ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun982

Kinetic Analysis of Cellulolytic Enzyme-Catalyzed Hydrolysis of Cocoyam Peel Biomass for Bioethanol Synthesis

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Publication Date: 2026/07/04

Abstract: This study investigated the kinetics of enzymatic hydrolysis of cocoyam peel (*Xanthosoma sagittifolium*) as a lignocellulosic feedstock for bio-ethanol production. *Aspergillus niger*, isolated from rotten cassava tuber and maintained on potato dextrose agar (PDA) slants, was used as the enzyme source. A crude cellulase preparation derived from this isolate was employed to hydrolyze the cocoyam peel substrate. Physicochemical characterization of the cocoyam peel was performed using Fourier Transform Infrared Spectroscopy (FTIR) and proximate analysis, which revealed a high crude fiber content of 10.8%, confirming its suitability as a fermentable substrate. Process parameter optimization showed that ethanol yield was favored at lower temperatures, while elevated temperature and alkaline pH conditions were inhibitory to ethanol production. The optimal hydrolysis conditions were determined to be a temperature of 35 °C, a pH of 5.5, and a hydrolysis period of 5.7 days. Kinetic analysis of the hydrolysis process demonstrated conformity with the Michaelis–Menten model, yielding a maximum reaction rate (*V*max) of 23.81 g·dm^{-3}·day^{-1}, a Michaelis–Menten constant (*K*m) of 20.56 g·dm^{-3}, and a coefficient of determination (R^2) of 0.99, indicating an excellent fit of the model to the experimental data.

Keywords: *Michealis-Menten, Lignocellulosic, Enzymatic Hydrolysis.*

How to Cite: Polycarp Ikechukwu Nwabuokei; Doris Onwenna Ngozi; Onyekachukwu Andrew Oginenwa (2026) Kinetic Analysis of Cellulolytic Enzyme-Catalyzed Hydrolysis of Cocoyam Peel Biomass for Bioethanol Synthesis. *International Journal of Innovative Science and Research Technology*, 11(6), 2353-2360. https://doi.org/10.38124/ijisrt/26jun982

I. INTRODUCTION

The dwindling of fossil fuel reserves and the increase in global demand for energy, coupled with environmental concerns associated with the utilization of fossil fuels, have intensified the search for renewable and sustainable energy sources (Balat & Balat, 2009). Among the various renewable energy alternatives, bioethanol stands out as a promising liquid biofuels due to its renewability, biodegradability, and potential to reduce greenhouse gas emissions (Sánchez & Cardona, 2008). Over time, bioethanol production has relied heavily on food-based feedstocks such as maize, sugarcane, and cassava. However, the use of these feedstocks raises concerns regarding competition with food supplies, increased production costs, and land-use conflicts (Hamelinck et al., 2005). Consequently, attention has shifted towards the utilization of agricultural residues and agro-industrial wastes as low-cost, renewable, and environmentally friendly substrates for bioethanol production (Sun & Cheng, 2002).

Cocoyam (*Colocasia esculenta* and *Xanthosoma sagittifolium*) is an important root crop cultivated extensively in tropical and subtropical regions, particularly in West Africa, where it serves as a major source of carbohydrates for millions of people (Falade & Okafor, 2015). During the processing and consumption of cocoyam, substantial quantities of peels are generated as waste materials. These peels contain appreciable amounts of starch, cellulose, hemicellulose, and other polysaccharides that can be converted into fermentable sugars and subsequently into bioethanol (Ogbonna et al., 2001). The effective utilization of cocoyam peels for bioethanol production offers a dual benefit of waste management and renewable energy generation while reducing environmental pollution associated with improper disposal of agricultural residues (Sánchez & Cardona, 2008).

A critical stage in the conversion of lignocellulosic and starchy biomass into bioethanol is the hydrolysis process, during which complex carbohydrates are broken down into simple fermentable sugars (Mosier et al., 2005). Hydrolysis

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun982

may be achieved through chemical or enzymatic methods; however, enzymatic hydrolysis has gained significant preference because it operates under milder conditions, exhibits high substrate specificity, and minimizes the formation of undesirable inhibitory compounds that may interfere with subsequent fermentation processes (Taherzadeh & Karimi, 2007). Enzymes such as cellulases, hemicellulases, and amylases catalyze the degradation of cellulose, hemicellulose, and starch fractions present in cocoyam peels, thereby enhancing sugar recovery and ethanol yield (Lynd et al., 2002).

