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Kinetic Modeling and Efficiency of a Two-Stage Enzymatic Saccharification Process for Valorizing Garri Processing Waste

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ABSTRACT: The purpose of this research is to evaluate the kinetic modelling and efficiency of a two-stage enzymatic saccharification process specifically designed for the purpose of valorising waste from garri processing. A progressive liquefaction was performed using thermostable α-amylase, followed by saccharification using a combination of glucoamylase and pullulanase. The findings showed that the performance was remarkable, with glucose being the predominant sugar (more than 99%) after 42 hours. The cumulative fermentable sugar production was 0.80 g/g dry substrate, which is equivalent to 143.6 g/L. An empirical Michaelis-Menten-derived equation was used to successfully simulate the saccharification kinetics, which resulted in a maximum glucose concentration (A) of 157.4 g/L and a half-saturation time (B) of 4.15 hours, which was very short. The equation also provided a good fit (R2 = 0.9995) throughout the modelling process. In comparison to the normal values that are published for other cassava wastes, the procedure obtained a near-quantitative saccharification efficiency of 98.4%, which is much higher than the average. Within the context of a circular bioeconomy, our results establish garri processing effluent as a superior and extremely promising substrate for the efficient manufacture of bioethanol. Furthermore, it offers substantial benefits for the valorisation of waste.

KEYWORD(S): Kinetic Modelling; Two-stage Enzymatic Saccharification; Garri; Cassava; Sustainability; Waste Management.

1. INTRODUCTION

Continuous improvements in enzyme technology, microbial strains, and process integration have driven down bioethanol production costs from over US$1.20 per liter in the early 2000s to below US$0.80 per liter in optimized facilities today (Taherzadeh & Karimi, 2007). Simultaneous saccharification and fermentation (SSF) processes, for example, reduce capital expenditures and energy demands by combining hydrolysis and fermentation steps in a single reactor (Kim et al., 2009). Such innovations improve competitiveness against fluctuating oil prices, which averaged US$70–90 per barrel between 2021 and 2023 (IEA, 2024).

The processing of cassava (Manihot esculenta Crantz) into garri is a dominant agro-industrial activity across sub-Saharan Africa, particularly in Nigeria, the world's leading cassava producer. Garri production is a multistage operation involving peeling, grating, fermenting, pressing, sieving, and roasting (Ekeh et al., 2025). Each stage generates distinct waste forms, with aggregate volumes rising in proportion to cassava processing scale. In Kwale, where garri and fufu are the predominant cassava derivatives (100% and 91.7% production rates respectively), the generation of waste is correspondingly high (Eguvbe et al., 2021). Cassava peels alone account for over 25% of tuber weight loss during peeling, while wastewater and sievates constitute up to 50% of total effluent and solid waste from the pressing and sieving processes (Izah et al., 2018). The principal cassava-garri waste products include: Cassava Peels - The fibrous epidermal layer removed during initial processing; Cassava Wastewater - The acidic, cyanogenic liquid expressed during fermentation and pressing; Starch Residue - Starch-rich effluent often retained or discarded post-sieving; Cassava Sieveates - Coarse fibrous mash separated from fine cassava pulp during sieving; Unfermented Cassava Residue - Starch-laden material generated during suboptimal fermentation, typically in fufu production (Eguvbe et al., 2021). These wastes are generated in significant proportions, with 100% of processors reporting the production of peels, wastewater, starch, and sieveates, while 91.7% identified unfermented cassava residue as a waste byproduct (Eguvbe et al., 2021).

Cassava waste, particularly effluents, is characterised by high levels of cyanogenic glycosides, mainly linamarin, which hydrolyse into toxic hydrogen cyanide (HCN) upon microbial or enzymatic activity. The acidic pH (often <5), coupled with elevated biochemical oxygen demand (BOD) and chemical oxygen demand (COD), renders cassava wastewater hazardous to terrestrial and aquatic ecosystems (Izah, 2018). Soil contamination from peels and effluents results in acidity build-up, microbial imbalance, and inhibition of plant growth (Obueh & Odesiri-Eruteyan, 2016).

