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Effects of Temperature, Substrate Concentration and Reaction Time on Reducing Sugar Yields from Hydrolysis of Cassava Peels

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KeyWords
Reducing Sugar, Acid, Crude Amylase, Temperature, Substrate Concentration, Time.

ABSTRACT

The effect of temperature, substrate concentration and time on reducing sugar yields from acid and enzyme hydrolysis of cassava peels was studied. Temperatures ranging from 40°C to 80°C were used. Substrate concentrations were varied between 5% and 30%. While the time for the hydrolytic process was between 1 hr and 6 hr. Crude amylase extract from potato was used for the enzyme hydrolysis. Results of the highest reducing sugar (RS) yields for enzyme hydrolysis at 40 °C, 50 °C, 60 °C, 70 °C and 80 °C were: 90 mg/mL, 238 mg/mL, 277 mg/mL, 388 mg/mL and 224 mg/mL, respectively. Similarly, highest RS yields for acid hydrolysis were: 36 mg/mL, 50 mg/mL, 74 mg/mL, 158 mg/mL and 507 mg/mL, respectively. Generally, RS yields increased with temperature for both hydrolytic processes. There was also a general increase in RS yields with substrate concentration up to 25% (substrate concentration). Reducing sugar yields also increased with time of hydrolysis for both treatments. The enzymatic hydrolysis peaked at 70°C, 25% substrate concentration and a reaction time of 6 hours, while the acidic hydrolysis (during this study) peaked at 80°C, 10% substrate concentration, at a reaction time of 6 hours.

Introduction

Reducing Sugars are very essential precursors in the production of ethanol, an alternative source of fuel. The search for alternative and cleaner energy sources, amidst the growing concerns of the environmental impact of greenhouse gas emissions, energy security, and increase in demand for fuel, is on. Among all alternatives so far, ethanol has proven to be a promising biofuel with various advantages [4].

Converting lignocellulosic biomass to ethanol involves four stages: pretreatment, hydrolysis, fermentation, and ethanol recovery by distillation [10]. Due to the complex and recalcitrant structure of lignocellulosic biomass, various pretreatment processes are required for carbohydrate hydrolysis; breakdown to reducing sugars. Pretreatment increases biomass digestibility for efficient fermentable sugar production, which reduces the cost of bioethanol production. Various pretreatment methods have been suggested, depending on the purpose of removing hemicellulose or lignin from the biomass [11]. Dilute acid pretreatment is a promising pretreatment capable of high solubilization of hemicellulose. This process degrades most of the hydrogen bonds in hemicelluloses and partially degrades cellulose and lignin [2]. In addition, acid pretreatment permits hemicellulose hydrolysis of pentoses and hexoses, removes some of the lignin, and makes the cellulose structure more accessible, so that a fraction can be converted to glucose enzymatically.

The production of ethanol from starch hydrolysis has become popular in recent years. Lignocellulosic feedstock represents an extraordinarily large amount of renewable bio-resource available in surplus on earth and is a suitable raw material for vast number of applications for human sustainability. The main composition of lignocellulosic feedstocks is cellulose, hemicellulose, and lignin. [1].

Cassava peels contain high amount of starch deposit constituting 20-35% of the tuber [7], it offer numerous advantages in comparison to other crop residues such as rice straw, wheat and sugarcane bagasse and can easily be attacked by micro-organisms [5]. This makes it a good choice of raw material for reducing sugar production. Nigeria is the highest producer of Cassava in the world,[7]. Industrial and local processing of cassava to food and other products has led to generation of enormous wastes that are dumped in drainages rather than transforming them to useful products. These wastes end up polluting the surface and underground water [6]. For example, about 2.96 million metric tons of cassava peels are generated and discarded annually in Nigeria from about 10 million metric tons of Cassava processed for Garri alone [9]. Therefore, exploring this abundant agricultural waste to produce enough ethanol to meet the world's energy needs is a worthy venture.

This work seeks to evaluate the effect of temperature, substrate concentration and reaction time of acid and enzyme hydrolysis on the Reducing Sugar (RS) yields from lignocellulosic waste (cassava peels).

Materials and Method

Collection of Samples: Freshly harvested cassava root tubers (*Mannihot esculenta Crantz)* used for this study were purchased from a local farmer in Egono, Etsako West Local Government Area of Edo State, Nigeria. They were identified in the Department of Agricultural Technology, Auchi Polytechnic, Auchi.