The efficiency of enzymatic hydrolysis is influenced by several factors, including enzyme concentration, substrate concentration, temperature, pH, reaction time, and the structural characteristics of the biomass substrate (Taherzadeh & Karimi, 2007). Understanding the kinetics of enzymatic hydrolysis is therefore essential for optimizing the conversion process and maximizing fermentable sugar production. Kinetic studies provide valuable information regarding reaction rates, enzyme-substrate interactions, substrate conversion efficiencies, and the effects of operating conditions on hydrolysis performance (Mosier et al., 2005). Furthermore, kinetic models can be employed to predict process behavior, facilitate reactor design, and support the scale-up of bioethanol production systems from laboratory to industrial levels (Sun & Cheng, 2002).

Therefore, this study focuses on the kinetic investigation of the enzymatic hydrolysis of cocoyam peels as a precursor step in bioethanol production. The study aims to evaluate the rate of sugar release under varying operational conditions and determine the kinetic parameters governing the hydrolysis process. The findings are expected to contribute to the development of efficient and economically viable bioethanol production technologies utilizing agricultural waste resources, thereby supporting sustainable energy development, environmental conservation, and the circular bioeconomy (Balat & Balat, 2009; Sánchez & Cardona, 2008).

II. MATERIAL AND METHODS

- *Sourcing of Raw Materials*

Cocoyam tubers were obtained from a daily market in Idumuje Ugboko, Aniocha North L.G.A of Delta State and the sample identified as *Xanthosoma sagittifolium* (cocoyam) in the Department of Botany, Dennis Osadebe University, Asaba.

- *Preparation of the Samples:*

The cocoyam corms were peeled to obtain the peels. The cocoyam peels were then washed and dried in an oven at 55 ^{0}C for 24 hours. The dried sample was then milled into fine flour using an electric grinding machine. It was then sieved and packaged in an air-tight polyethylene bags for analysis.

- *Characterization of the Cocoyam Peel Sample*

Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed on the cocoyam peel sample to identify and characterize the functional groups present in the material. The analysis was conducted using a Shimadzu Analytical FTIR-8400S spectrophotometer

- *Determination of the Proximate Composition of Cocoyam Peel*

Crude protein content was determined using the Kjeldahl method, while fats and oils were quantified via the Soxhlet extraction method. Lignin and ash contents were assessed by treating the peels with 72% w/w H_2SO_4 for 4 hours. The resulting suspension was filtered through a crucible, and the solid residue was dried at 105°C for 24 hours and recorded as W1. The dried residues were subsequently transferred to pre-weighed porcelain crucibles and subjected to combustion at 600°C for 6 hours. Upon cooling, the remaining mass was recorded as the ash content (W2), and the lignin content was derived from the difference W1 − W2 (Wrolstad et al., 2006).

Cellulose and hemicellulose concentrations were determined using the Kurschner-Hanack method (Wrolstad et al., 2006; Igbokwe et al., 2016), a gravimetric approach in which nitric acid and acetic acid are used to dissolve all other components of the cocoyam peel, leaving behind the acid-insoluble cellulose and hemicellulose fractions. One gram of each peel sample was weighed into separate round-bottom flasks, to which 15 mL of 80% acetic acid and 1.5 mL of concentrated nitric acid were added. The flasks were fitted with reflux condensers and heated using a heating mantle for 2 hours. Following heating, the solutions were filtered, and the residues were thoroughly washed and oven-dried at 105°C. The mass of the dried residues was recorded as the cellulose/hemicellulose content of the sample.

- *Identification of Microorganism (Aspergillus Niger (A. Niger))*

Aspergillus niger was isolated from rotten cassava tubers and maintained on Potato Dextrose Agar (PDA) slant at 4^{0}C using prescribed methods by (Wrolstad et al., 2006). The isolation was carried out by serially diluting 0.1g of rotten cassava tuber in 1ml of sterile water, followed by plating out 0.1ml of 10^{-4}dilution on PDA substrate in a Petri dish. The culture wa cultivated upside down for 3 days under 37 °C. Furthermore, the single colony was separated and inoculated on plate medium cultivated for another 3 days. Pure colony of *Aspergillus niger* was obtained and stored on PDA at 4^{0}C.

- *Enzymatic Hydrolysis using Aspergillus Niger*

The enzymatic hydrolysis of cocoyam peels was conducted under varying temperature, pH, and reaction time conditions to evaluate their effects on sugar production. A 250 cm³ conical flask containing 50 cm³ of a 5% inoculum suspension of *Aspergillus niger* was used for each experiment, with different dosages of cocoyam peel substrate added for hydrolysis. The reaction mixtures were incubated in a shaker incubator at an agitation rate of 300 rpm to ensure adequate mixing and enzyme–substrate interaction. At the end of each hydrolysis period, the mixtures were filtered to separate the hydrolysate from the residual solids. The soluble sugar content of the filtrate was determined using a

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun982

refractometer, while the reducing sugar concentration was quantified by the 3,5-dinitrosalicylic acid (DNS) method, which is widely employed for the determination of reducing sugars released during enzymatic hydrolysis (Kongkiattikajorn, 2012; Igbokwe et al., 2016). The results obtained were subsequently used to evaluate the efficiency of the hydrolysis process and the influence of the operating parameters on sugar yield.

