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A Comparative study on Glucose Production from Sorghum Bicolor and Manihot Esculenta Species in Nigeria

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ABSTRACT

The two-step enzymatic hydrolysis of *Manihot esculenta* and *Sorghum bicolor* using α-amylase and amyloglucosidase were studied. The starch substrate were gelatinized at 80^{o}C and then liquefied with the amylase enzyme at a temperature of 90^{o}C. The samples are then cooled to 60^{o}C and saccharified with amyloglucosidase. The effects of temperature and pH on saccharification process were considered. Results indicated that the optimum temperature for the conversion of starch to fermentable sugar (glucose) was at 60^{o}C and the amount of glucose produced after 4 hours in sorghum was greater than that obtained when cassava was used. It was therefore concluded that optimum conditions for the production of glucose from both Manihot esculenta and Sorghum bicolor for bioethanol production are obtained at pH of 4 and saccharification temperature of 60ºC, with Sorghum bicolor giving higher yield.

Keywords: *Manihot esculent; Sorghum bicolor; Starch; α-amylase; Amyloglucosidase; Glucose.*

1. INTRODUCTION

The need for glucose has greatly increased due to expansion in the food and pharmaceutical industries. Glucose, being a sweet and simple sugar, is mainly added to products like confectionery products, canned food, beverages, to improve the qualities of such products in food industries [1]. In dealing with global warming and other ecosystemic problems of carbon compounds emission from fossilized fuels, new interlinked research directions on renewable energy sources are being charted [2]. With the inevitable decline in the world energy supply, there has been an increasing worldwide interest in renewable sources of energy. One of such renewable alternatives is ethanol obtained from glucose. The demand for ethanol has been increasing in recent years because of its wide use in chemical, portable and motor fuel industries [3]. Ethanol produced from glucose through starch hydrolysis, a combined dual-processing through liquefaction and saccharification, and sugar fermentation processes is known as bioethanol [4].

Starch- and cellulose- containing polysaccharide materials are the two raw material classifications used in the production of glucose [5]. It has been reported that glucose production from starch-containing polysaccharide raw materials like *Manihot esculenta* and *Sorghum bicolor* are optimized through enzymatic hydrolysis while acid or alkali hydrolysis is used for cellulose-containing polysaccharides materials [6].

Starches are generally insoluble in water at room temperature due to complex α-1,4- and α-1, 6-glucosidic linkages. An un-branched, single chain polymer of 500 to 2000 glucose units with only α-1,4- glucosidic links or bonds is called amylose. On the other hand, the presence of α-1,6 glucosidic linkages results in a branched glucose polymer called amylopectin [7]. The breaking down of the α-1, 4- and the α-1, 6- linkages to small units of monosaccharide is made possible by the actions of α-amylase and amyloglucosidase respectively. The starch substrates include cereal grains such as corn, sorghum, wheat and starchy root plants like cassava.

Sorghum bicolor ranks fifth in global cereal production [8]. Due to high content of starch in *Sorghum bicolor*, it has become a potential feedstock for ethanol production. Interest in using grain sorghum for bio-industrial applications is growing in the United States [9]. Its adaptability to most agricultural regions of the world, its resistance to draught and its efficient utilization of soil nutrients are some of the evident attributes of *Sorghum bicolor* that make it relevant for large-scale bioethanol production [10].

Cassava, also known as Manihot esculenta Crantz, is a perennial woody shrub and its root tuber is another good source of glucose production. It is a cheap substrate that is easily available in tropical countries [11]. It can be grown in the land with 0-250 meter above the sea level and the minimum annual rainfall of 1,000 mm [12]. Besides, it has high tolerance to drought because it can survive even during the dry season when soil moisture is low and humidity is high. Also, it can survive low soil quality or nutrient as it thrives better in poor soils than any other major food plant [13]. An important role of cassava roots is that it is an energy store for plant's growth. The energy is preserved in a form of carbohydrate, mainly as starch, which is the greatest component of dry matter in fresh roots. The advantages of cassava as an energy crop for fuel alcohol production include its potential for high alcohol yields per hectare of land, its high tolerance to drought and plant disease and its lower soil quality required than for sugarcane. This, among others, makes vast areas of land, not suitable for most crops, available for its cultivation [10]

Enzymatic hydrolysis is essential for the production of glucose syrups from starch because of the specificity of the linking bonds that characterize its physical and chemical properties. The advantage of enzymatic hydrolysis is the reduced number of side reactions that accompanies acid or alkali hydrolysis [6]. The aims of this study are to compare the amount of fermentable sugar (glucose) obtained from cassava and sorghum grains and to observe the effects of pH and temperature on the action of the enzymes involved.