Processing of Cassava Peels: The cassava root tubers were washed thoroughly with tap water and peeled. The peels were again washed and air-dried for 48 hr and milled into flour using a disc-attrition mill. The resulting flour was then sieved (using a 250µm sieve) to obtain uniform particle size. The sieved flour and residue were packed in clean plastic containers for further analysis.

Preparation of Crude Enzyme Extract: Crude enzyme was extracted from freshly harvested potato. Three medium-sized sweet potato storage roots were thoroughly washed in water and sliced, 100 g were then homogenized in a warring blender for three minutes with 300 ml of cold extraction buffer consisting of 20 mM Sodium phosphate (pH 6.0). The mixture was allowed to stand at

room temperature for 1 hr, with intermittent shaking. The extract was then filtered through two layers of cheesecloth. This extract was centrifuged at 12,000 x g for 10 minutes, and the supernatant removed and kept on ice.

Acid Hydrolysis: A 0.02M Sulfuric acid (**H_2SO_4**) solution was used for the hydrolysis. Processed CP (5 g) was placed in a test-tube (30 mL). To this, 10 mL of the 0.02m sulfuric acid solution was added and mixed vigorously. The set-up was then placed in a water-bath set at 40°C. Twelve test-tubes were used for this set-up, with two analyzed every 1 hr for reducing sugar content, and the mean recorded. This was done for a 6 hr period. This procedure was repeated at 50 °C, 60 °C, 70 °C and 80 °C, and RS results recorded.

Enzyme Hydrolysis: Processed CP (5 g) was placed in a test-tube (30 mL). To this, 10 mL of the crude amylase extract was added and mixed vigorously. The set-up was then placed in a water-bath set at 40°C. Twelve test-tubes were used for this set-up, with two analyzed every 1 hr for reducing sugar content, and the mean recorded. This was done for a 6 hr period. This procedure was repeated at 50 °C, 60 °C, 70 °C and 80 °C, and RS results recorded.

Estimation of Reducing Sugar Content: Estimation of RS was carried out for cassava peel hydrolyzed using H_2SO_4 and crude amylase enzyme. DNS Method was followed. Standard graphs have been plotted by using Glucose solution (200µg/mL of working standard). To the above samples 2 mL of DNS reagent was added into a lightly capped test tube. The mixture was heated at 90ºC for 15 minutes to develop the red brown color. Thereafter 16 mL of distilled water was added to stabilize the color. After cooling it to room temperature in a cold water bath, absorbance was measured with a spectrophotometer at 540nm. The above procedure was repeated for 1.0 mL of extract and 1.0mL of water was taken for unknown estimation [3]. The standard graph for the estimation of reducing sugar have been plotted by using glucose as working standard solution (200ug/mL). The sugar content of sample extracts was calculated by comparing their optical density values at A 540 with the standard graph. The individual values were taken in duplicates.

Results

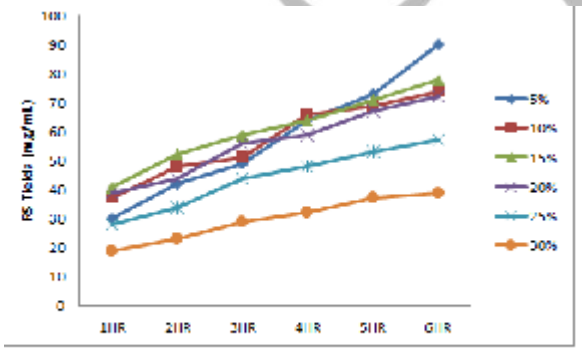

Fig. 1: Hourly RS Yields of the Hydrolysis of CP using amylase extract at 40°C for 6 Hr

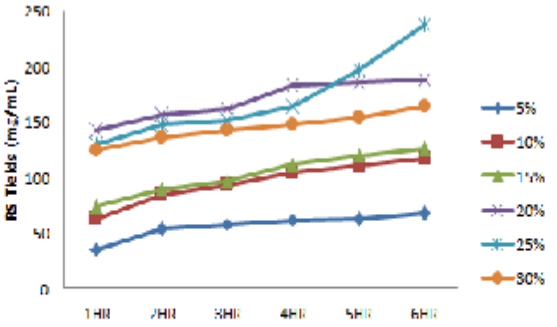

Fig. 2: Hourly RS yields of the Hydrolysis of CP using amylase extract at 50°C for 6 Hr

Results of reducing sugar (RS) concentration from Cassava Peels (CP) hydrolyzed using amylase extract is shown in figures 1-5. At 40°C RS yields ranged from 19 mg/mL to 90 mg/mL. The highest yield was recorded for CP at 5% substrate concentration and a reaction time of 6 hours. At 50°C, there was a gradual increase in RS yield with increase in substrate concentration. The lowest RS yield (35 mg/mL) was observed at 5% substrate concentration, hydrolyzed for 1 Hr. The highest yield, of 238 mg/mL, was recorded for 25% substrate concentration and a reaction time of 6 hours.

