

Original Research Article

Hydrolysis and Analysis of Agricultural Wastes Using Mineral Acids for Possible Glucose Synthesis.

Abstract

This work focuses on conversion of agricultural wastes into fermentable sugar via acid hydrolysis. Proximate and ultimate analysis was carried out to ascertain the physiochemical properties of the biomass feedstock and fermentable sugar. Mineral acid such as hydrochloric acid (HCl) and sulfuric acid (H₂SO₄) was used as the media for the conversion of plantain peels and water yam peels into fermentable sugar. Modelling of the process was done using three established kinetic models to determine the rate determining step. Results showed that untreated plantain peels and water yam peels contained 30.1% and 6.4% cellulose but increased significantly to 55.1% and 10.03% after hydrolysis indicating increase in fermentable sugar. In comparing the same conditions of plantain-HCl, plantain-HS, water yam-HCl and water yam-HS at 80°C with acid concentration/sample ratio of 0.5:2 at time 90 mins, plantain peels with HCl yield highest concentration of 4.09mg/l fermentable sugar while at the same condition water yam produced 2.031mg/l. Second order kinetic model appears to be the best fit for the process with better correlation coefficient $R^2 > 99\%$ with HCl and plantain peels at a reaction temperature of 60°C conforming to the rate determining step. The model demonstrated an increasing rate constant with increase in temperature which agrees with the magnitude of the hydrolysis process. Plantain peels and water yam peels demonstrated good potential as a source of fermentable sugar but plantain peels performed better.

Key words: Fermentable sugar, hydrolysis, agricultural wastes, kinetics

1.0 Introduction

Generally, agro-wastes are defined as the residues from the growing and processing of raw agricultural products [1-2]. Examples of these agro-waste include peels of plantain and water yam peels. The major components of agro-wastes are lignin, cellulose and hemicelluloses. These

peels from plantain and water yam contain primarily hemicellulose for which pentoses and hexoses are the polymers [3]. Agricultural waste provides a unique and sustainable resource for environmentally friendly organic fuel and chemicals. Furthermore, due to the abundance of agricultural waste, its conversion to fermentable sugar is considered one of the most important uses of biomass as an energy source in the modern world [4].

Agricultural wastes possess both advantages and disadvantages. Agricultural slurring for example, can be converted to fertilizer, the acid hydrolysis of agricultural-wastes can be used to produce ethanol. Exposure of workers to agricultural dust for those who are experts in the removal of waste products poses as some of the disadvantages of agricultural waste. Moreover, when agricultural waste decays, greenhouse gases such as methane and CO₂ are released into the atmosphere [5-8] and this will automatically defeat the 2030 target of the united nation regarding greenhouse gas emission.

There is an increase at the level of energy consumption globally [9-10]. Therefore, to improve the energy security of the world, there is a growing interest to meet the current economical sustainable energy demand, devoid of environmental contamination generated by fossil fuels. Since the use of food grains and tuber (yam, corn, cassava, rice, sugarcane etc.) to produce bioethanol can cause significant stress on food prices and food security, what comes to mind therefore, is the production of bioethanol from 'another' lignocellulose biomass like agricultural wastes such as plantain peels, watermelon peels, yam peels etc as viable alternatives. However, because of the rich carbohydrate nature of some of the agricultural wastes, which has wide spread abundance in Nigeria with its procurement having been relatively cost free, there is therefore, a need to employ a systematic study on the manufacture of ethanol from these agricultural wastes to meet the current energy demand, devoid of environmental pollution caused by fossil fuels.