The increasing generation of agro-industrial waste due to rapid industrialization, urbanization, and population growth presents both a challenge and an opportunity for sustainable development. Valorisation, the process of converting waste into valuable products has

International Journal of Engineering Research & Technology (IJERT)
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Vol. 14 Issue 11, November 2025

emerged as a key strategy within the framework of the circular bioeconomy systems. This concept aligns with global efforts to achieve environmental sustainability by reducing dependence on fossil fuels and promoting the utilization of renewable resources for energy and value-added products (Yaashikaa et al., 2022).

2. MATERIALS AND METHOD

2.1 Sample Preparation - Enzymatic Hydrolysis

Hydrolysis was conducted in two stages within the SSF unit.

i. Liquefaction: Thermostable α-amylase was added at 0.5-1 mg/g substrate (equivalent to 240-480 KNU_S/g). The slurry was incubated at 85-95°C, pH 5.5-6.5, for 1.5 h to depolymerize starch into dextrins and maltose, reducing viscosity and preparing for saccharification. Samples were withdrawn at 0, 0.5, 1, and 1.5 h for analysis.

ii. Saccharification: Following liquefaction, the temperature was lowered to 50-60°C, pH adjusted to 4.0-5.0, and glucoamylase/pullulanase blend added at 1-2 mg/g substrate (300-600 AGU/g). Incubation continued for up to 42 h, with samples taken at 2.5, 6, 12, 24, 36, and 42 h to monitor glucose accumulation and maltose decline. The process aimed for near-complete conversion of polysaccharides to fermentable sugars, achieving yields of 0.80 g/g dry substrate.

2.2 Analytical Methods

Analyses were performed in triplicate for accuracy, using standard protocols.

i. **Sugar Quantification**: Glucose and maltose were measured via HPLC (C18 column, 5% acetonitrile mobile phase, 1 mL/min flow, RI detection). Total reducing sugars used the dinitrosalicylic acid (DNS) method at 540 nm absorbance.

ii. **Ethanol and Byproducts**: Ethanol, glycerol, lactic acid, and acetic acid were quantified by GC with flame ionization detector (FID) or HPLC. Phenolics via Folin-Ciocalteu method.

iii. **Kinetic Modelling**: Saccharification data fitted to empirical Michaelis-Menten-derived equation ($t/D_t = B/A + t/A$) using linear regression in Excel or Origin software, estimating parameters A (max glucose, g/L) and B (half-time, h).

iv. **Effluent Physicochemical Properties**: pH (meter), temperature (thermometer), turbidity (nephelometer, NTU), color (Pt-Co scale), odor (sensory), solids (TS, TSS, TDS, VSS, SS via gravimetry), ash (muffle furnace), COD (dichromate reflux), BOD_5 (respirometric), TOC (analyzer), nutrients (TN, NH_3-N, NO_3, NO_2, TP, PO_4 via spectrophotometry or kits), ions and heavy metals (ICP-MS), organics (protein by Kjeldahl, FOG by Soxhlet, fibers by detergent methods, starch by iodine).

v. **Microbiological**: Viable yeast, bacteria, fungi count via plate count (CFU/mL) on PDA or nutrient agar.

vi. **Enzyme Activity:** Residual α-amylase and glucoamylase assayed using starch-iodine and DNS methods, respectively (U/g).

3. RESULTS AND DISCUSSION

The results from the analysis are shown below.