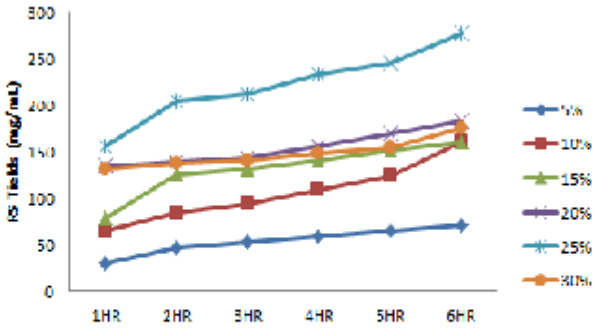

Fig. 3: Hourly RS yields of the Hydrolysis of CP using amylase extract at 60°C for 6 Hr

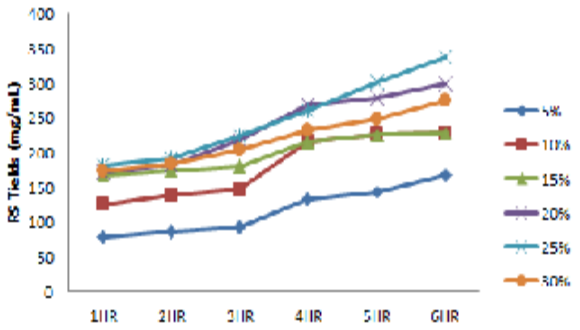

Fig. 4: Hourly RS yields of the Hydrolysis of CP using amylase extract at 70°C for 6 Hr

RS yields during hydrolysis at 60°C also showed increase with substrate concentration and reaction time. The lowest yield of 30 mg/mL was observed with 5% substrate concentration, at 1 hr treatment duration. The highest yield for the 1 hr treatment duration was 156 mg/mL, observed at 25% substrate concentration. The range of RS yield for 25 % substrate concentration was 156 mg/mL (at 1 hr) to 277 mg/mL (at 6 hr), the highest for this set-up. The RS yield for hydrolysis carried out at 70°C using crude amylase enzyme also increased with substrate concentration and reaction time. For the 1 hr treatment, 5% substrate concentration, RS yield was 78 mg/mL. As the duration increased, there was a gradual increase in yield from 87 mg/ml, to 167 mg/mL (at 5%, 6 hr). The highest RS yield of 338 mg/mL, was observed at 25% substrate concentration, 6 hr period.

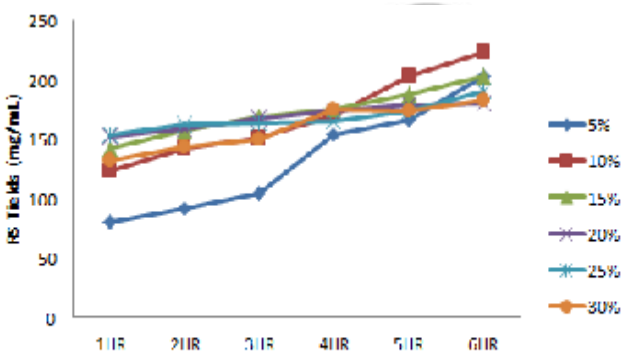

Fig. 5: Hourly RS yields of the Hydrolysis of CP using Sulfuric acid at 80°C for 6 Hr

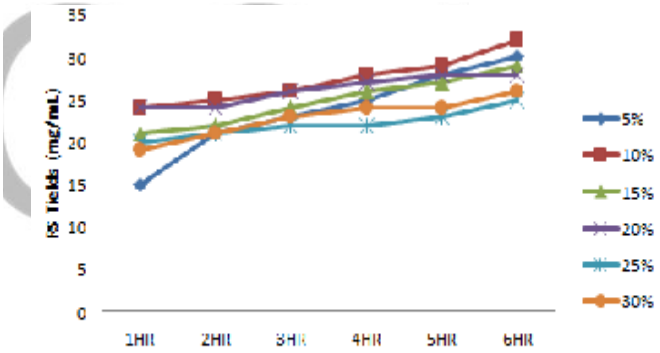

Fig. 6: Hourly RS yields of the Hydrolysis of CP using Sulfuric acid at 40°C for 6 Hr