Acid hydrolysis is the breakdown of larger molecules into simpler substances by the addition of acid. When agro-waste are hydrolyzed, fermentable sugars are obtained [11]. Glucose is a simple sugar with the molecular formula C₆H₁₂O₆. Glucose circulates in the blood of animals as blood sugar. Fermentable sugars are sugars that are easily fermented in the digestive system, which include oligosaccharides, disaccharides, monosaccharides and polyols. They are composed of short chains of sugar molecules making them easy to break down [12]. fermentable sugars such

as glucose are simple sugars with the molecular formula $C_6H_{12}O_6$. Glucose is the key to keeping the mechanisms of the body in top working order. When our glucose level is optimal, it often goes unnoticed. But when they stray from recommended boundaries the unhealthy effect it has on normal functioning begins to show [13-14]. Fermented sugar produced from the acid hydrolysis of agricultural waste can be used to produce ethanol. The ethanol produced from the food crops were called first generation bioethanol whereas the ethanol produced from lignocellulosic biomass were called second generation biomass [15]. This study therefore, is aimed at converting these agro-wastes such as (peels of plantain and water yam) to fermentable sugar via acid hydrolysis.

2.0 Materials and Method

2.1 Sample collection and processing

All the agro-wastes (plantain and water yam peels) were collected from household kitchen wastes, sorted, washed with distilled water to remove sand and other dirt and cut into pieces before sun-drying. The samples were sun-dried for three weeks with constant turning to ensure proper drying and to a constant weight. They were then milled using a laboratory milling machine. Thereafter, the samples were then sieved using a $250\mu\text{m}$ sieve in order to get a smooth sample of uniform size and stored in a labeled container for further analysis. The dried samples were put into two different conical flasks of 500ml with 20% NaOH and placed in a water bath for 3hrs at 90°C . After which it was washed several times with distilled water to neutrality and dried for 2days.

2.2 Preparing the Dinitrosalicylic Acid (DNSA) solution

The DNS solution was prepared by following the method according to Erdei et al.,[15]. 10ml of 0.5g NaOH was added to 0.5g DNSA with 0.1ml of phenol, stirred to complete dissolution in order to dissolve the DNSA. Then 10ml of water was used to dissolve 10g of Potassium Sodium tartrate (NaK). The NaK tartrate solution was then added to the DNSA solution and the mixture was made up to 50ml mark with distilled water.

2.3 Hydrolysis of plantain peels and water yam peels

This was achieved by following critically the procedure giving by Oyeleke et al. [16]. 2g sample of the blended plantain and water yam peels were weighed differently into different conical flasks containing an acid concentration of 0.5% HCl and H₂SO₄ with constant volume of 20ml each. Sterile distilled water was added to make up to 200 cm³ mark and the flasks were plunged with sterile cotton wool wrapped in aluminum foil to avoid contamination, the samples were then heated for 1hour 30 minutes in a water bath at 60°C, followed by sterilization in an autoclave at 121 °C for 15 minutes and the samples were allowed to cool and were filtered through a No 1 Whatman filter paper. The pH of the filtrate sample was adjusted to pH of 5 using 8 % NaOH. The residual samples were washed with distilled water to obtain a neutral pH for all treatment. The glucose concentration was determined by the DNS method according to Itelima et al. [1]. After the hydrolysis, the solution is centrifuged then placed in a water bath of temperature 90°C for 6minutes. Then cooled with cold water, after which the absorbance was obtained with the use of spectrophotometer at a wavelength of 575 nm. Effect of different factors such as time, temperature, concentration and dosage of the feedstock on glucose yield was analyzed at varying parameters.

2.4 Characterization of the samples

The method as described by Association of Pure and Applied Chemistry according to Horwitz et al. [17] was adopted for the analysis of crude protein using the Kjeldal method while the fats and oils was measured using the Soxhlet method. Gravimetric procedure as prescribed by Enejo et al. [18] was adopted in the analyses for the concentration of cellulose/hemicelluloses in the water yam peels plantain peels. Weight of cellulose/hemicelluloses was measured when nitric acid and Acetic acid dissolved every other component of the peels. 2g gram of each of the peel samples was measured in a round bottom flask. 10 ml of 60% acetic acid and 2.5 ml nitric acid were added into the flask. The flask was connected to a reflux condenser and heated with a heating mantle for 1 hour. 30 minutes. Note: Cellulose and hemicelluloses are insoluble in water, acetic acid and nitric acid. The solution after heating was filtered and the residue washed and dried in an oven at 100°C. The dried residue was weighed as the cellulose/hemicelluloses content of the sample. The lignin and ash contents of the peels were measured according to Igbokwe et al. [19]. This was done by treating the peels with 75% w/w H₂SO₄ for 3.5 hrs. The suspension after the treatment with the acid was filtered through a crucible and the solid residue dried at 100°C for 23 hrs and weighed (R₁). The residue was then transferred to a pre-weighed dry porcelain crucible