➢ *The Kinetic Modeling of the Enzymatic Hydrolysis*

The kinetics model was based on the following theorems:

The rate of the reaction is given by the Equation (1)

$$r_A = \frac{dC_A}{dt} \quad (1)$$

Where C_A is the concentration of the limiting reactant, in this case the limiting reactant is the cellulose and hemicellulose content of the cocoyam peels and banana peels.

The differential, $\frac{dC_A}{dt}$ can be calculated by numerical method (Fogler, 1999).

The numerical differential formula is used when the data points in the independent variable are equally spaced, such as: $t - t = t - t = \Delta t$. The three-point differential formula was applied in this work. Typically, the tabulation of the concentration variation with time is as shown in Table 1.

The three-point differential formulae are presented in Equations (2) - (5):

Initial point: $\left(\frac{dC_A}{dt}\right)_{t0} = \frac{-3C_{Ao} + 4C_{A1} - C_{A2}}{2\Delta t}$ (2)

Interior points: $\left(\frac{dC_A}{dt}\right)_{t1} = \frac{1}{2\Delta t}\left[C_{A(i+1)} + C_{A(i-1)}\right]$ (3)

Table 1 Concentration Variation Against Time

Time (hrs)	t_o	t_1	t_2	t_3	t_4	t_5
C_A(g/dm^3)	C_{Ao}	C_{A1}	C_{A2}	C_{A3}	C_{A4}	C_{A5}

$$\left[\mathbf{e.g}\ \left(\frac{dC_A}{dt}\right)_{t3} = \frac{1}{2\Delta t}\left[C_{A(4)} + C_{A(2)}\right]\right] \quad (4)$$

Last point: $\left(\frac{dC_A}{dt}\right)_{t5} = \frac{1}{2\Delta t}\left[C_{A3} - 4C_{A4} + 3C_{A5}\right]$ (5)

Equations (2) - (5) were used to calculate the change in the reactant concentration with time ($\frac{dC_A}{dt}$).

- *The Michaelis-Menten Model*

The form of the Michaelis-Menten kinetic equation is given as in Equation (6) (Fogler, 1999):

$$r_A = \frac{V_{max}(S)}{K_m + (S)} \quad (6)$$

Where r_A = the rate of the enzymatic reaction;

V_{max} = the maximum rate of the reaction for a given total enzyme concentration;

K_m = the Michaelis - Menten constant;

S = the substrate concentration.

The linear form of the model is given in Equation (7)

$$\frac{1}{r_s} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}}\left(\frac{1}{s}\right) \quad (7)$$

The plot of - $\frac{1}{r_s}$ against $\left(\frac{1}{s}\right)$ gives a straight line where V_{max} and K_m can be calculated from the intercept and the slope respectively (Igbokwe et al., 2016).

III. RESULTS AND DISCUSSION

➢ *Characterization of the Cocoyam Peels*

- *Proximate Analysis of Cocoyam Peels*

The results from the proximate analysis of the cocoyam peels were presented in table 3.1. From the result it could be observed that the cocoyam peels has a lignin content of 0.002% which is negligible and in agreement as reported in literature (Debabandya et al., 2010; Parveen et al., 2012).

The cocoyam peel fibre was reported to be 10.8%, which is higher than the crude fibre content value of 1.9% reported for cocoyam tuber (Apata and Babalola, 2012).

The variation in fibre content value implies that cocoyam peels have greater fibre content than the cocoyam tuber itself. The fat content values of 0.72% and 6.2% were determined for cocoyam and banana peels, respectively. The values are consistent with the fat content values of 0.7% (Apata and Babalola, 2012) and 6% (Alula and Mebrahtom, 2014) for cocoyam and banana peels, respectively, reported in the literature.

Aash content values of 6.05% was recorded for cocoyam. This is consistent with the ash content value of 5.2% reported by Apata and Babalola, (2012). The cocoyam peels have a moisture content values of 6.4%.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun982

Table 2 Proximate Composition of the Cocoyam Peels (%)