2. MATERIALS AND METHODS

Fresh *Manihot esculenta* and *Sorghum bicolor* were obtained from a local market at Ifo, Ogun State and Ikotun, Lagos, Nigeria respectively. α–amylase and amyloglucosidase were obtained from the culture collection unit of the Department of Biotechnology, Federal Institute of Industrial Research, Oshodi (FIIRO) Lagos, Nigeria. The reagents used during the course of this study include hydrochloric acid solution which works to adjust the pH level, Anhydrous D–Glucose, Sodium Hydroxide solution, Distilled water, 3,5- Dinitrosalicyclic acid (DNSA) and Potassium Sodium Tartrate (Rochelle Salt).

Preparation of Cassava Starch: The tubers were peeled and washed. The tubers were grated to break the plant cells and then mixed with water. After which it was pressed using a muslin cloth to extract the starch. The starch was allowed to settle down and the water was decanted. The resulting white starch residue was sun-dried to remove all the moisture in it and then it was stored. The result showed a twenty-two percent starch composition by mass.

Preparation of Sorghum Starch: The sorghum grains were soaked in water and left to stand for 48hrs. During this shell-softening period, the water was changed every six hrs. After soaking, the seeds were then comminuted and mixed with water. The mixture was decanted and the supernatant is separated from the precipitate (containing starch). The precipitate was sun dried and stored.

Preparation of 2M Sodium Hydroxide solution: 4g of NaOH was weighed and dissolved in 50mL of distilled water.

Preparation of DNSA Reagent: DNSA reagent was prepared by dissolving 1g of 3, 5 – dinitrosalicyclic acid in 20mL of 2M NaOH which gave a yellowish colour. Then 30g of Na – K Tartrate was added to the mixture and mixed. When the Na – K Tartrate was total dissolved, distilled water was added to make it up to 100mL.

Procedure: 50g each of Cassava starch and Sorghum starch was mixed in 150mL water respectively. The slurries were left to gelatinize at a temperature of 80^{o}C in a water bath for 30 minutes. The slurries were maintained at a temperature of 90^{o}C. 5mL of α- amylase was added to each sample and left to liquefy for 1 hour. During this period at 15 minutes interval, the glucose content was determined. After this period, the sample is cooled to 60^{o}C. Then the values of the pH were adjusted within the range of 4.0 – 7.0 using Sodium Hydroxide solution and Hydrochloric acid. The temperature of the samples were then varied within the range of 60^{o}C – 100^{o}C. 5.0mL of amyloglucosidase was added to each sample and left to stand for 4 hours for saccharification to take place. During which at one hour interval, the glucose content was determined. At the end of saccharification process, clear glucose solution was obtained from the slurries.

Determination of Glucose content: To determine the glucose content, the first step is to plot a standard curve for glucose. A stock solution of D – glucose with concentration, 0.2mg/ml was prepared. After which different dilutions were prepared by adding distilled water. 1ml of each dilution was transferred to different test tubes and 2ml of DNSA reagent was added to each of them. Each tube was placed in a boiling water bath for 5 minutes and cooled in cold water. Then the absorbance was read at a wavelength of 540nm. Then the graph of absorbance against concentration of glucose was plotted and the slope was determined using Beer-Lambert relationship. This was used in calculating the concentration of the samples obtained.

To measure the concentration of glucose in the samples, 1ml of each sample was extracted from the slurries and transferred into test tubes. 2ml of DNSA reagent is added then to each sample and the tubes were placed into a water bath of 100^{o}C and boiled for 5 minutes for colour change. They are then cooled. The colour change was measured in a spectrophotometer at a wavelength of 540nm and the absorbance was read.

3. RESULTS AND DISCUSSION

The standard glucose curve **(Figure 1)** was used to determine the concentration of glucose obtained at different pH and temperature.

