Original Research Article

STUDY OF THE EFFECT OF PROCESS PARAMETERS ON THE YIELD OF FERMENTABLE SUGAR FROM TUBER PEELS VIA ACID AND ENZYME HYDROLYSIS

ABSTRACT

The aim of this work is to study the acid and enzymatic hydrolysis of water yam peels using HCl, H_2S0_4 acids and cellulase enzyme. The cellulase was secreted from Aspergillus Niger (A.niger) fungi. The proximate analysis of the substrate showed that water yam peel is a lignocellulosic biomass with a cellulose composition of 48%. The effect of the process parameters (time, temperature, acid concentration and pH) on the yield of glucose in acid and enzymatic hydrolysis of the water yam peel was respectively investigated. Maximum glucose yield of 44.5% was obtained after 3 days of enzymatic hydrolysis at 30°C and pH 5. The HCl acid hydrolysis showed a maximum glucose yield of 27.3% at 70°C, 5% HCl after 180 minutes. The glucose yield in H_2S0_4 hydrolysis was relatively lower than that of the HCl with a maximum yield of 26.5% at 70°C, 5% H_2SO_4 after 180 minutes. In addition to, the functional groups present in the glucose synthesized from ground water yam peels and the standard glucose were evaluated using Fourier Transformed Infrared (FTIR). The FTIR results showed similarities in the functional groups present in both sugars. Yam peel can be used for the production of glucose and further fermentative process to produce ethanol.

Keywords: *Acid hydrolysis, Enzyme hydrolysis, Glucose, pH, Temperature*

1.0 INTRODUCTION

In years passed, increasing research and development efforts have been directed to reducing the use of fossil fuels and decreasing the emission of carbon dioxide. Bio-ethanol is made mostly from sugar cane, maize, wheat, and barley. However, the use of these crops to produce bio-ethanol competes with their use as food sources [1]. Hence, a special attention is actually being paid to the use of renewable resources, which are mainly agricultural and industrial by-products. Examples of the agricultural wastes are (corn stover, sugar cane bagasse, yam, water yam, cocoyam, flux straw, potato pulp, cassava bagasse, cowpea husk, rice husk, soya bean husk), forestry (beech bark, beech wood, populous tremuloides wood) and herbaceous materials (e.g. reed grass, switch grass, rye grass). These agricultural wastes biomass tend to dominate and pollute the environment. Many of these agro-wastes are allowed to rot away and not utilized [2]. These wastes biomass consist of cellulose, hemicelluloses, lignin and other materials called extractive, [3, **4**]. Among all the constituents of agricultural waste biomass, cellulose constitutes relative high percentage, because it is a strong elastic material that forms cell wall of nearly all plants, **[4].** The cellulose can be hydrolyzed to produce glucose for human needs which can be used as substrates for fermentative production of useful product like alcohols, [5, 6]. As mentioned above, all forms of plant materials that can be used for energy are derived from agricultural waste [7, 8. 9].

Water Yam is the third most important root crops (after potato and cassava) cultivated in West Africa. More than three quarters of world yam production comes from Africa with Nigeria and Ghana being the world's leading producers [10]. In Egypt, Hawaii and Japan they are also important crops, [11]. In general, they are stem tubers that are widely cultivated in both tropical and subtropical regions of the world **[12]**. Among the species of the family *Dioscoreaceae* which originated from Asia and other species of genus *Dioscorea* which is from America, Indian and China, the species mostly grown in West Africa particularly in Nigeria is the *Dioscorea alata*, which are either red or white **[12].**

The *Dioscorea alata* variety in Nigeria is hard and highly starchy which makes it very easily used for fufu preparation. The young leaves and the cormels of the *Dioscorea alata* variety serve as leafy vegetables in some diets, in Nigeria [13]. The substrate used in this study is *Dioscorea alata* (water yam). *Dioscorea alata* can be processed in several ways to produce food and feed products similar to that of potatoes in the Western world. Water yam can be processed via the following; boiling, roasting, frying, milling and conversion to "fufu", as earlier mentioned, soup thickeners, flour for baking, chips, beverage powder, porridge and speciality of food for gastrointestinal disorder [13, **10,** 14, 15].