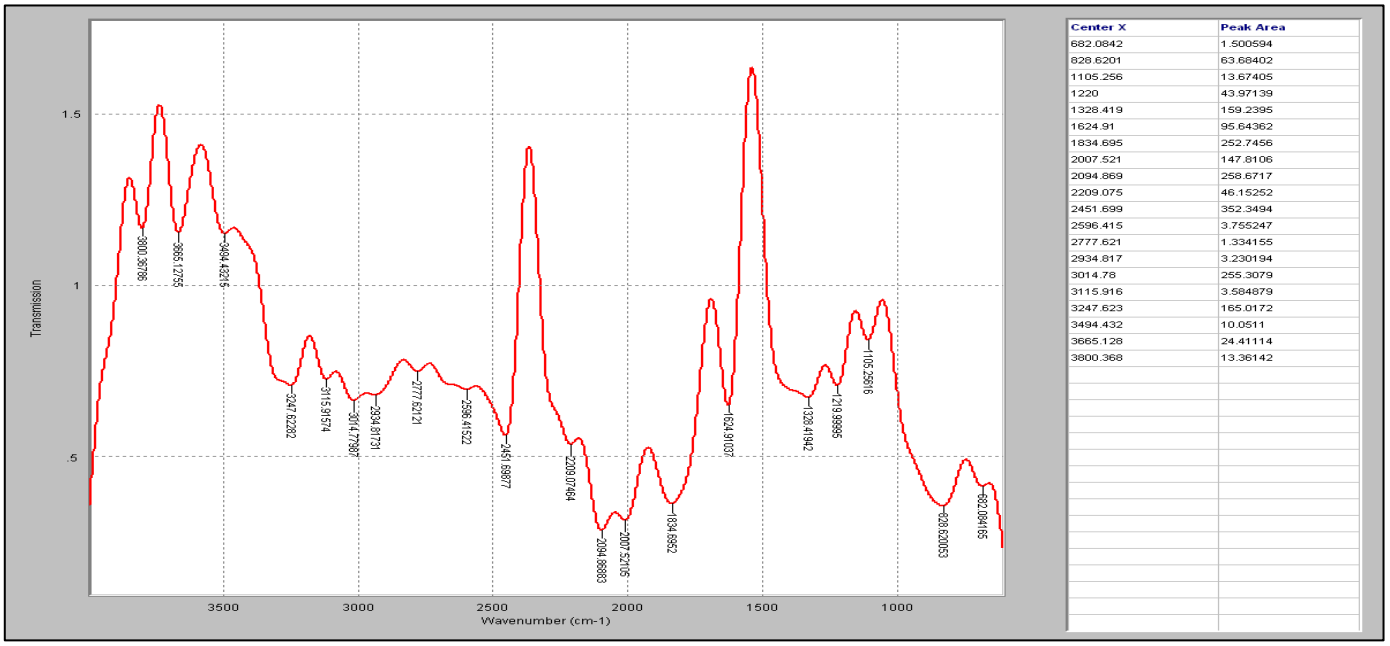
Parameters	Percentage composition (%)
Protein	6.40±0.10
CHOs	41.20±0.10
Fibre	10.80±0.10
Fats	0.72±0.10
Tannins	1.46±0.10
Moisture content	6.08±0.10
Sugar	12.01±1.0
Lignin	0.001 ± 0.002
Ash	6.05± 0.10

- *FTIR Studies of Cocoyam Peels*

The FTIR spectrum of the cocoyam peels is presented in Figure 1, while the corresponding functional group assignments are shown in Table 3.1. The spectrum was interpreted using standard absorption band assignments reported by Robert M. Silverstein et al. (1981). The analysis revealed the presence of several characteristic functional groups associated with lignocellulosic biomass.

A broad absorption band corresponding to hydroxyl (–OH) groups was observed, indicating the presence of alcohols, phenols, and carboxylic acids. These functional groups are characteristic constituents of cellulose, hemicellulose, and lignin, which are the major structural components of cocoyam peels. The abundance of hydroxyl groups suggests a high potential for enzymatic hydrolysis, as these groups contribute to the hydrophilic nature and reactivity of the biomass.

In addition, absorption peaks attributed to amines, amides, aldehydes, esters, ethers, alkenes, and di-substituted benzene rings were identified in the spectrum. The presence of these functional groups further confirms the complex organic composition of the biomass and indicates the existence of lignin-derived aromatic structures as well as carbohydrate-related compounds. These findings are consistent with the chemical characteristics of lignocellulosic materials commonly utilized as feedstocks for bioethanol production. The identified functional groups demonstrate that cocoyam peels possess the necessary chemical constituents for effective enzymatic degradation and subsequent fermentation into bioethanol.