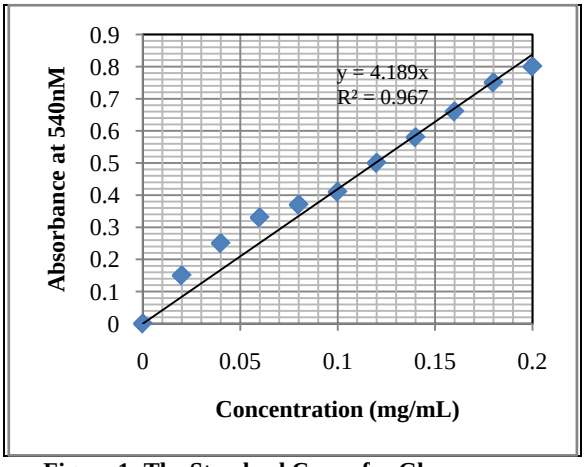

Figure 1: The Standard Curve for Glucose

Effect of pH: From **Figure 2, 3 & 4**, the concentrations of glucose were the highest at pH 4 compared to any other pH (pH 5, 6 and 7) when cassava was used, at the different temperatures considered. This is due to the fact that amyloglucosidase has optimum pH range of 4 – 5 [14]. Also, the same trend was noticed when sorghum was used as well. Also, from **Figure 2,** sorghum at pH 4 gave the highest concentration of glucose when compared to that of cassava after 4 hours, at constant temperature of 60^0C. It is also submitted in this work that the concentrations of glucose obtainable in sorghum were more than that in cassava. These same facts are confirmed when the experiments were run at temperatures of 80^0C and 100^0C (see **Figure 3 and 4**).

Effect of Temperature: Having established an optimum pH of 4 for maximum yield of glucose, another set of experiments were carried out to obtain optimum temperature for saccharification process. From **Figure 5**, it can be observed that the highest amount of glucose was obtained at a temperature of 60^oC. At this temperature, sorghum gave the highest concentration of glucose when compare to that of cassava, after 4 hours of operations. The concentration of glucose declined with increasing temperature (from 60^oC to 80^oC to 100^oC) for both cassava and sorghum. This is due to the fact that the enzymes are denatured at high temperatures. Therefore, the higher the temperature, the lower the amount of glucose obtained and vice versa. These findings agree with previously published work [14, 15].

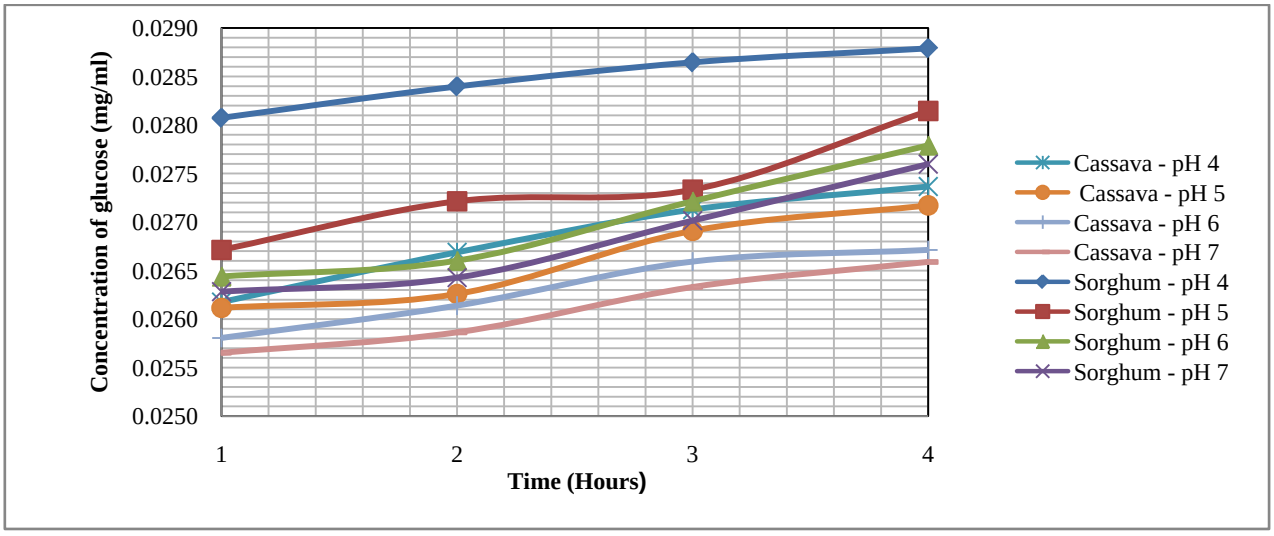

Figure 2: Graph of the Effect of pH on the obtained Glucose Concentration from Cassava and Sorghum at 60^oC