Saccharification of Water yam peels to produce reducing sugar is important, owing to the fact that reducing sugars are essential raw material for the production of bio-ethanol (bio-fuel). Saccharification is basically achieved via acidic and enzymatic hydrolysis of polysaccharides or cellulose. The Large quantities of these wastes produced annually in Nigeria are under-utilized. The practice is usually, to leave residues to decomposed or burnt. However, studies have shown that these residues could be processed into liquid fuels or combusted/gasified to produce electricity and heat **[7, 8, 9].** Conversion of these waste products such as glucose, xylose, arabinose, etc. provides a more efficient means of waste management.

This study therefore, focused on the production of bioethanol from Water yam peels that are readily available in the country in large quantities as agrowastes. The use of waste biomass like water yam peel to generate energy can reduce problems associated with waste management such as pollution, greenhouse gaseous emissions and fossil fuels use. The rate of global warming can be reduced drastically through the use of bioenergy derived from municipal or agricultural wastes. According to a recent past report, it was proposed that by the year 2020, constant use of biomass will produce 19 million tons of petroleum equivalents. Out of this, 46% will be obtained from bio-wastes like farm waste, agricultural waste, municipal solid waste and other biodegradable waste [16].

2.0 MATERIALS AND METHOD

2.1 Acid hydrolysis of Water yam peel

The water yam peels were collected and pretreated by washing, drying in an oven at 105°C for 4 hours and grinding before sieving to fine particle size of 250µm. Thereafter, the dilute acid hydrolysis was carried out using the method adopted and described by [17, **18**]. The ground water yam peels were first soaked in ethanol for 24hrs after which they were washed repeatedly with distilled water until the residues were free of the solvent. 1.0g of the pretreated biomass was weighed into a 250mL conical flask. 20mL of 1% HCl (0.1M) was added into the flask. The flask was covered with cotton wool and aluminum foil and put into a water bath set at 30°C for 30 minutes. The mixture was thereafter filtered with filter paper, neutralized with drops of 6M NaOH and the concentration of the simple sugar obtained was measured using DNS method. The experiment was repeated at different concentrations of HCl (3% and 5%) for different durations (60, 90, 120, 150, 180 minutes) and at different temperatures (50°C and 70°C). H_2SO_4 at different concentrations of (1%, 3%, 5%) for duration (60, 90, 120, 150, 180 minutes) and at temperatures (30°C, 50°C and 70°C) was also used for the experiment. The yield of simple sugar (glucose) was calculated using Equations 2.1 and 2.2, while the percentage conversion of cellulose/ hemicelluloses to simple sugars at each run was calculated using Equation 2.3 as described by **[18].**

$$yield\ (\%) = \frac{M_{glucose}}{M_{initial}} \times 100 \qquad (2.1)$$

$$M_{glucose}(g) = Vol_{Hydrolysate} \times Conc_{glucose}\ (g/100ml) \qquad (2.2)$$

$$E(\%) = \frac{M_{glucose} \times f}{M_{initial} \times y} \times 100 \quad (2.3)$$

Where

$yield$ (%) = yield of simple sugar (glucose) based on the total weight of the biomass

$M_{glucose}(g)$ = total mass of simple sugar (glucose) after hydrolysis

$Vol_{hydrolysate}$ = total volume of the hydrolysis mixture (mL)

$Conc_{glucose}$ = percentage concentration of simple sugar (glucose) obtained from the standard graph