and heated at 500°C for 7 hrs. After cooling down, it was weighed (R_2) as the ash content. The lignin was calculated by the difference ($R_1 - R_2$)

2.5 Kinetics study of cellulose hydrolysis to glucose

The kinetic hydrolysis of cellulose to glucose was done by constant concentration of the two mineral acids used (0.5%) with a 2g of each sample in a conical flask at varying reaction temperature and hydrolysis time of 2 hours at 30minutes intervals in a gyratory shaker. Reaction kinetics also called chemical kinetics is the study of the rates of chemical processes or system which includes investigations how time dynamics, concentrations and different conditions can influence the speed of reaction and glucose yield according to Igbokwe et al, 2016. First order kinetics model describes the change in bulk concentration of the system using a simple order equation as shown below in equation (1)

$$C_t - C_o e^{-k_1 t} \quad (1)$$

Equation (1) can be rearranged to obtain a linear form as shown in equation (2)

$$\log C_t = \frac{K_1}{2.303} t + \log C_o \quad (2)$$

Where C_t and C_o are the concentration of the solute at time t and initial concentration of glucose produced (mg/l) respectively, K_1 is the first order rate constant (min^{-1}). The application of this order will give a linear relationship plotting $\log C_t$ versus t with $K_1/2.303$ and $\log C_o$ as slope and intercept respectively. Second order kinetic model is a typical second order equation is as shown in equation (3)

$$dc/dt = -K_2 C_t^2 \quad (3)$$

Integrating equation (3) with boundary conditions will yield equation (4)

$$\frac{1}{C_t} = K_2 t + \frac{1}{C_o} \quad (4)$$

C_t and C_o are the concentration of the solute at time t and equilibrium (mg/l) respectively, K_2 is the order rate constant ($1/\text{mg} \cdot \text{min}$). A plot of $1/C_t$ versus t to give a linear relationship with K_2 and $1/C_o$ as slope and intercept respectively. Seaman pseudo-first order model was designed for the hydrolysis of cellulose according to Ahmad et al. [20]. The change in the concentration of waste (cellulose) either positively or negatively at a constant temperature over a period of time can be described by a relationship as given in equation (5)

$$C_o - X = C_o \exp(kt) \quad (5)$$

Where C , the total initial waste cellulose concentration, k , specific rate constant (min⁻¹), cellulose concentration at time t (g/l), X ,glucose content (g/l), and t , time (min). On the basis of a first-order reaction for the hydrolysis, equation (5) becomes:

$$\ln \frac{C_o}{C_o - X} = -kt \quad (6)$$

Thus, equation (6) allows the natural logarithmic plots of experimental values of $\frac{C_o}{C_o - X}$ versus process time (t) in which straight lines obtained are indications of the validity of first-order reaction kinetics for the cellulose acid hydrolysis.

3.0 Results and Discussion

3.1 Characterization of treated and untreated peels

The impurities left after base treatment was soluble in ethanol extract as it aids the polymer extraction loosening it packed surface. The raw material for the untreated plantain peel and water yam peel gave 30.1% and 6.4% respectively for cellulose while the after treatment the plantain peel and water yam peel gave an increase in cellulose content with 55.1% and 10.03%, this is in agreement with the report according to Kongkiattikajorn.[21]. The percentage component in Table 1 supports the theory that the depolymerization of the lignin makes the cellulose fraction more accessible according to Reddy et al.[22], also reported that the degradation of the lignin and hemicelluloses makes the carbohydrate polymer soluble in water. The treatment decreased the lignin content by 70%, the hemicelluloses by 60% and increased the cellulose by 50% showed the both peels are good substrate for the glucose production but plantain peels showed more potential than water yam peels.