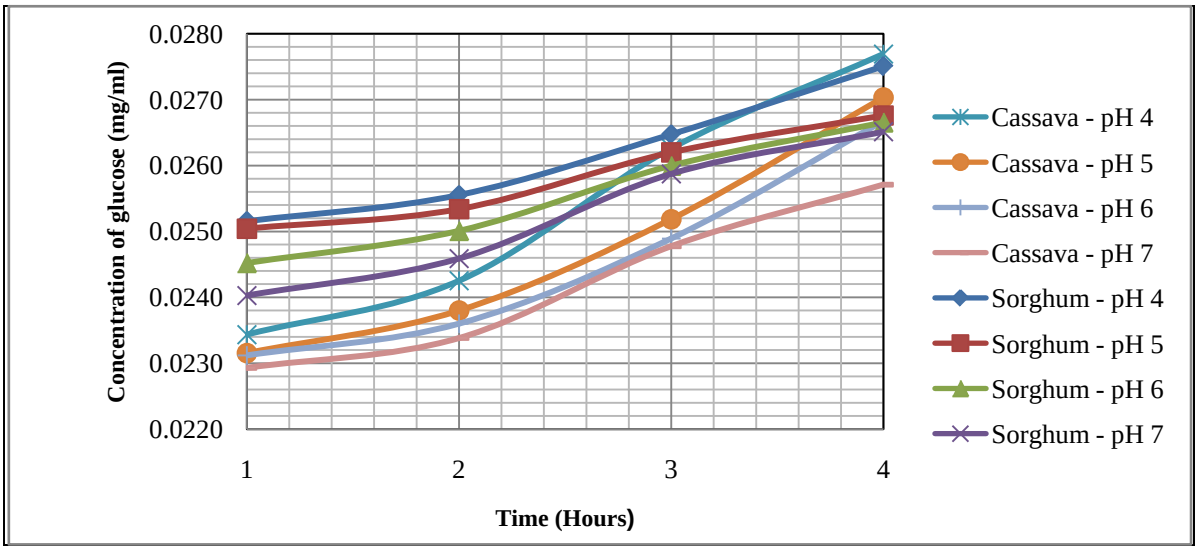

Figure 3: Graph of the Glucose Concentration obtained from Cassava and Sorghum at different pH at 80^oC