$E(\%)$ = simple sugar (glucose) percentage conversion

$M_{Initial}$ = mass of the extractive free biomass

F = conversion factor (0.9 for cellulose)

y = fraction of cellulose /hemicelluloses in the biomass

2.2 Cellulase synthesis and Enzyme assay

2.2.1 Isolation of Aspergillus niger

The fungi Aspergillus niger (A.niger) was isolated and characterized at Microbiology Department of Enugu State University of Science and technology (ESUT) Nigeria following the method described by [19]. Soil obtained from groundnut husk dump site was crushed, sieved and diluted serially using sterile distilled water. Different dilutions of the soil was inoculated on the surface of slant Potato Dextrose Agar (PDA) medium in test tubes and incubated for 7days. Spores of A.niger were harvested by vortex. Cellulase production was detected by the disappearance of the red colour of the Congo red solution around microbial colonies. Evaluation of the clear zones of each colony was estimated as radius (mm) of the clear zone minus the radius of the colony. A. niger colonies producing large clear zones were picked up [20].

2.2.2 Inoculums preparation

Inoculums for enzyme production were prepared by adding 10mL of citrate buffer (pH of 5.0) to each test tube containing fully grown spores of A.niger. The inoculums were estimated to have $2.8x10^6$ spores/ml **[20].** The inoculums were stored in a refrigerator for future use.

2.2.3 Cellulase enzyme production

Enzymes production was carried out in 250 mL Erlenmeyer flasks with 50 mL medium as described by [21]. The ingredients of culture medium included 30 g/L alkaline pretreated cocoyam shell (dry biomass), 1 g/L glucose, 6 g/L ammonium sulfate, 2.0 g/L KH_2PO_4, 0.3 g/L $CaCl_2$, 0.3 g/L $MgSO_4$, 0.005 g/L $FeSO_4$, 0.0016 g/L $MnSO_4$, 0.0014 g/L $ZnSO_4$ and 0.0037 g/L $CoCl_2$. The initial pH value was adjusted to 4.8 by adding 2.5 mL citrate buffer solution (1 mol/L) to the medium. Then the prepared medium was autoclaved at 121°C for 30 min. The submerged fermentation started by inoculating the 50mL medium with 10ml of the fungi inoculums in a 250mL Erlenmeyer flask. The flask was incubated under shaker for 7days. The fermentation was terminated when the glucose level was zero. The medium was filtered and centrifuged to obtain the supernatant, which is referred to as the crude enzyme.

Cellulase assay was done following the procedure described by [22]. One milliliter of 1% Carboxyl Methyl Cellulose (CMC) in 0.1M citrate suffer (pH 5.5) was placed in a test tube and 1ml of culture filtrate was added. The reaction mixture was incubated at 50˚C for 30mins and the reaction terminated by adding 1.5ml of DNS reagent. The tubes were heated at 100 ˚C in a boiling water bath for 15 minutes and then cooled at room temperature. The absorbance was read at 540nm. Enzyme activity is expressed as mmol glucose released per sec ml of culture filtrate. The result after 7 days of incubation gave 2.4×10^{-4} µg/ml.

2.3 Enzymatic hydrolysis

The enzymatic hydrolysis was performed in 250 mL Erlenmeyer flasks with a 20 mL mixture of 0.05 M citrate buffer solution (pH 5.0), and enzymes. 1g of the alkaline (NaOH) pretreated ground yam peels was added in the mixture and the flask was incubated in an orbital shaker (140 rpm) at 30°C [21]. Sampling was conducted at 1 day interval for analysis. The glucose yield was analyzed using the DNS method. The different pH used for the hydrolysis process was 3.0, 5.0 and 7.0. The pH was adjusted using citrate buffer and the temperatures used were 30, 50 and 70°C [23].