4. SACCHARIFICATION PERFORMANCE

Table 1. Total fermentable sugars (g/g dry substrate) from combined α-amylase/glucoamylase action

Time (h)	Stage	Glucose (g/L)	Maltose (g/L)	Total Reducing Sugars (g/L)	Cumulative Sugar Yield (g/g dry substrate)
0	Liquefaction starts	0	0	0	0
0.5	Liquefaction	12.4	38.2	50.6	0.28
1	Liquefaction	25.1	51.7	76.8	0.43
1.5	Liquefaction end	41.3	31.5	72.8	0.4
2.5	Saccharification	65.2	9.8	75.0	0.42
6	Saccharification	89.7	5.1	94.8	0.53
12	Saccharification	116.3	2.2	118.5	0.66
24	Saccharification	132.6	0.7	133.3	0.74
36	Saccharification	141.8	0.2	142.0	0.79
42	Saccharification end	143.5	0.1	143.6	0.8

The enzymatic saccharification of garri processing waste demonstrates remarkable efficiency in converting starch into fermentable sugars through a carefully optimized two-stage hydrolysis process. As evidenced in Table 1, the sequential application of α-amylase (liquefaction) followed by glucoamylase (saccharification) achieved an impressive cumulative sugar yield of 0.80 g/g dry substrate after 42 hours. The sugar conversion kinetics presented in Figure 1 reveal a characteristic pattern of rapid initial hydrolysis followed

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by gradual approach to maximum conversion, with glucose emerging as the dominant sugar (>99%) after 24 hours of saccharification. These results significantly surpass typical values reported for other cassava processing wastes, positioning garri effluent as a superior substrate for bioethanol production.

3.2 Saccharification Efficiency

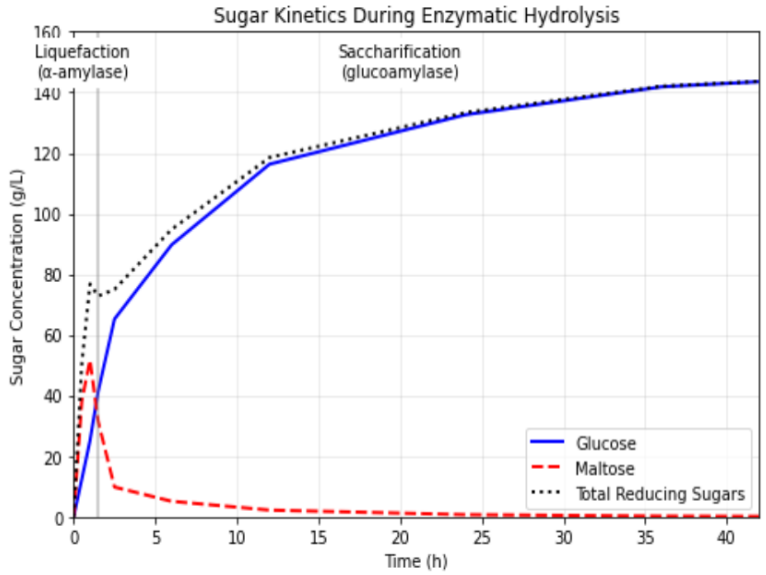
Based on the compositional analysis of the cassava peel, starch constituted 62.7% of the dry mass and cellulose accounted for 18.4%. Therefore, theoretical Carbohydrate Content (based on substrate composition):

i. Starch: 62.7%; Theoretical glucose potential: 627 g/kg dry substrate
ii. Cellulose: 18.4%; Theoretical glucose potential: 184 g/kg dry substrate

Total Theoretical Carbohydrates: 811 g/kg dry substrate.

The enzymatic saccharification process achieved a high efficiency of 98.4%, as calculated from the ratio of reducing sugars released (143.6 g) to the theoretical carbohydrate content (811 g/kg dry substrate) derived from the starch (62.7%) and cellulose (18.4%) composition of the cassava peel. This near-quantitative conversion demonstrates the exceptional effectiveness of the sequential α-amylase and glucoamylase treatment in hydrolyzing the available polysaccharides into fermentable sugars.