Center X	Peak Area
682.0842	1.500594
828.6201	63.68402
1105.256	13.67405
1220	43.97139
1328.419	159.2395
1624.91	95.64362
1834.695	252.7456
2007.521	147.8106
2094.869	258.6717
2209.075	46.15252
2451.699	352.3494
2596.415	3.755247
2777.621	1.334155
2934.817	3.230194
3014.78	255.3079
3115.916	3.584879
3247.623	165.0172
3494.432	10.0511
3665.128	24.41114
3800.368	13.36142

Fig 1 FTIR Result of Cocoyam Peels

Table 3 Cocoyam Peels FTIR Interpretation Table

Wave number	Peak Intensity	Functional group
3665.128	Strong/weak	O-H (free) for Alcohols, Phenols
3494.432 3247.623	Sharp, broad	O-H or N-H (H- bonded) for Alcohols, Phenols, Amines, Amides
3115.916 3014.78	Medium, Strong	O-H for Acids, Carboxylics
2777.621	Medium	CHO for Aldehydes

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun982

1624.91	Medium	C=C stretch for Alkenes
1220	Strong	C-O Stretch for Acids, esters
1105.255	Strong	C-O for Ethers
828.6201	Strong	C-H bond for 1,4-di-substituted benzene

➢ *Effect of Temperature on Hydrolysis Process for Reducing Sugar Yield*

Varying different incubation temperatures with pH, reducing sugar yield from the cocoyam peel was investigated. This was achieved by varying temperatures 30°C, 35°C and 40°C with pH to monitor the reducing sugar yield. The plot of the effect of incubation temperature on reducing sugar yield is presented in Figure 2.

An inverse relationship was observed between temperature and reducing sugar yield. Yields declined as temperature increased, with the optimum recorded at approximately 35°C. Within the range of 30°C to 35°C, glucose yield rose progressively, before falling off at temperatures beyond this point.. This is due to enzyme denaturation. The effect of temperature on the reducing sugar yield was however significant. It was also observed at the different temperatures under study, that there was an increase in reducing sugar yield with pH up to pH 5.5, after which the yield decreases with pH. The maximum reducing sugar yield was recorded at the temperature of 35°C and pH 5.5. The maximum reducing sugar yield were 95.8mg/mol, 96.5mg/mol and 95.1mg/mol recorded against 30°C, 35°C and 40°C respectively.

The results of the study on the effect of incubation temperature on the reducing sugar yield from the peel implies that low temperature favours reducing sugar yield while high temperature and high pH are unfavourable for maximum reducing sugar yield. This is in agreement with the report of Itelima, et al. (2013).

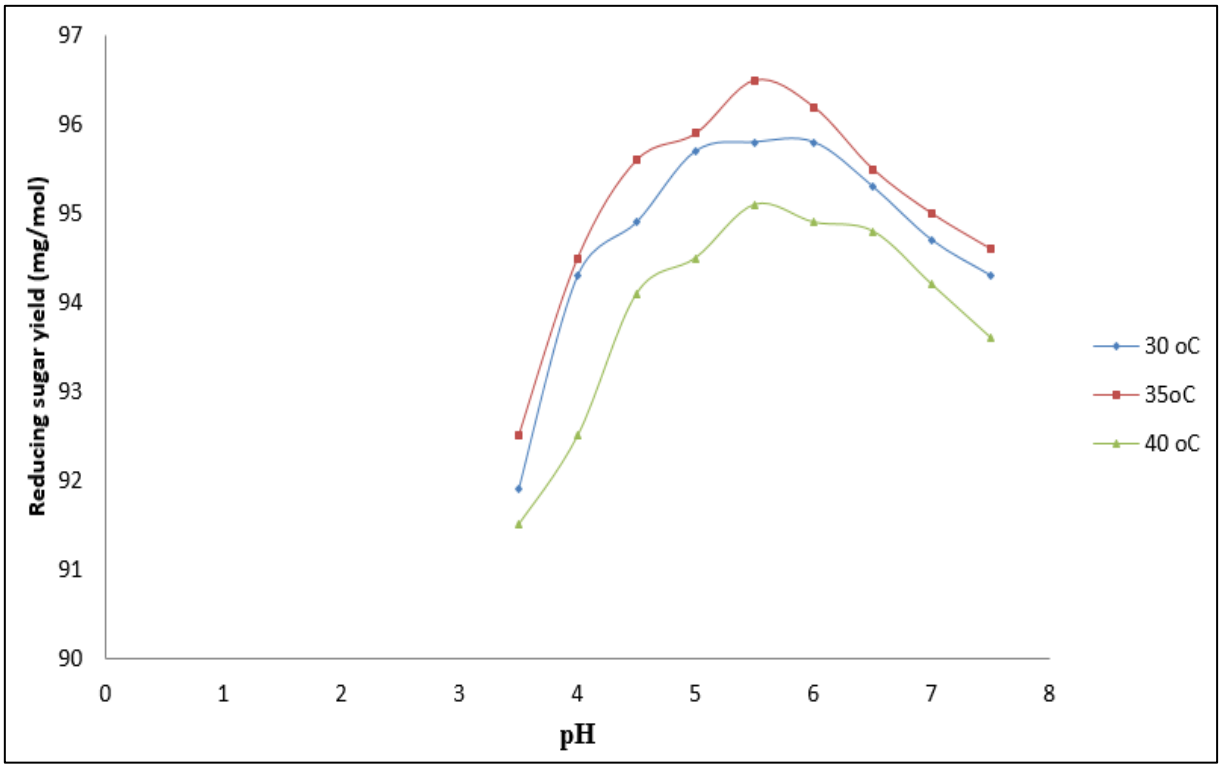


Fig 2 Effect of Incubation Temperature on Hydrolysis of Cocoyam Peels for Reducing Sugar Yield

