Study of Cassava Starch Hydrolysis by *α-Amylase* and an Airborne Mould Culture

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Introduction

Ethanol is an excellent source of energy which can be produced by microbial fermentation of glucose. Starch can be used as a precursor in glucose processing by chemical or biological hydrolysis (Amutha and Gunasekaran, 2001). Cassava is a woody shrub containing starch that is extensively cultivated as an annual crop in tropical and subtropical regions for its edible tuberous root (Encyclopedia Britannica, 2007).

It was reported that *α-amylase* is capable of breaking down starch to a mixture of sugars and amyloglucosidase to produce glucose from dextrin in the sugar mixture (Waliszweski *et al.*, 1992). This study investigates the use of a commercially available *α-amylase* and an airborne mould culture (as a source of amyloglucosidase) for the hydrolysis of cassava starch at different processing conditions.

Materials and methods

Materials

Cassava and *α-amylase* were purchased from the local market. *Aspergillus niger* was produced in the microbiological laboratory via a mixed airborne fungal culture. Dinitrosalicylic acid reagent, anthrone, 95% H_2SO_4, 72% perchloric acid, anhydrous glucose, 0.1M HCl, agar, potassium sodium tartrate, Na_2SO_3 and potato extract were supplied by the Institute of Chemistry, Ceylon. All chemicals used were of analytical grade.

Methods

The study involves several laboratory experiments. Methods are briefly given below. Starch contents were estimated by the method of McCready *et al.*, (1950). Glucose contents were measured using the dinitrosalicylic acid (DNS) method (Wang, 2007).

Cassava Flour Processing: Cassava flour was produced by the wet milling of fresh cassava roots. The process involved washing, rasping, pulping, grating, starch washing, dewatering, drying, and milling of cassava.

Liquefaction: Liquefaction was carried out by the method proposed by Amutha and Gunasekaran (2001) using heat stable *α-amylase*. Initially, starch slurries were prepared by dissolving cassava flour in distilled water and pH was adjusted to 5.0- 5.5 using HCl. Slurries containing starch concentrations; 20% (samples A), 10% (samples B) and 5% (samples C) were prepared. Then, *α-amylase* was introduced into samples contained in titrimetric flasks. Enzyme to flour weight volume ratio was maintained at 1:20. $CaCl_2$ was added in small amount (0.3 g/l) in order to stabilize the enzyme. Glucose concentrations in above samples were measured after subjected to following heat treatment (HT) processes:

HT 1: Maintained at 65 ^{0}C for two days then heated up to 100 ^{0}C for 10 min and kept at 90 ^{0}C for 3 hours.

HT 2: Heated up to 75 ^{0}C and held at this temperature for 3 hours with agitation.

HT 3: Heated up to 100 ^{0}C and held for 10 min and at 90 ^{0}C for 3 hours with agitation.

Saccharification: After liquefaction, the pH of the slurry was reduced to 4.5- 4.8 and solutions were cooled to 30 ^{0}C. The liquefied starch containing flasks were inoculated with *Aspergillus niger* and incubated at 30 ^{0}C for 2-5 days.

Growing of Aspergillus niger: A microbial culture identified as *Aspergillus niger* was obtained on potato dextrose agar (PDA) medium, pH 4.8, after selectively isolating them from a mixed airborne fungal culture grown on PDA medium at room temperature. *Aspergillus niger* was identified by referring to the Commonwealth Mycological Institute descriptions of pathogenic fungi and bacteria. The slants of *Aspergillus niger* were stored at

4 ^{0}C in the refrigerator and were sub-cultured twice per month.

Results and discussion

When starchy slurry is heated above 60 ^{0}C, the starch containing granules get swelled and rupture and are gelatinized. The gelatinized cassava starch gets partially hydrolyzed rapidly by heat stable *α-amylase* in liquefaction. The partially degraded starch chains are called dextrins, which are suitable as starting materials for the later steps in ethanol production.

For this study cassava powder was prepared from cassava root by a wet milling process. The average cassava powder yield was 375 g/kg of fresh cassava. It was estimated that the starch and glucose contents in dry cassava powder are 19.55 and 3.48 g/100g, respectively.

Hydrolysis of cassava starch was studied under none different conditions using *α-amylase* as explained before; three different starch concentrations under three thermal processing methods. The selected sample size was 30 ml. After the liquefaction stage, the air borne fungal culture which was identified as *Aspergillus niger* was introduced to each of the sample. The change in glucose concentration was monitored after liquefaction and saccharification. Results are given in Table 1. Samples are denoted by an English letter and a number. The letter refers to starch concentration and the number indicates the thermal treatment process. Conversion of starch to glucose in each test sample is shown in Table 2.

According to Table 2, the highest glucose conversions were found among samples 'C' (above 54%) in which the initial starch concentrations are only 5%, while samples with highest initial starch concentrations (A) have reported the minimum Conversion Yields (less than 21%). The best result; 57.6% was obtained with sample C3 which was prepared by the method explained before.

Decreasing glucose conversion yield with increasing initial starch concentration is attributed to a possible inhibition activity taking place in starch solutions. Vasquez *et al.*, (2004) reported that plants contain anti bacterial and anti fungal substances (such as polyphenolics, cyanogenic compounds and peroxidases) in order to inhibit the bacterial and fungal attacks. Therefore, it seems that low concentrated starch solutions are more suitable for the

Table 1. Concentration of glucose at different stages of the process

Process Stage	Concentration of glucose in samples, mol dm^{-3}								
After Liquefaction	0.19	0.19	0.18	0.14	0.09	0.13	0.06	0.07	0.08
Saccharification									
After 2 days	0.22	0.17	0.20	0.17	0.14	0.17	0.11	0.13	0.10
After 5 days	0.22	0.22	0.23	0.17	0.18	0.16	0.15	0.15	0.16
Sample	A 1	A2	A3	B1	B2	B3	C1	C2	C3

Table 2. Starch to Glucose Conversion yields

Process Stage	Conversion Yield, g glucose/100 g starch								
	A 1	A2	A3	B1	B2	B3	C1	C2	C3
After Liquefaction	17.1	17.1	16.2	25.2	16.2	23.4	21.6	25.2	28.8
Saccharification									
After 2 days	19.8	15.3	18.0	30.6	25.2	30.6	39.6	46.8	36.0
After 5 days	19.8	19.8	20.7	30.6	32.4	28.8	54.0	54.0	57.6

enzymatic hydrolysis in order to produce ethanol. However, the use of diluted solutions in a biological reactor could be uneconomical as large quantities of reactants are needed to be handled per unit production of ethanol.

Conclusions

The best sugar converting percentage in the liquefaction process was obtained when, 5% starch slurry was processed at 100 ^{0}C for 10 minutes followed by 90 ^{0}C for 3 hours with agitation. The microbial strain that cultured in the laboratory was found capable of converting liquefied cassava starch into glucose. Sugar concentration continued to rise even after 5 days of inoculating the fungus to the medium. The maximum yield obtained after 5 days was 57.6 g glucose/100 g starch.

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