2.4 Dinitrosalicylic (DNS) method of simple sugar analysis [24]

Dinitrosalicylic acid reagent solution, 1%

- Dinitrosalicylic acid: 10 g
- Phenol: 2 g (optional, see Note 1)
- Sodium sulfite: 0.5 g
- Sodium hydroxide: 10 g
- Potassium sodium tartrate solution, 40%

Procedure:

i. Add 3 ml of DNS reagent to 3 ml of glucose sample in a lightly capped test tube. (To avoid the loss of liquid due to evaporation, cover the test tube with a piece of paraffin film if a plain test tube is used.)

ii. Heat the mixture at 90º C for 5-15 minutes to develop the red-brown color.

iii. Add 1 ml of a 40% potassium sodium tartrate (Rochelle salt) solution to stabilize the color.

iv. After cooling to room temperature in a cold water bath, the absorbance was recorded with a spectrophotometer at 540 nm

Note1: Phenol, up to 2g/l, intensifies the color density. It changes the slope of the calibration curve of absorbance versus glucose concentration but does not affect the linearity. The above procedure yield an absorbance of 1 for 1 g/l of glucose in the original sample in the absence of phenol in the reagent, as opposed to an absorbance of 2.5 for 1 g/l of glucose in 2 g/l of phenol. This property can be exploited to achieve the maximum sensitivity for dilute samples. See Fig 1 at for the glucose standard graph.

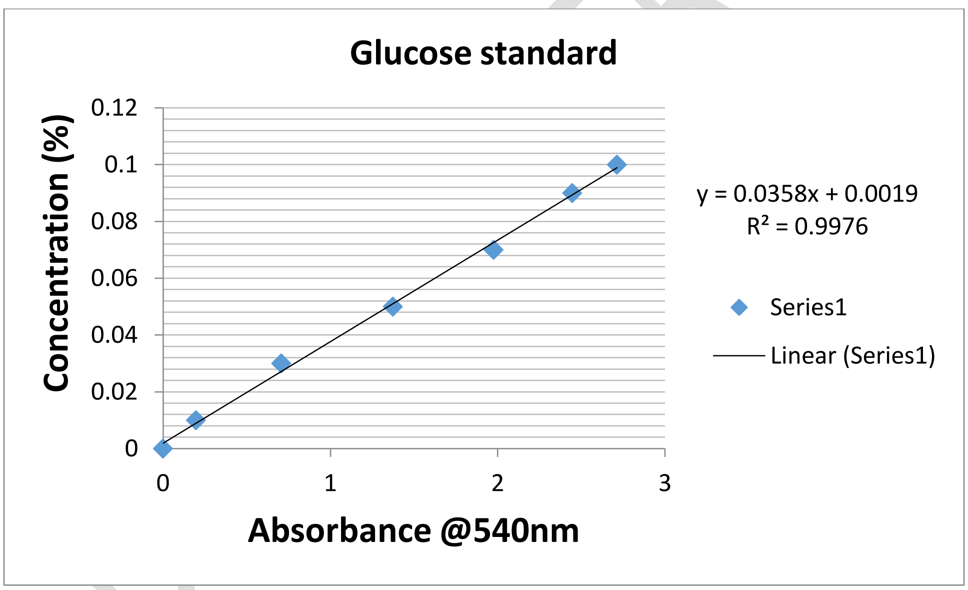

Figure 1: Glucose standard graph of concentration against absorbance

3.0 RESULTS AND DISCUSSION

The results obtained for the study of the effects of time, acid type/concentration and temperature on glucose yield for acid hydrolysis of ground yam peels are shown on Tables 3.1, 3.2, and 3.3 below. It was observed that glucose yield for HCl hydrolysis was higher than that of H_2SO_4 hydrolysis. At high temperature and acid concentration, higher glucose yield is achieved faster than at low temperature and low acid concentration **[18**, 25].

However, the result showed a maximum sugar yield of 27.3% and 26.5% for 5% HCl and 5% H_2SO_4 hydrolysis respectively at 70˚C and at 180 minutes. This result trend was in consonance with results obtained in similar studies of acid hydrolysis of lignocellulosic biomass from literatures. Increase in contact time and increase in acid concentration will enhance adequate access of the acid molecules to the cellulose content of a lignocellulosic biomass, and thus, break adequately the crystalline bonds (hydrogen bonds) of the cellulose polymer into glucose monomers, thereby leading to higher glucose yield. **[25]** in their study reported higher glucose yield at higher temperature.

