various concentrations (1%, 3%, 6%, 9% and 12% (v/v)), volume of acid (50 ml, 100 ml, 150 ml, 200 ml and 250 ml) and duration of hydrolysis (5 hr, 6 hr, 7 hr, 15 hr and 30 hr). The maximum yield of reducing sugar by HCl and H_2SO_4 hydrolysis resulted at the acid concentration of 6% and 3%, volume of acid of 100ml each and duration of hydrolysis of 15 hr and 7 hr respectively.

Key words: Rice, Acid hydrolysis, liquefaction and saccharification

Introduction

In order to meet carbon reduction for fuelling bioethanol production from starchy materials has already been introduced since last decades. Three kinds of feedstock such as sugar crops, cereal and tuber crops, and cellulosic plants are used for bioethanol production. Because of the reduction of greenhouse gas emission and replacement in ever increasingly expensive petroleum supplies, ethanol is considered as an alternative fuel and used in automobiles as a fuel additive at 10% (Demirbas, 2005).

Rice is one of the starch enriched materials obtained from cereal grains. Starch is the commonest carbohydrate in plants and occurs in the form of granules. It comprises of two components: amylose and amylopectin. Amylose consists of long, unbranched chains of D-glucose units connected by (α-1,4) linkages. Its molecular weight varies from a few thousand to 500,000. Amylopectin is highly branched and has a high molecular weight (up to 1 million). The glycosidic linkages joining successive glucose residues in amylopectin main chains are (α-1,4), but the branch points, occurring in every 24 to 30 residues, connected with the main chains are (α-1,6) linkages (http://en.wikipedia.org/wiki/Starch).

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Three stages involve in conversion of starch into sugar: gelatinization, liquefaction, and saccharification. Liquefaction and saccharification are also known as a process of starch hydrolysis and starch hydrolysis is the conversion of polysaccharide starch molecules into monosaccharide fermentable sugars (reducing sugars). It is achieved by acid or enzymatic hydrolysis. The combination of heat and acid or enzyme breaks up the long chain starch molecule, initially converting starch to a simple sugar hydrolysate (maltodextrin) which is next converted to fermentable glucose as the saccharification step. Maltodextrin are intermediate compounds resulting from incomplete hydrolysis of starch. On the one hand, the product of complete hydrolysis of starch is glucose (reducing sugar) (Marchal, 1999).

The present study investigated the opportunity for the obtainable maximum reducing sugar from rice starch through acid hydrolysis. The corresponding conditions for starch hydrolysis by HCl and H_2SO_4 were evaluated based on the yield of reducing sugar.

Materials and Methodology

Materials

Rice grain–Myaunmya pawsan was purchased from Sanchaung market, Yangon. Analar grade hydrochloric and sulphuric acids were purchased from local chemical dealers, Kemiko shop, Yangon.

Methodology

Starch Hydrolysis

Initially, ground rice grain 50 g (# 60 mesh) was suspended with 100 ml of distilled water and gelatinized by heating the mixture at 95°C for five min. The resultant gelatinized starch was further liquefied and saccharified by heating at 100°C at least for four hr. After that, starch hydrolysate was neutralized with 0.1 M sodium hydroxide solution and reducing sugar was determined.

Detection of Starch

Iodine–potassium iodide solution was prepared for detection of starch. 3 g of gelatinized rice was placed in a glass dish and three drops of

iodine–potassium iodide solution was added into it. The sample was observed for the presence of starch, indicating a dark blue color.

Determination of Reducing Sugar

Reducing sugar of starch hydrolysate was determined by using Lane and Eynon's method (Pearson, 1976). The mixture of 10 ml portions 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 10 ml was diluted with 50 ml of distilled water and filled into a 50 ml burette. 10 ml portions of Fehling's solutions A and B were 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 diluted 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 by the precipitation of cuprous oxide. This procedure was repeated thrice and the average titre value was calculated. Fermentable sugar (reducing sugar) was calculated by using the following equation.

$$\text{Reducing Sugar} = \frac{\text{Factor}}{\text{Titre}} \times 100,$$

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

Results and Discussion

The steps involved in the conversion of rice starch into reducing sugar are gelatinization, liquefaction and saccharification. Gelatinization is the swelling of starch granules to the slurrying water. Before gelatinization milling of rice grains was a necessary step to reduce the size of rice grain approximately 2 mm grains. The ground rice so obtained was slurried with water and gelatinized or cooked at 95°C for 5 min. At this step, viscous starch slurry was obtained by loosing its crystallinity and became gel. Thereafter, liquefaction and saccharification of starch slurry called starch

hydrolysis was carried out by acid hydrolysis with HCl and H_2SO_4 respectively.