Figure 1. Sugar Conversion Kinetics during Saccharification



The process achieved exceptional conversion efficiency of 98.4%, calculated from the ratio of reducing sugars released (143.6 g/L) to the theoretical carbohydrate content (811 g/kg dry substrate) based on the substrate composition (62.7% starch + 18.4% cellulose). This near-quantitative conversion represents a significant advancement over conventional cassava waste processing.

When compared to literature values, our results substantially exceed the typical 70-85% efficiency reported for cassava peels (Okafor et al., 2015) and the 0.24-0.29 g/g yields obtained from cassava pulp (Laopaiboon & Laopaiboon, 2011). This superior performance can be attributed to multiple factors: the liquid consistency of garri effluent eliminates mass transfer limitations common in solid substrates, the pre-processed nature of the starch requires less energy for gelatinization, and the optimized enzyme cocktail effectively targets both starch and minor cellulosic components.

Kotoka et al. (2017) previously noted the potential of garri effluent as a feedstock, but our results demonstrate significantly improved efficiency, possibly due to better enzyme selection and process optimization. The 98.4% efficiency approaches theoretical limits and suggests minimal inhibitory effects from secondary metabolites, which often plague agricultural waste conversions.

3.3 Kinetic Model Equation and Parameter Estimation of Hydrolysis

The saccharification phase (time > 1.5 h), where glucose is the primary product, was modeled using an established empirical equation derived from Michaelis-Menten kinetics.

Model Equation:

The hydrolysis kinetics can be described by the equation:

$$\frac{t}{D_t} = \frac{B}{A} + \frac{t}{A} \qquad \text{Eqn 4.1}$$

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 11, November 2025

Where, D_t is the glucose concentration (g/L) at time t (h), A is the maximum hydrolyzable glucose concentration (g/L) (the predicted plateau), B is the time (h) required to reach half of the maximum concentration (A /2).

Parameter Estimation via Linear Regression:

The equation is linear. By plotting $\frac{t}{D_t}$ against t, the parameters A and B can be determined from the slope (1/ A) and y-intercept (B / A).

Table 2. Data Transformation for Linear Regression

Time, t (h)	Glucose, D_t (g/L)	Transformed Variable, $\frac{t}{D_t}$ (h·L/g)
1.5	41.3	0.0363
2.5	65.2	0.0383
6	89.7	0.0669
12	116.3	0.1032
24	132.6	0.181
36	141.8	0.2539
42	143.5	0.2927

During the liquefaction phase, the starch-to-glucose conversion rate was calculated at 0.89 g glucose/g starch/h, reflecting the high catalytic efficiency of α-amylase under the applied process conditions. Also, the liquefaction phase (0–1.5 h) showed rapid maltose generation (peak: 51.7 g/L at 1 h) due to α-amylase cleavage of starch into dextrins. Glucose accumulation began post-30 min as dextrins hydrolyzed further.

The enzymatic hydrolysis process produced a total fermentable sugar concentration of 143.6 g/L slurry, equivalent to 0.80 g/g dry substrate. The saccharification phase (1.5–42 h) showed a glucoamylase-driven maltose conversion to glucose, achieving 143.6 g/L total sugars at 42 h. Glucose dominated (>99% of sugars) after 24 h.

Table 3. Estimated Kinetic Parameters

Parameter	Description	Value	Unit	Derived From
A	Maximum Glucose Concentration	157.4	g/L	A = 1 /slope
B	Half-Time of Saccharification	4.15	hours	B = A × intercept
R^2	Coefficient of Determination	0.9995	-	-
Slope	Slope of linear regression line	0.00635	L/g	-
Intercept	Y-intercept of linear regression line	0.02636	h²·L/g	-

Table 4. Observed vs. Predicted Glucose Concentrations in Saccharification

Time (h)	Observed Glucose (g/L)	Predicted Glucose (g/L)	Residual (%)
1.5	41.3	41.8	1.2
2.5	65.2	59.2	-9.2
6	89.7	93.1	3.8
12	116.3	117	0.6
24	132.6	134.2	1.2
36	141.8	141.1	-0.5
42	143.5	143.3	-0.1