Table 3.1: Percentage glucose yield with different acids and varying acid concentrations at 30˚C.

			At 30˚C			
Time	**1% HCl**	**1% H_2SO_4**	**3% HCl**	**3% H_2SO_4**	**5% HCl**	**5% H_2SO_4**
(hrs)	% glucose	% glucose	% glucose	% glucose	% glucose	% glucose
0	0	0	0	0	0	0
30	0.5	0.5	1.1	0.8	2.2	1.4
60	1.4	0.9	1.6	1.9	2.9	3
90	1.75	1.2	2.1	2.3	3.4	3.4
120	2.1	1.5	2.6	2.7	3.9	3.8
150	2.6	1.95	3.5	3.4	5.45	5
180	3.1	2.4	4.4	4.1	7	6.2

Table 3.2: Percentage glucose yield with different acids and varying acid concentrations at 50˚C.

At 50˚C						
Time (hrs)	**1% HCl** % glucose	**1% H_2SO_4** % glucose	**3% HCl** % glucose	**3% H_2SO_4** % glucose	**5% HCl** % glucose	**5% H_2SO_4** % glucose
0	0	0	0	0	0	0
30	1.2	1.1	2.3	1.8	2.1	2.8
60	2.3	2.2	3.8	3.6	5.1	3.6
90	3.65	3.25	5	4.95	7	5.5
120	5	4.3	6.2	6.3	8.9	7.4
150	5.9	5.1	7.8	7.45	10.1	9
180	6.8	5.9	9.4	8.6	11.3	10.6

Table 3.3: Percentage glucose yield with different acids and varying acid concentrations at 70˚C.

At 70˚C						
Time (hrs)	**1% HCl** % glucose	**1% H_2SO_4** % glucose	**3% HCl** % glucose	**3% H_2SO_4** % glucose	**5% HCl** % glucose	**5% H_2SO_4** % glucose
0	0	0	0	0	0	0
30	2.1	1.97	3.99	2.93	5.72	5.373
60	4.1	3.5	5.8	5.67	14.2	11.421
90	5.75	4.9	8.41	8.31	19.15	15.7705
120	7.4	6.3	11.02	10.95	24.1	20.12
150	8.71	7.695	13.11	12.775	25.7	23.31
180	10.02	9.09	15.2	14.6	27.3	26.5

Table 3.4: The Effect of pH, Time and Temperature on the Percentage Yield of Glucose in Enzymatic Hydrolysis

Temp.	Time	pH 3		pH 5		pH 7	
(˚C)	(hour)	Yield (%)	E (%)	Yield (%)	E (%)	Yield (%)	E (%)
30	24	15.7	21.74	32.4	44.86	23.4	32.4
	48	21.9	30.32	43.8	60.65	39.0	54.0
	72	22.2	30.74	44.5	61.62	40.4	55.94
50	24	12.3	17.03	27.9	38.63	20.1	27.83
	48	15.9	22.02	38.6	53.45	28.9	40.02
	72	19.8	27.42	38.9	53.86	30.9	42.78
70	24	8.5	11.77	10.5	14.54	8.6	11.91
	48	10.2	14.12	10.9	15.09	9.4	13.02
	72	10.3	14.26	11.1	15.37	9.8	13.57

In the study of the effects of process parameters (time, pH and temperature) on the yield of glucose via enzymatic hydrolysis, the results as presented on Table 3.4 showed the maximum glucose yield of 44.5% obtained at 30°C at pH of 5 and 72 hours (3 days). The table also contains the conversion efficiency (E) based on the total concentration of cellulose and hemicelluloses in the water yam peel.

The yield of glucose was observed to decrease as the temperature increased from 30 to 70˚C. [26] in their study reported an optimum pH range of 5-6 for cellulase activity and at 40˚C. Also, [27] reported an optimum pH range of 5.5-7 and 45˚C for cellulase activity. The results obtained in this study were in agreement with the results of similar studies from the literatures. The yield of glucose from enzymatic hydrolysis of the yam peels actually depends on process parameters investigated.