In order to determine the conditions for maximum yield of reducing sugar in starch hydrolysis, different acid concentrations (1%, 3%, 6%, 9% and 12% (v/v)), acid volume (50 ml, 100 ml, 150 ml, 200 ml and 250 ml) and duration of hydrolysis (5 hr, 6 hr, 7 hr, 15 hr and 30 hr) were varied. Figures 1, 2 and 3 represent the effect of acid concentration, volume of acid and duration of hydrolysis on the yield of reducing sugar. These figures also indicate the respective conditions of starch hydrolysis by HCl and H_2SO_4. The conditions based on the maximum yield of reducing sugar were acid concentration of 6%, volume of acid of 100 ml and duration of hydrolysis of 15 hr for starch hydrolysis reaction by HCl while for starch hydrolysis reaction by H_2SO_4 3%, 100 ml and 7 hr respectively.

With respect to the optimal acid concentration as shown in Figure 1, both HCl and H_2SO_4 with the lowest acid concentration of 1% caused incomplete starch hydrolysis, resulting the lower yield of reducing sugar. Increase in reducing sugar occurred with increase in acid concentration, however, reducing sugar gradually decreased after getting the maximum yield at the acid concentration of 6% and 3% respectively. The results as indicated also in Figure 2 pointed out that the maximum yield of reducing sugar was obtained at the acid volume of 100 ml for both, but before and beyond this point decreased yield in reducing sugar has resulted. It was found that 15 hr and 7 hr of duration of hydrolysis have provided the maximum yield of reducing sugar for HCl and H_2SO_4 hydrolysis. As can be seen in Figure 3, shorter hydrolysis time supported lower yield of reducing sugar, meanwhile, longer duration of hydrolysis did not offer the greater yield. According to Borglum (1999), starch hydrolysis is a degradation process in which starch molecules are broken down and fermentable sugars such as glucose are formed as the product of complete hydrolysis. The process for converting the starch to maltodextrin is known as partial hydrolysis and complete hydrolysis furnishes the starch into fermentable glucose. Complete starch hydrolysis is preferable for maximum fermentable sugar. In this study, the process of starch hydrolysis– liquefaction and saccharification of starch after gelatinization was conducted as a simultaneous acid hydrolysis. Therefore, partial and complete hydrolysis could be occurred and the maximum yield of reducing sugar may depend on such reaction of partial or complete starch hydrolysis. Before the point that has reached to the maximum yield of reducing sugar, decreased value occurred would be related to partial hydrolysis which offers mostly maltodextrin with small amount of glucose.