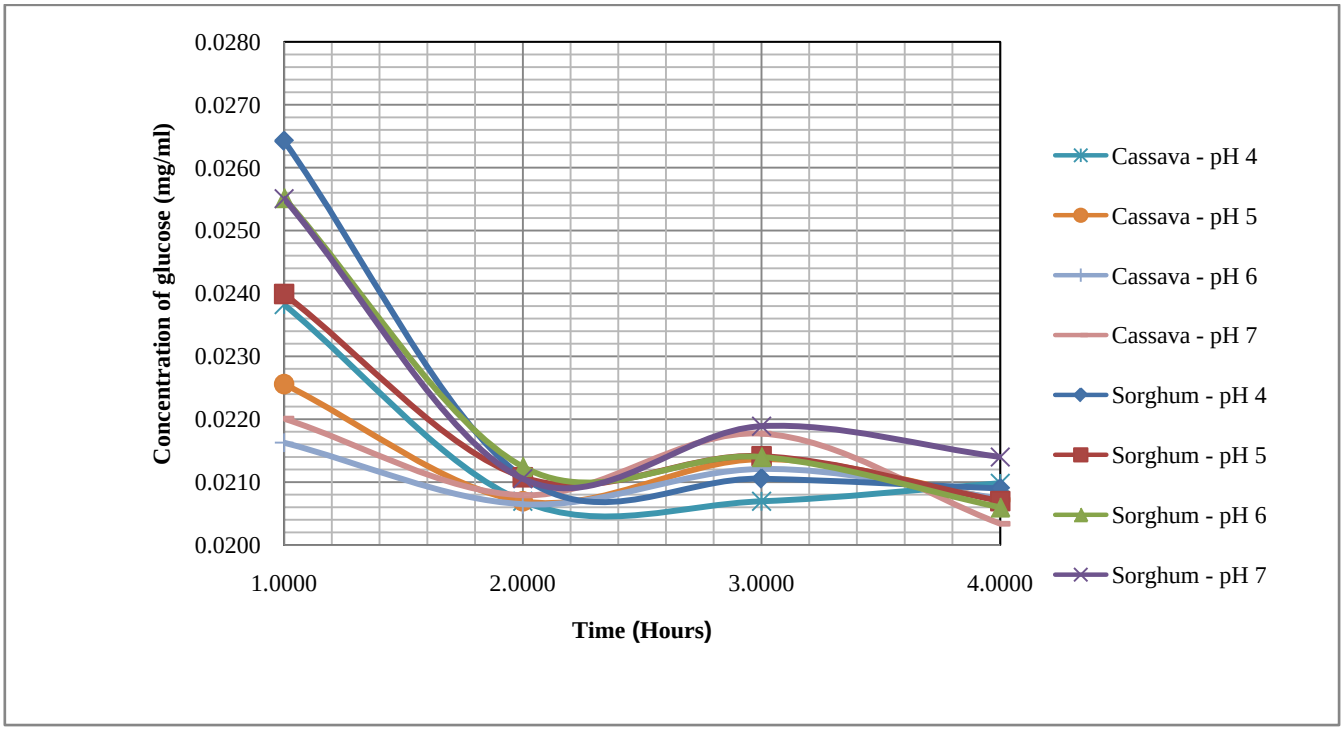

Figure 4: Graph of the Glucose Concentration obtained from Cassava and Sorghum at different pH at 100^{o}C

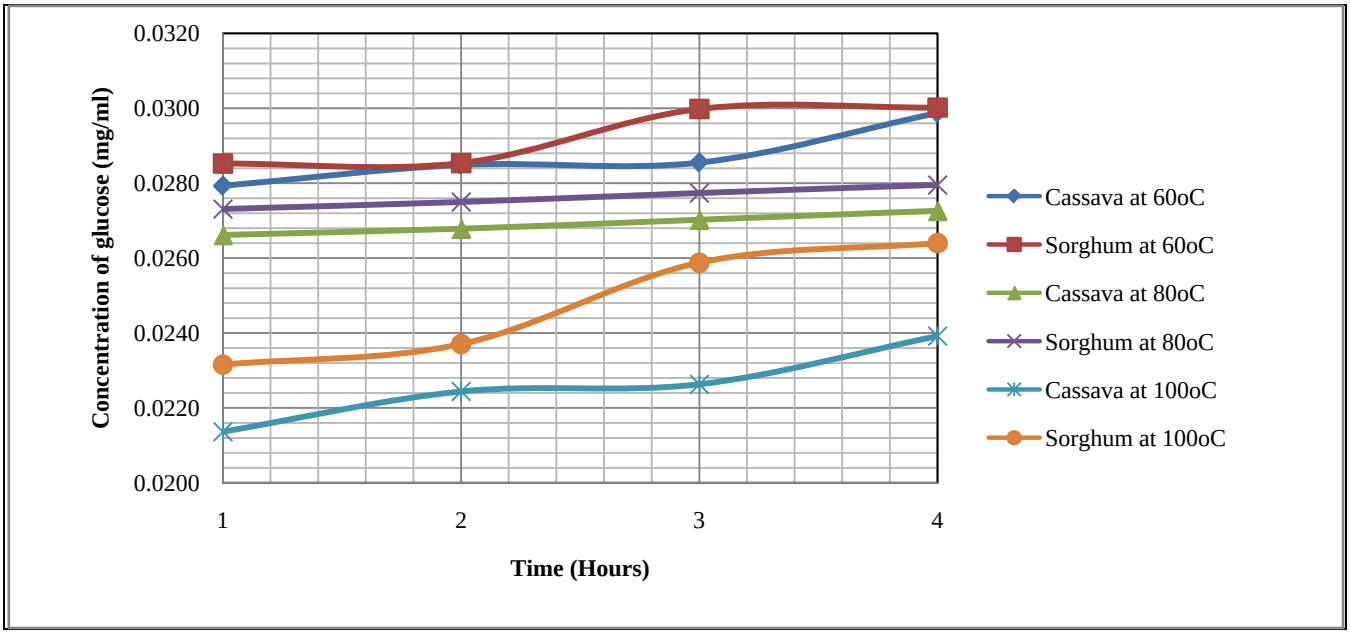

Figure 5: Graph of the Glucose Concentration obtained from Cassava and Sorghum at different temperatures at pH 4.

4. CONCLUSIONS

It was found that sorghum produced a higher concentration of glucose than cassava and that at a temperature of 60^{o}C of saccharification, the optimum yield of glucose was obtained after 4 hours of operation. The optimum pH was 4 because at this pH, the highest yield of glucose was produced as compared to pH 5, 6 and 7. Therefore, it is advisable to use sorghum for glucose production. Another advantage is that sorghum can be grown twice in a year as compared to cassava which is an annual crop [11].

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