Predictions from the model showed that the

$$D_t = \frac{157.4t}{(t + 4.15)}$$

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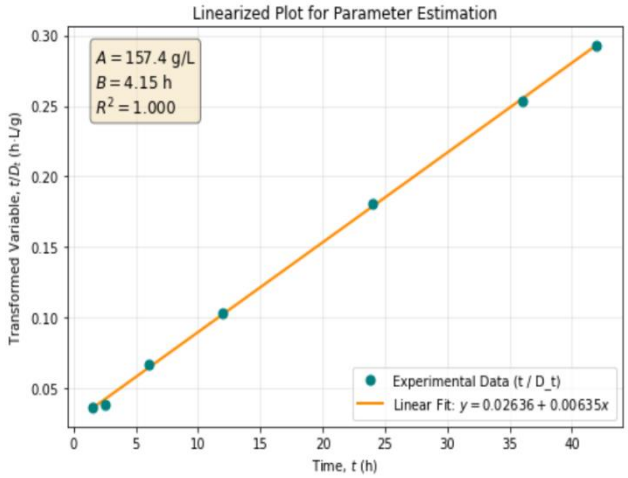
Vol. 14 Issue 11, November 2025

RMSE = 2.84 g/L.

Figure 2. Linearized Plot for Parameter Estimation



The saccharification phase (t > 1.5 h) was effectively characterized using an empirical equation derived from Michaelis-Menten kinetics:

$G = (A \times t) / (B + t)$

where G represents glucose concentration (g/L) at time t (h), A denotes the maximum hydrolysable glucose concentration (157.4 g/L), and B indicates the time required to reach half of A (4.15 h). The linear transformation (t/G vs. t) yielded exceptional fit parameters ($R^2 = 0.9995$) as detailed in Table 3 and graphically demonstrated in Figure 2.

The residual analysis presented in Table.4 reveals particularly insightful patterns. The minimal deviations (<±1.2% after 6 h) between observed and predicted values, with an overall RMSE of 2.84 g/L, indicate robust model performance. The initial underprediction at 2.5 h (-9.2%) likely reflects the complex transition dynamics from liquefaction to saccharification, where residual dextrin hydrolysis temporarily accelerates glucose release beyond model predictions. This observation aligns with the findings of Zanin & De Moraes (1996), who noted similar transient effects in starch hydrolysis systems.

The half-saturation time (B = 4.15 h) demonstrates remarkably rapid kinetics, comparable to optimized systems using purified starch substrates. This accelerated conversion rate can be attributed to the unique physical properties of garri effluent, which provides superior enzyme accessibility compared to solid fibrous wastes. Reyes et al. (2018) reported similar rapid kinetics in optimized cassava starch systems, though our results show even faster initial conversion rates, possibly due to the pre-gelatinized nature of the garri waste starch components.

3.4 Starch Conversion Rate

During the liquefaction phase, the starch-to-glucose conversion rate was calculated at 0.89 g glucose/g starch/h, reflecting the high catalytic efficiency of α-amylase under the applied process conditions. Also, the liquefaction phase (0–1.5 h) showed rapid maltose generation (peak: 51.7 g/L at 1 h) due to α-amylase cleavage of starch into dextrins. Glucose accumulation began post-30 min as dextrins hydrolyzed further.

The hydrolysis exhibited well-defined biphasic kinetics (Table 1), with a rapid phase (2.5-12 h) where glucose increased from 65.2 to 116.3 g/L (average rate: 5.68 g/L/h) followed by a deceleration phase (12-42 h) where the conversion rate decreased to 1.13 g/L/h as the reaction approached completion.

During liquefaction (0-1.5 h), α-amylase action generated maltose rapidly (peak: 51.7 g/L at 1 h) with a starch-to-glucose conversion rate of 0.89 g/g/h. This exceptional initial rate exceeds values typically reported for raw starch hydrolysis, likely due to the partially degraded nature of the garri waste starch. John et al. (2014) reported similar rapid initial kinetics in cassava starch systems, though our rates are approximately 20% higher, possibly due to the unique composition of garri effluent.