Table 1: Characterization of the treated and untreated plantain and water yam peels

Parameters	Untreated plantain peel	Treated plantain peel	Untreated water yam peel	Treated water yam peel
Ethanol extraction	6.7444	2.8085	1.9568	0.3827
Cellulose	30.0654	55.1213	6.4097	10.026
Hemicelluloses	25.7440	14.0452	10.7061	6.5821
Lignin	12.9968	5.4451	16.4222	6.8115

3.2 Fourier Transform Infra-red analysis of Treated and Untreated Peels

From Table 2, the untreated peel with wave-number of 760.4cm^{-1} and 1375.4 cm^{-1} representing the presence of chlorine and nitrogen oxide indicates the high presence of lignin polymer. The high presence of carbon to carbon bonds (C-C) and carbon to oxygen(C-O) bonds with wave-number 1420.1 cm^{-1} and 2076.1cm^{-1} indicates the composition of lignocelluloses complex in the untreated peels [23]. The highest transmission percentage in the plantain peel with 95.4% agrees strongly with Rawinder & Himanshu.[24] postulate on the composition of lignocelluloses in the sample. From Table 3, the treated peels with high transmission percentage of 94.4% and 96.6% represent the ether bond that appears to be the most interesting among the functional groups because it holds the glucose monomers in a polymer chain (glucosidal linkage) and it is by far the most predominant bond in the lignin polymer according to Rawinder & Himanshu.[24]. The wave-number of 3276 cm^{-1} of untreated peel increased to 3287 cm^{-1} in the treated peel showing that O-H stretching band after treatment has more hydrogen bond indicating high cellulose composition of the sample. This is in line with the works of Oh & Balint.[25] and Amir et al. [26] on the hydrolysis of sweet sorghum bagasse.

Table 2. FTIR spectra of untreated plantain and Water yam peel

Plantain peel			Water yam peel		
Wave-number (cm ⁻¹)	Transmission (%)	Functional groups	Wave-number (cm ⁻¹)	Transmission (%)	Functional groups
3276.3	60.730	O-H	3276.3	57.24	O-H
2922.2	67.279	O-H	2922.2	74.789	O-H
2091	95.407	C=C	1636.3	70.657	C-O-C ether bond
1591.6	60.196	C=C	1244.9	73.848	C-O-C ether bond
1375.4	62.079	NO ₂ stretch	1408.9	73.949	C-F
1148	62.921	C-O-C ether bond	1364.2	71.179	NO ₂
1013.8	37.191	ring C-O	1148	60.372	C-O-C Ether bond
			928.1	55.573	C-O
			1073.5	48.723	C-O-C Ether bond
			760.4	55.395	C-Cl
			991.5	28.867	C-O

Table 3. FTIR spectra of Treated plantain and Water yam peel

Plantain peel		Water yam peel	
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Wave-number (cm ⁻¹)	Transmission (%)	Functional groups	Wave-number (cm ⁻¹)	Transmission (%)	Functional groups
3283.8	66.471	Water OH/ Alcohol O-H	3287.5	80.856	Water OH/ Alcohol O-H
2922.2	72.811	=C-H stretch Alkene	2918.5	87.518	O-H carboxylic acid
2098.5	94.399	C=C	2076.1	96.423	C=C
1364.2	74.613	CH ₃ bend	1994.1	96.604	C-O-C Ether bond
1606.5	74.712	C=C alkene Ether bond	1625.1	85.769	Ether bond
1315.8	73.949	C-O	1420.1	84.113	C=C Aromatics
1420.1	74.633	C=C	1364.2	83.921	C-O
1151.7	70.371	C-O-C Stretch Ether bond	1148	80.953	C-O-C Ether bond
1017.6	42.311	C-O	849.8	80.905	C-H RING
			1010.1	65.214	C-O