The highest temperature treatment used (80°C) displayed a different trend. For 1 hr treatment, at 5% substrate concentration, RS yield of 80 mg/mL was the lowest (Fig. 5). The yield peaked at 25% substrate concentration (154 mg/mL) at this treatment. However, as duration increased, the RS yield at 5% substrate concentration peaked at 204 mg/mL at the 6 hr treatment duration. The highest RS yield of 224 mg/mL was observed at 10% substrate concentration and a reaction time of 6 hours. The yield for 25% substrate concentration, which has been consistently high, was 154 mg/mL (at 1 hr duration) and 191 mg/mL (at the 6 hr treatment duration).

Results of the hydrolysis carried out using sulfuric acid for the various treatments are showed in figures 6-10. At 40°C, RS yields were relatively low, ranging from 15 mg/mL (at 5% substrate concentration; 1 hr treatment duration) to 36 mg/mL (at 20% substrate concentration; 6 hr treatment duration). Hydrolysis carried out at 50°C recorded 22 mg/mL (at 30 % substrate concentration; treatment duration) and highest yield of 50 mg/mL (at 10% substrate concentration; 6 hr treatment duration). There was a slight improvement in RS yields in the hydrolysis carried out at 60°C; with lowest yield of 26 mg/mL (at 30% substrate concentration; 1 hr treatment duration) and highest yield of 74 mg/mL (at 10% substrate concentration; 6 hr treatment duration). The RS yield continued to increase

with temperature, as yield range from 59 mg/mL (at 20% in 1 hr) to 158 mg/mL (at 15% in 6 hr) at 70°C. At 80°C, RS yield ranged from 104 mg/mL (at 5% in 1 hr) to 507 mg/mL (at 10% in 6 hr).

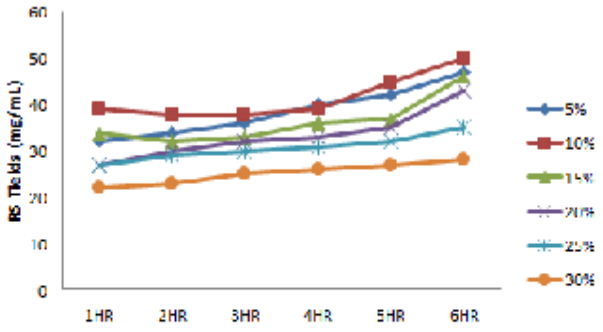

Fig. 7: Hourly RS yields of the Hydrolysis of CP using Sulfuric acid at 50°C for 6 Hr

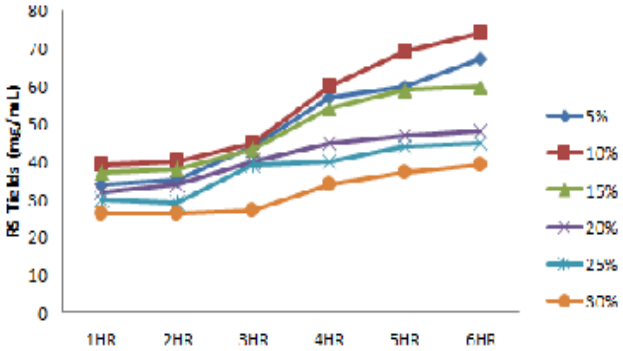

Fig. 8: Hourly RS yields of the Hydrolysis of CP using Sulfuric acid at 60°C for 6 Hr

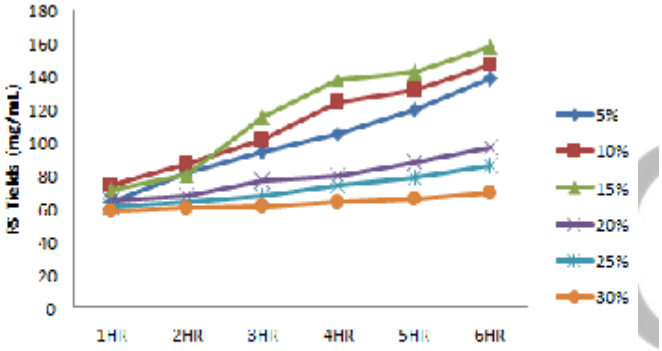

Fig. 9: Hourly RS yields of the Hydrolysis of CP using Sulfuric acid at 70°C for 6 Hr