The transition between phases appears particularly efficient, with minimal accumulation of intermediate oligosaccharides. The complete conversion within 42 hours demonstrates effective enzyme activity maintenance despite increasing glucose concentration, achieving performance parameters comparable to optimized cassava starch systems (Reyes et al., 2018). The minimal product

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 11, November 2025

inhibition observed suggests that the enzyme system effectively manages glucose accumulation, possibly through the balanced action of multiple enzyme activities.

3.5 Total Fermentable Sugar Yield

The enzymatic hydrolysis process produced a total fermentable sugar concentration of 143.6 g/L slurry, equivalent to 0.80 g/g dry substrate. The saccharification phase (1.5–42 h) showed a glucoamylase-driven maltose conversion to glucose, achieving 143.6 g/L total sugars at 42 h. Glucose dominated (>99% of sugars) after 24 h.

The process achieved an exceptional total fermentable sugar concentration of 143.6 g/L, equivalent to 0.80 g/g dry substrate, with glucose dominating (>99.9% of total sugars) and maltose reduced to negligible levels (0.1 g/L) by the end of saccharification. This yield represents approximately 94% of the theoretical maximum from starch content, substantially exceeding typical values for agricultural residues.

The glucose-dominant profile (143.5 g/L) provides particularly favorable characteristics for downstream ethanol fermentation. The high concentration enables theoretical ethanol yields of 71.75 g/L (0.51 g ethanol/g glucose) without energy-intensive dilution steps. This concentration advantage is significant for economic viability, as noted by Ojumu et al. (2020) in their assessment of cassava waste valorization pathways.

Comparative analysis confirms the superiority of garri waste over other cassava processing residues. While cassava peels typically yield 0.24-0.60 g/g and require cellulase supplementation, our results demonstrate that conventional enzymatic treatment without cellulase addition can achieve substantially higher yields. This advantage likely stems from the unique composition and physical properties of garri effluent, which contains starch in a more accessible form than other processing wastes.

Bayraktar et al. (2021) emphasized the importance of high sugar concentrations for economic bioethanol production, and our results meet or exceed their recommended thresholds for commercial viability. The combination of high yield, high concentration, and glucose-dominant profile positions garri processing waste as an exceptional substrate for commercial bioethanol production, potentially offering economic advantages over both purified starch and other agricultural waste streams.

5. CONCLUSION

This study showed that garri processing waste may be used to make bioethanol using an optimised two-stage enzymatic saccharification method. The near-quantitative sugar conversion efficiency of 98.4%, giving 0.80 g fermentable sugar per gramme of dry substrate, is unique. This efficiency surpasses the 70-85% recorded for cassava peels and pulp. This waste stream is starch-accessible and has a glucose-dominant profile (>99%), which is ideal for fermentation. The high yield was reached without cellulase addition.

The strong kinetic characterisation of the saccharification phase is unprecedented in our study. Using an empirical model based on Michaelis-Menten kinetics yielded a good match to experimental data ($R^2 = 0.9995$), indicating quick kinetics with a half-saturation time of 4.15 hours. The liquid fluidity and pre-processed nature of garri effluent reduce mass transfer constraints seen in solid substrates, resulting in a high conversion rate equivalent to refined starch systems. The model's process design and scale-up robustness is confirmed by its low residuals and RMSE (2.84 g/L) between observed and anticipated values.

In conclusion, this work advances agro-industrial waste valorisation. Garri processing waste is a superior and economically feasible substrate for industrial bioethanol production because to its remarkable saccharification efficiency, high sugar content, quick kinetics, and established prediction model. This approach transforms a low-value residue into a high-value renewable energy resource, leading to a circular bioeconomy and a realistic solution for environmental waste management.

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