3.3 Effect of Feedstock dosage on glucose yield

From figure.1 shows a significant increase in concentration of glucose with 2.5g giving highest yield in all samples. The higher the degree of grinding of the cellulosic feedstock, the greater the contact areas for the acid penetration. This could be the reason for the increase in dosage resulting to increase in glucose yield. The high yield in dosage increment is also as a result the liquid-to-solid ratio of 2–20 ml/g chosen for the conversion process according to Adetunji et al.[27]. The hydrolysis with HCl shows higher yield than the hydrolysis with H₂SO₄ for all samples. The plantain peels feedstock yielded much better glucose produce than water yam peel with highest yield of 4.095mg/l and 2.85mg/l. The hydrolysis process from the effect of dosage indicates desorption from the raw sample into the acid solution which also shows increased rate of diffusion.

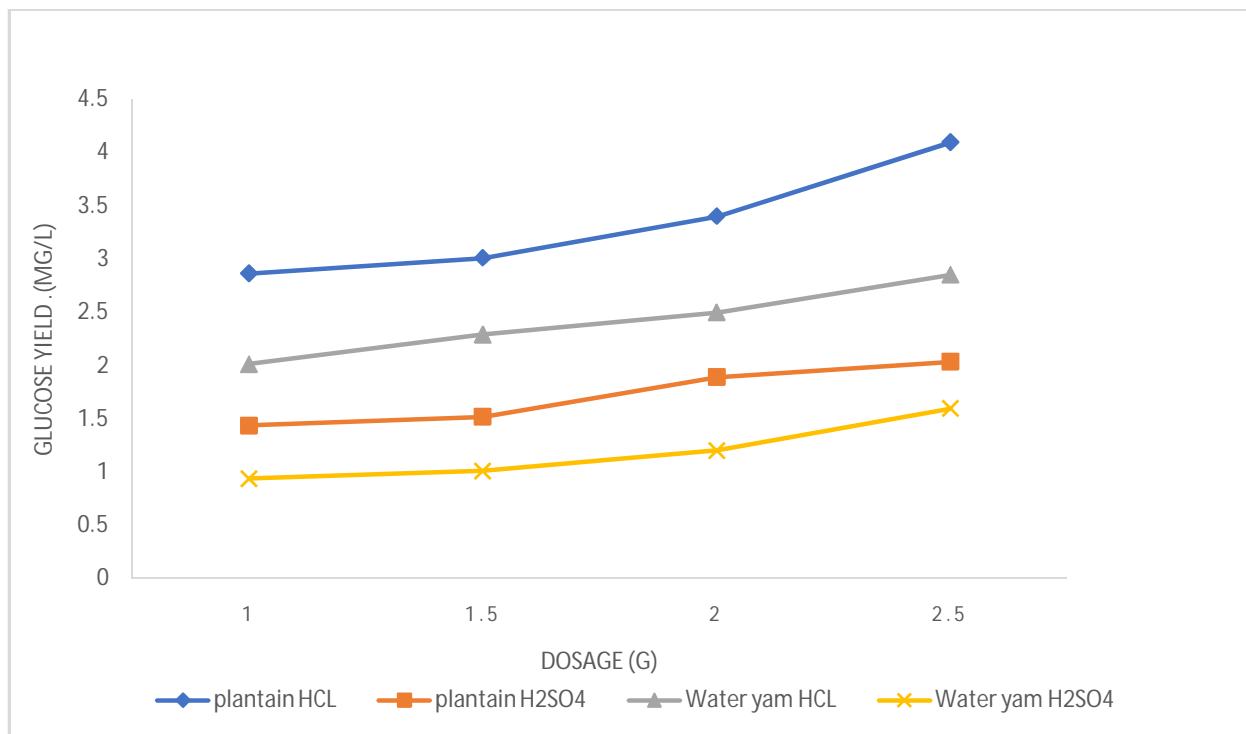


Figure.1: Effect of feedstock dosage on the acid hydrolysis of plantain and water yam peel