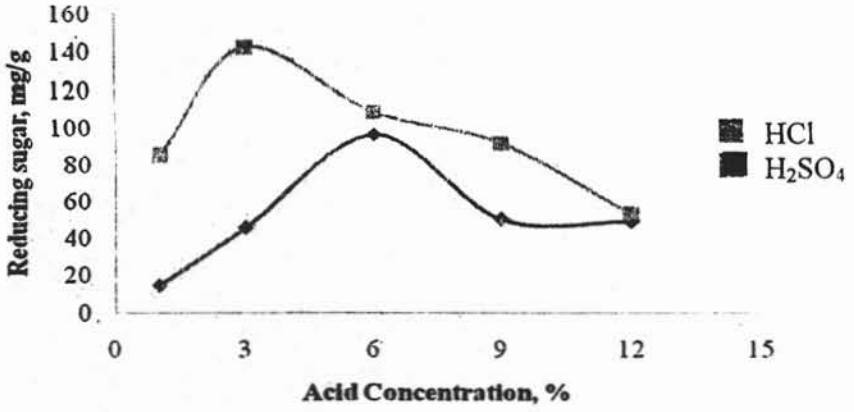

Figure 1 Effect of Acid Concentration on the Yield of Reducing Sugar

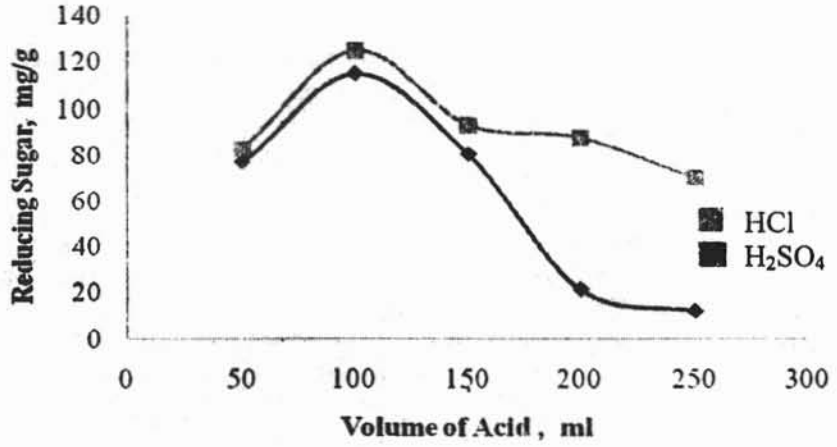

Figure 2 Effect of Acid Volume on the Yield of Reducing Sugar

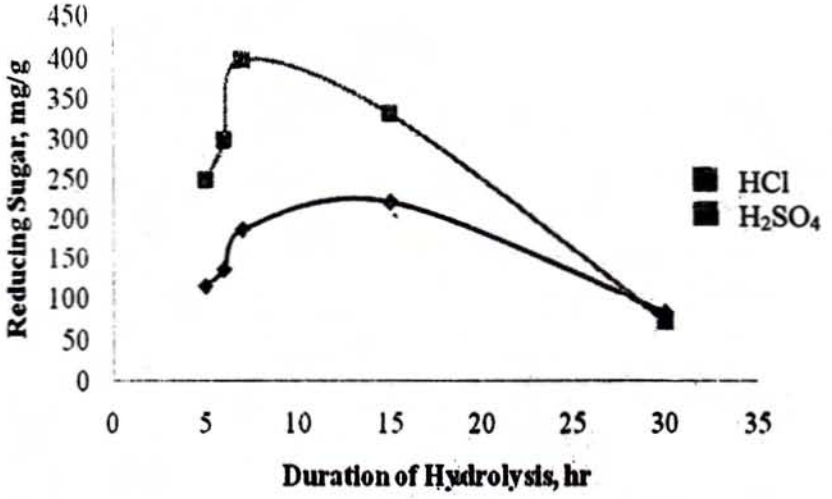

Figure 3 Effect of Duration of Hydrolysis on the Yield of Reducing Sugar

In addition, van Dam, Kieboom & van Bekkum (1986) found that glucose decomposed in acidic media under moderate reaction conditions and by then 5-hydroxymethylfuraldehyde (HMF) was formed. Therefore, the resulting glucose may decompose in acid media with longer hydrolysis time.

Figure 4 shows the comparison between starch hydrolysis by HCl and H_2SO_4 as regards the maximum yield of reducing sugar. It was apparent that starch hydrolysis by H_2SO_4 has resulted higher yield of reducing sugar of 399 mg/g than that by HCl in which 221.7 mg/g was obtained. Moreover, starch hydrolysis by H_2SO_4 occurred at lower acid concentration and shorter hydrolysis time when compared with starch hydrolysis by HCl.

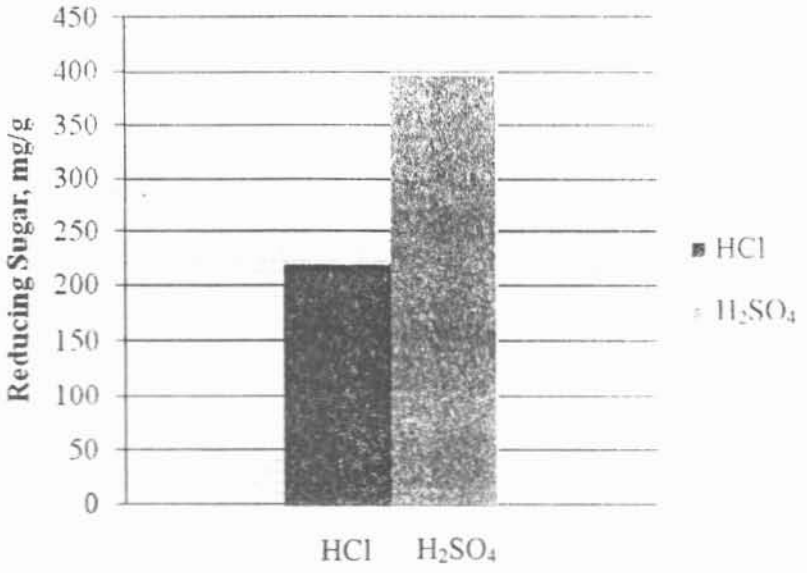

Figure 4 Comparison of the Yield of Reducing Sugar by HCl and H_2SO_4 in Starch Hydrolysis

Conclusion

Starch hydrolysis –liquefaction and saccharification of gelatinized rice was carried out as a simultaneous acid hydrolysis by HCl and H_2SO_4 respectively. Starch hydrolysis by H_2SO_4 has resulted the higher yield of reducing sugar at acid concentration of 3% (v/v), volume of acid of 100 ml and duration of hydrolysis of 7 hr whereas starch hydrolysis by HCl revealed at acid concentration of 6%, volume of acid of 100 ml and duration of hydrolysis of 15 hr.

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