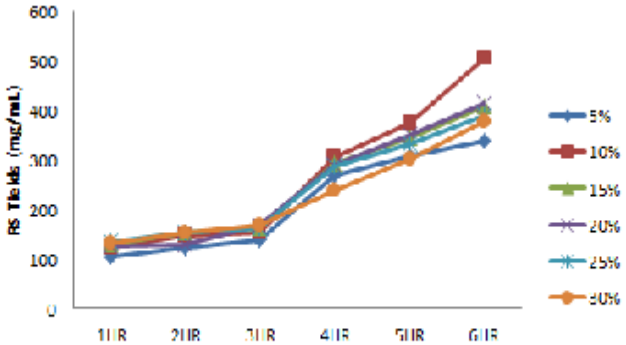

Fig. 10: Hourly RS yields of the Hydrolysis of CP using Sulfuric acid at 80°C for 6 Hr

Discussion

The increase in Reducing Sugar (RS) yields with temperature for enzyme hydrolysis may be attributed to increase solubilization of the lignocellulosic substrate. This inference is consistent with that of [10] who analyzed the conditions for H_2SO_4 hydrolysis. They reported an increase in the release of total sugars during the chemical hydrolysis, reaching a maximum concentration of 0.274 g total sugars/g biomass at a H_2SO_4 concentration of 1.5% at 80°C. There was a significant increase in the solubilization of cellulose for obtaining total sugars. The reduction in RS yields observed at 80°C for enzyme hydrolysis may be as a result of denaturation of the enzyme protein structure due to high temperature, which was above the optimum operational range.

Increase in RS yields with temperature for acid hydrolysis may be attributed to a decrease in the degree of polymerization of the substrate leading to a more equal distribution between the possible products formed and a general increase in product output. Higher temperatures also leads to increased solubilization of lignocellulosic substrate, thereby increasing the surface area of interaction with the hydrolytic agent.

Higher yields of RS with increased substrate concentration, for both enzyme and acid hydrolysis, may not be unconnected to the increased available lignocellulosic biomass for saccharification. However, both hydrolytic treatments at 30% substrate concentration gave relatively lower yields. This may portend that the optimum substrate concentration range was exceeded. Increasing substrate concentration increases the rate of reaction to a certain point. Once available enzymes have bound, any substrate increase will have no effect on the rate of reaction, due to saturation [11].

Comparing the RS yields for both acid and enzyme hydrolysis for the various temperature treatments, it was observed that at lower temperatures, enzyme hydrolysis gave better results. At 40^{o}C, RS yield for amylase hydrolysis ranged from 19 mg/mL to 90 mg/mL. This was higher than acid hydrolysis carried out at the same temperature, with RS yields ranging from 15 mg/mL to 32 mg/mL. At 50^{o}C, RS yields from enzyme hydrolysis ranged from 35 mg/mL to 238 mg/mL, while for acid hydrolysis, it ranged from 22 mg/mL to 50 mg/mL. Similarly, at 60^{o}C and 70^{o}C, better RS yields were observed with enzyme hydrolysis with ranges of 30 mg/mL to 277 mg/mL and 78 mg/mL to 388 mg/mL respectively, as compared to acid hydrolysis with 26 mg/mL to 74 mg/mL and 59 mg/mL to 158 mg/mL respectively. The trend was, however reversed at 80^{o}C, with RS yield for acid hydrolysis recording higher yields, ranging from 104 mg/mL to 507 mg/mL as compared to 80 mg/mL to 224 mg/mL from enzyme hydrolysis respectively. It can be opined that the hydrolytic process for crude amylase extract peaked at 70^{o}C, 25% substrate concentration and a reaction time of 6 hours, while the acid hydrolysis peaked at 80^{o}C, 10% substrate concentration and a reaction time of 6 hours.

Conclusion

This study was carried out to evaluate the effect of temperature, substrate concentration and duration of hydrolysis on the reducing sugar yield from Cassava Peels, using acid and crude amylase extract. The results have shown that RS yields, generally, increased with temperature for both acid and enzyme treatments, with exception to 80^{o}C, which showed a relatively slight reduction in yield. The study also showed that there was a relative increase in RS yields with increase in substrate concentration for enzyme hydrolysis, which peaked at 25% substrate concentration (at 70oC, 6 hr), while for both treatments, RS yields, generally increased with time.

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