3.4 Effect of time on the hydrolysis of plantain and water yam peel

As time increased from 30minutes to 120minutes as shown in figure 2, there was a gradual increment observed in all peels with different acids at temperature 80°C. The peak sugar yield occurred at 120minutes reaction time indicating that longer time of hydrolysis increases the yield of fermentable sugar because when the reaction rate is fast, monosaccharide can be removed from the solid surface in a timely manner favoring the breaking of bonds, release of energy and formation of covalent bond that aid the sugar production according to Schell et al.[28]. Reaction time at moderate temperature therefore aids the transition of the cellulose to a fermentable sugar.

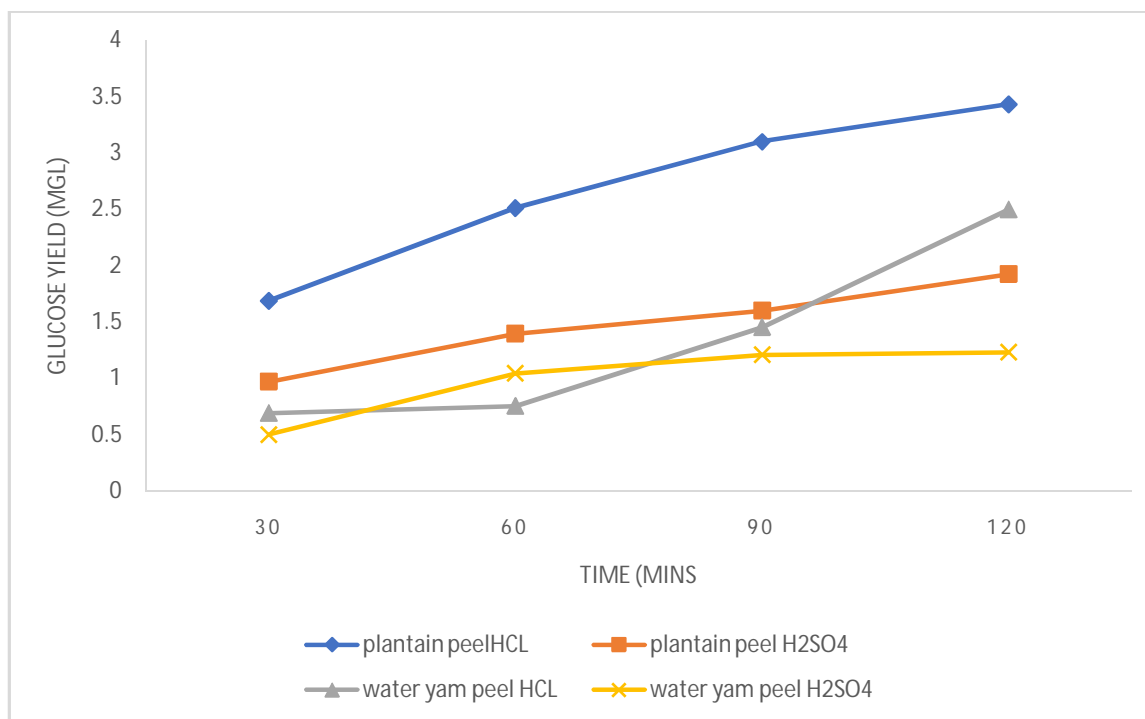


Figure 2: Effect of time on the hydrolysis of plantain and water yam peels

3.5 Effect of Temperature on the hydrolysis of plantain and water yam peel

From figure 3, it was seen that the fermentable sugar yield from each of the agricultural waste increased with increase in temperature. Generally, when the temperature rises by 10°C, the hydrolysis rate will increase by 0.5–1 time as observed in this work. However, higher temperature accelerates the decomposition rate of the monosaccharide. Hence a moderate temperature was used to achieve a maximum yield according to Adiotomre.