➢ *Effect of Hydrolysis Time on the Yield of Reducing Sugar*

To determine effect of hydrolysis period/time on the yield of reducing sugar from the cocoyam peels, hydrolysis time of 3days, 5days and 7days was varied with respect to pH. The plot of the effect of hydrolysis time on reducing sugar yield is presented in Figure 3. The results show that reducing sugar yield increases with time up to day 5 after which a decrease in reducing sugar yield with time follows. This is in agreement with the report of Itelima et al. (2013). Optimum reducing sugar yield was observed at day 5 after which a decrease was observed. Reducing sugar yield also increased with pH up to PH 5.5 after which a reduction was recorded. This suggests that hydrolysis is dependent on the growth kinetics of the fungus. The maximum reducing sugar yield was recorded at pH 5.5 at the different fermentation period. Maximum reducing sugar yields of 93.6mg/mol, 95mg/mol and 94.2 mg/mol were recorded for the cocoyam peels against 3days, 5days and 7days, respectively.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun982

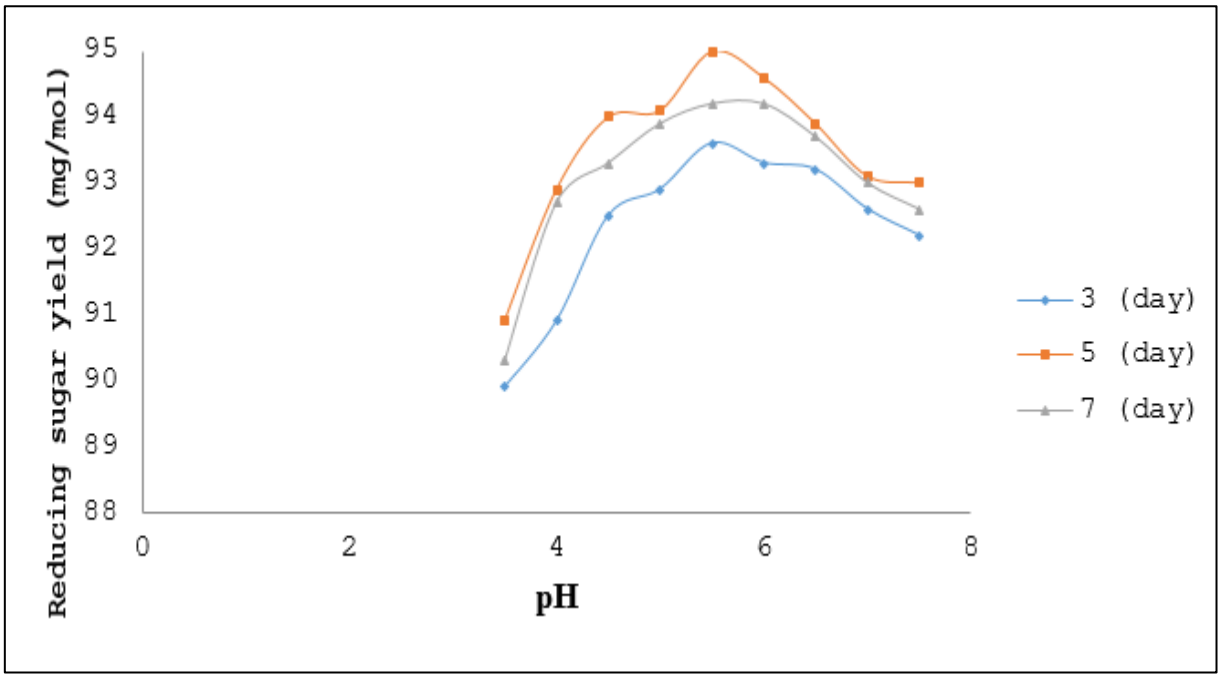


Fig 3 Effect of Time on Hydrolysis of Cocoyam Peels for Reducing Sugar Yield

➢ *Effect of pH on the Hydrolysis of Cocoyam Peels for Reducing Sugar Yield*

The plots of the effect of pH on reducing sugar yield are presented in Figure 4 The effect of pH on reducing sugar yield was studied by conducting experiment at different pH of 3.5, 5.5 and 7.5 with time using the cocoyam peels as the substrate.