Table 3.5: Fourier Transform Infrared (FTIR) Characterization of Pure Glucose

Serial No	Frequency (cm-1)	Peak Area	Assigned functional Group
1	686.7681	0.2967709	Meta disubstituted aromatic
2	790.2341	0.2970884	Para disubstituted aromatic
3	880.1656	0.346344	Geminaldisubstituted alkene
4	1073.869	0.694572	Alkoxy C-O
5	1330.733	0.464741	Nitro symmetric group N=O
6	1608.217	0.5667408	Nitro asymmetric group N=O
7	1862.701	0.354259	Ketones group C=O
8	2058.185	0.3656095	Alkene C-H bend region
9	2144.022	0.4011851	Aromatic group
10	2246.856	0.407736	Acyl, strong C-O
11	2454.593	0.7394771	Acid O-H, very broad (overlap C-H stretch)
12	2515.718	0.7147146	Thio group S-H
13	2665.982	0.5441699	Thio group S-H

14	2856.719	0.526873	aldehyde C-H stretch (two bands)
15	2986.8	0.49722	Alkene group C-H stretch
16	3123.093	0.6279666	Alkene group C-H bend
17	3215.532	0.689305	Amides strong NH2 (two bands)
18	3356.835	0.7904052	Amides weak N-H (one band)
19	3,616	1.153365	alcohol O-H stretch
20	3798.235	1.010345	alcohol O-H stretch

Table 3.6: Fourier Transform Infrared (FTIR) characterization of Glucose from yam peel

Serial No	Frequency (cm-1)	Peak Area	Assigned functional Group
1	708.8468	0.3298843	Meta disubstituted aromatic
2	881.0667	0.3106069	Geminaldisubstituted alkene
3	1064.352	0.7379492	Alkoxy C-O
4	1229.854	0.5999861	Acid C- O (acyl, strong)
5	1396.559	0.4440224	Alkanes C-H bend
6	1624.1	0.8195098	Amides N-H bend Stronger than Amines
7	1783.406	0.4915262	Ketones group C=O
8	1863.412	0.4766571	Anhydrides group C=O
9	2000.229	0.3603461	Aromatics C-H bend (weak overtone)
10	2123.247	0.4286211	Acid C- O (acyl, strong)
11	22223.606	0.5377615	Acid C- O (acyl, strong)
12	2459.971	0.5180169	Acids O-H very broad, (overlap C-H stretch)
13	2742.079	0.376213	Aldehydes C-H weak
14	2952.291	0.5454173	Alkanes C-H Stretch
15	3133.967	0.7419297	Aromatics C-H Stretch

16	3301.147	0.8750531	Alkene group C-H Stretch
17	3441.368	0.8156928	Amides weak N-H (one band)
18	3680.039	1.364788	alcohol O-H stretch
19	3,806	1.08593	alcohol O-H stretch

CONCLUSION

The hydrolysis of ground yam peels with HCl and H_2SO_4 at different temperatures, time and concentrations gave a maximum glucose yield of 27.3% and 26.5% respectively at 70˚C, 180 minutes and 5% acid concentration. Also in enzymatic hydrolysis, a maximum glucose yield of 44.5% was obtained at 30˚C, pH 5 and 3 days. Furthermore, the functional groups present in the cocoyam peel glucose and the standard glucose was evaluated using FTIR. The FTIR results showed similarities in the functional groups present in both sugars as shown in Tables 3.5 and 3.6.

Finally, the results obtained in this study have shown the suitability of water yam peel for the production of fermentable sugar that can be fermented to synthesize ethanol (biofuel).

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The agrowaste used for this research is commonly and predominantly used substrate in our area of research and country. There is absolutely no conflict of interest between the authors. Also, the research was funded by Tertiary Education Fund (TETFund) of Nigeria Ministry of Education.

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