[29]. Highina et al. [30], reported that in the acid hydrolysis of orange peels at low temperature range, sugar yield increased with increase in temperature and at very high temperature range, sugar yield declines. However, Megawati et al. [31] also reported that in the acid hydrolysis of rice husk, at high temperature range (160 – 220°C), total sugar concentrations increased with increase in temperature. Igbokwe et al. [19] has also observed an increase in glucose yield with temperature for acid hydrolysis of plantain peels and sugar bagasse respectively. Furthermore, the plantain peel had a steady increase from temperatures 50-80°C and at 80°C, plantain peel produced higher yield with HCl. Water yam peel increased from 50-70 with HCl and starts decreasing in yield and with H₂SO₄ increased from 50-60°C and reach an equilibrium point. In all, temperature has significant effect in the production of fermentable sugar because heat render cell walls of substrate to become permeable leading to increase in diffusion rate.

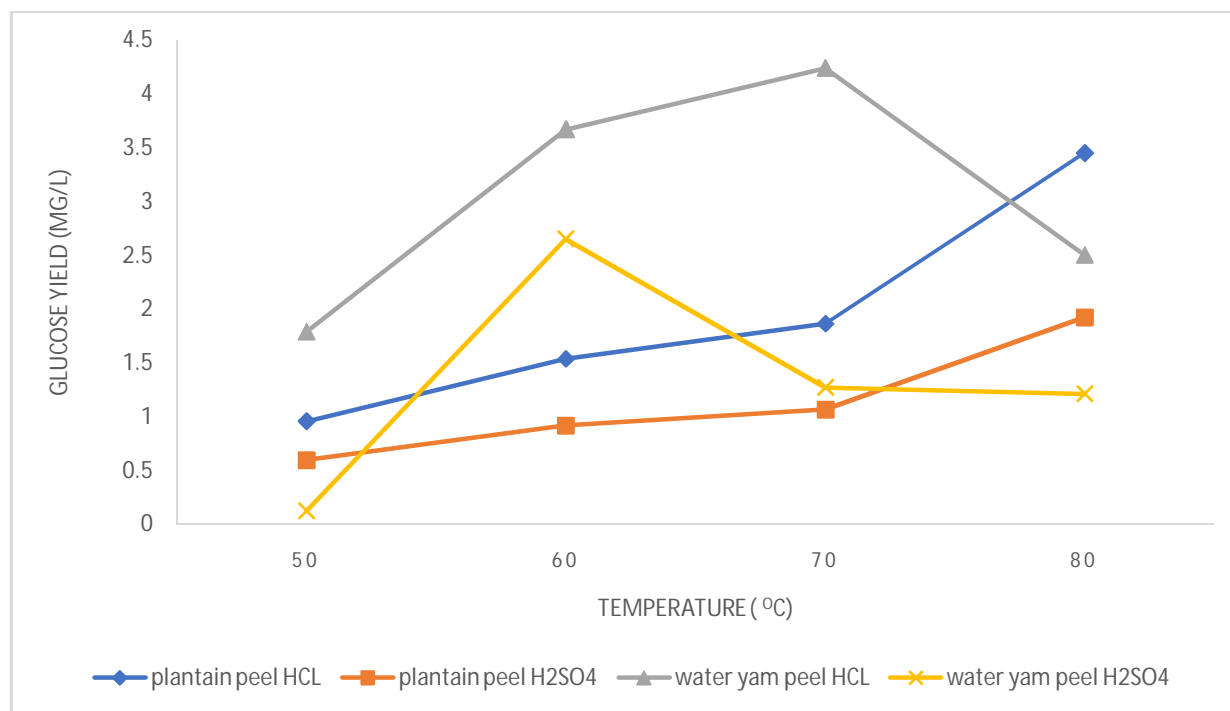


Figure 3: Effect of temperature on the hydrolysis of plantain and water yam peels