Figure 4 shows that the pH of the solution affected the reducing sugar yield during hydrolysis**.** The maximum reducing sugar yield was observed at pH 5.5 at the different fermentation period after which a decrease was observed. It can be seen that reducing sugar yield increases with time up to 7 days after which there was no further increase in reducing sugar yield with time. This is in agreement with the report of Hiren et al. (2012): Akponah and Akpomie (2011). This informed that the pH of the solution has effects on the structure and activity of the enzyme. The maximum reducing sugar yield of 94.1mg/mol, 95.5mg/mol and 94.7mg/mol were recorded for cocoyam peels against pH of 3.5, 5.5 and 7.5, respectively.

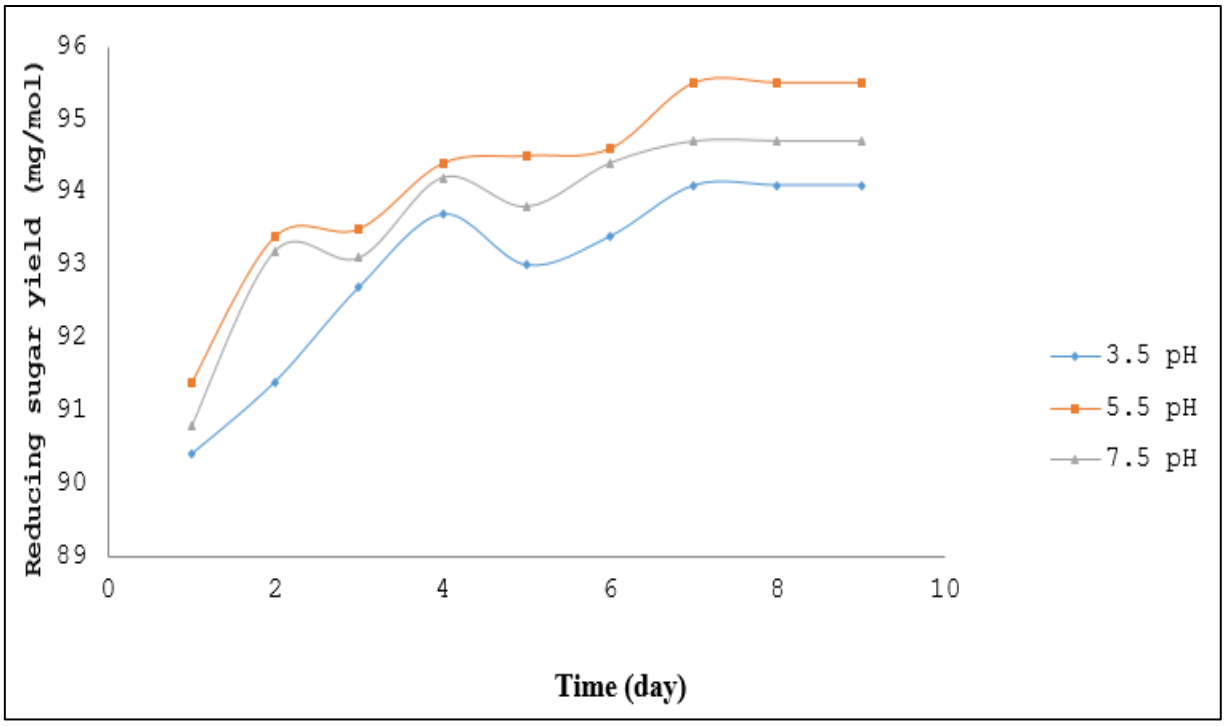


Fig 4 Effect of pH on Hydrolysis of Cocoyam Peels for Reducing Sugar Yield

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun982

➢ *Kinetics of the Enzymatic Hydrolysis for Cocoyam Peels*

The Michaelis-Menten Model was employed to model the kinetics of the enzymatic hydrolysis for cocoyam peels. This was carried out using the model Equation (8)

$$\frac{1}{r_A} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \left(\frac{1}{C_A}\right) \quad (8)$$

The kinetic parameters governing the enzymatic hydrolysis of cocoyam peels were determined using the linearized Michaelis–Menten plot presented in Figure 3.6. The coefficient of determination (R^2) obtained was close to unity, indicating a strong agreement between the experimental data and the Michaelis–Menten kinetic model. This suggests that the model adequately describes the enzymatic conversion of cocoyam peel cellulose into reducing sugars under the investigated conditions. As shown in Table 4, the reaction rate gradually decreased with increasing hydrolysis time, which can be attributed to substrate depletion, product inhibition, and possible enzyme deactivation. The hydrolysis rate approached a nearly constant value after the fifth day, indicating that the reaction was nearing equilibrium or that the available hydrolysable substrate had been substantially consumed. Using the calculated Michaelis–Menten kinetic parameters, namely the maximum reaction velocity (Vmax) and Michaelis constant (Km), the kinetic equation describing the enzymatic hydrolysis process was developed by substituting these parameters into Equation (2.6), resulting in the hydrolysis rate expression presented in Equation (9).