3.6 Kinetics modelling of the hydrolysis process

The kinetic parameters in Table 4 demonstrated the performance of the three models in the analysis of data generated during the hydrolysis of plantain peels and water yam peels. The hydrolysis process of cellulose to glucose was repeatedly carried out at four different times and temperatures with same dosage of each sample to investigate how different experimental conditions can influence the speed of a process/yield. With various times varying from 30minutes to 120minutes, time (t) against concentration (C) was plotted and fitted on three models to confirm the most suitable model for the process. First-order kinetic model showed a good correlation with sulfuric acid and water yam peels as evident in the correlation coefficient $R^2 > 0.9$ at temperature between 60-70°C as shown in Table 4 but shows a slower rate of reaction from its smaller slope and intercept and as such is not a good fit for the hydrolysis process. The application of the seaman's pseudo-first order showed a much smaller slope and an intercept with very low correlation coefficient (R^2) at every change in temperature signaling that it does not follow a determining step for the reaction of cellulose to glucose conversion [31]. The second order kinetic model created from the seaman's model appears to be the best fit for rate of reaction of the process with better correlation coefficient $R^2 > 0.99$ with HCl and plantain peels at

a reaction temperature of 60°C conforming to the rate determining step. The model demonstrates an increasing rate constant as temperature increases agreeing with the magnitude of the hydrolysis process [32].

Table 4 Kinetics studies on the hydrolysis of plantain and water yam peel

		Second order kinetic model			
		50	60	70	80
Plantain peels H ₂ SO ₄	K	-0.022	-0.017	-0.012	-0.005
	R ²	0.852	0.943	0.735	0.895
Plantain peels HCl	K	-0.013	-0.011	-0.007	-0.003
	R ²	0.851	0.999	0.736	0.895
Water-yam peels HCl	K	-0.471	-0.009	-0.007	-0.012
	R ²	0.605	0.760	0.806	0.962
Water-yam peels H ₂ SO ₄	K	-0.566	-0.01	-0.005	-0.013
	R ²	0.752	0.886	0.96	0.752
		First-order kinetic Model			
Plantain peels H ₂ SO ₄	K	0.0069	0.0092	0.0069	0.0069
	R ²	0.905	0.988	0.988	0.944
Plantain peels HCl	K	0.0069	0.0092	0.0069	0.0069
	R ²	0.905	0.988	0.988	0.944
Water-yam peels HCl	K	0.0437	0.016	0.014	0.014
	R ²	0.680	0.857	0.896	0.948
Water-yam peels H ₂ SO ₄	K	0.0207	0.0115	0.0046	0.0092
	R ²	0.904	0.901	0.901	0.821
		Pseudo-first kinetic order model			
Plantain peels H ₂ SO ₄	K	0.007	0.00	0.0046	0.02
	R ²	0.011	0.00	0.006	0.022
Plantain peels HCl	K	0.0046	0.00	0.00	0.0023
	R ²	0.015	0.00002	0.000009	0.001
Water-yam peels HCl	K	0.1	0.096	0.099	0.08
	R ²	0.449	0.448	0.461	0.369
Water-yam peels H ₂ SO ₄	K	0.13	0.099	0.096	0.096
	R ²	0.523	0.465	0.45	0.472

Conclusion

Characterization using ethanol extract decreased the lignin content by 70%, the hemicelluloses by 60% and increased the cellulose by 50% showing that both peels are great substrate for the fermentable sugar production. The alkali pre-treatment aided in releasing the ionic bonds from a complex matrix, thus significantly improving the efficiency of hydrolysis and the cellulose content. In comparing the same conditions of plantain-HCl, plantain-HS, water yam-HCl and water yam-HS at 80°C with acid concentration/sample ratio of 0.5:2 at time 90mins, plantain

peel with HCl yield highest with 4.09mg/l concentration, followed by plantain-HS then water yam-HCl and water yam-HS. The results demonstrated that utilization of these biomass (plantain peels and water yam peels) could add significant value to the energy need and greatly improve our environment sanitation and also revealed that the plantain and water yam peels are a potential resource for fermentable sugar.

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