$$-r_A = \frac{23.81\, C_A}{20.36 + C_A} \quad (9)$$

Table 4 Michaelis-Menten Kinetics Parameters for Cocoyam Peel Hydrolysis

	K_m (g·dm^{-3})	V_{max} (g·dm^{-3}·day^{-1})	R^2
Cocoyam peels	20.36	23.81	0.99

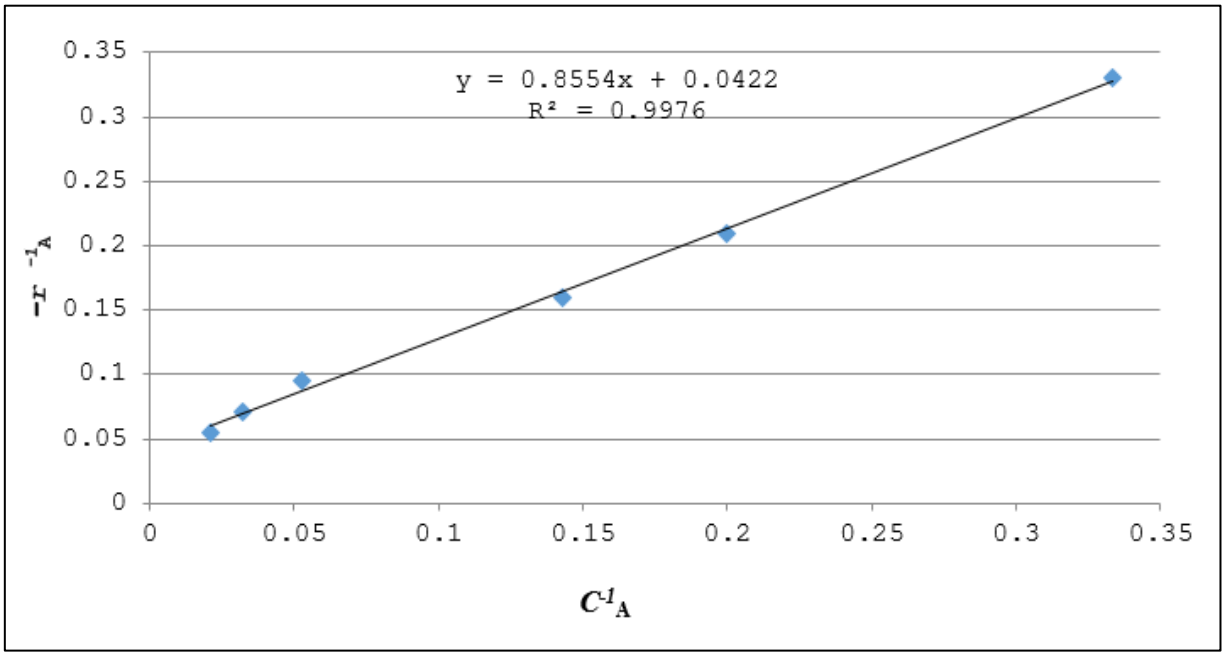


Fig 5 The Michaelis-Menten Kinetic Model for Enzymatic Hydrolysis of Cocoyam Peels.

Table 5 Variation in the Concentration of Cellulose with Time During Hydrolysis Process

Time(day)	Cellulose conc. (g/L) C_A	Rate of reaction $-r_A$	C^{-1}_A	$-r^{-1}_A$
0	51	17.5	0.02	0.06
1	35	14.5	0.03	0.07
2	22	11	0.05	0.09
3	13	7	0.08	0.14
4	8	4.5	0.13	0.22
5	4	3.5	0.25	0.29

IV. CONCLUSIONS

The kinetics of enzymatic hydrolysis of cocoyam peels for bioethanol production was investigated. Characterization of the raw material confirmed the presence of cellulose and hydroxyl (–OH) functional groups associated with alcohols and phenols, establishing cocoyam peels as a suitable lignocellulosic feedstock for bioethanol production.

Crude enzyme preparations from *Aspergillus niger* proved effective in hydrolyzing lignocellulosic materials,

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun982

releasing fermentable reducing sugars suitable for downstream ethanol production.

Reducing sugar and ethanol yields were significantly influenced by temperature, reaction time, pH, and substrate concentration. Yields improved with increasing substrate concentration under low-temperature, low-pH conditions. Conversely, elevated temperature and high pH were unfavorable for maximum product yields.

Kinetic analysis confirmed that the hydrolysis process conforms to the Michaelis–Menten model, providing a robust mathematical framework for characterizing and predicting the enzymatic reaction behaviour.

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