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Bioethanol Production from Garri Processing Waste Via Simultaneous Saccharification and Fermentation: Performance and Inhibition Analysis

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Abstract— This study investigates the feasibility of producing bioethanol from garri processing waste via Simultaneous Saccharification and Fermentation (SSF). A pilot-scale SSF bioreactor was used to process cassava peel hydrolysate under controlled conditions. The process yielded an ethanol concentration of 62.3 g/L, with a volumetric productivity of 1.48 g/L·h and a process yield of 0.46 g/g sugar consumed. Comprehensive effluent characterization revealed significant residual starch (35.82%) and inhibitory levels of heavy metals, particularly chromium (19.81 mg/L), which likely contributed to incomplete fermentation and reduced yeast viability. The study highlights the potential of garri waste as a viable feedstock for bioethanol while identifying key inhibition factors that must be addressed for process optimization and environmental sustainability.

Keywords— Simultaneous Saccharification And Fermentation (SSF), Agricultural Waste, Fermentation Inhibition, Bioethanol Production, Sustainability.

I. INTRODUCTION

Global bioethanol demand has risen sharply over the past two decades, driven by policy mandates, market incentives, and growing concerns over energy security and climate change. In 2000, global ethanol production stood at approximately 17 billion litres; by 2023 it surpassed 140 billion litres, underscoring a more than eightfold increase [1].

This upward trajectory reflects both supply-side advances such as the development of high-yield feedstocks and improved bioconversion technologies and demand-side stimuli, including blending requirements, carbon pricing mechanisms, and consumer preferences for cleaner fuels [2, 3].

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 [4, 5].

Simultaneous saccharification and fermentation (SSF) processes, for example, reduce capital expenditures and energy demands by combining hydrolysis and fermentation steps in a single reactor [6].

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Such innovations improve competitiveness against fluctuating oil prices, which averaged US$70–90 per barrel between 2021 and 2023 [1].

First-generation feedstocks compete with food crops, potentially raising food prices and driving land-use change [7]. Water use, agrochemical runoff, and biodiversity loss are additional environmental risks [8].

As a result, policy frameworks increasingly emphasize advanced biofuels those derived from non-food feedstocks or waste materials and incorporate robust sustainability criteria, such as the EU's high-ILUC (indirect land-use change) risk feedstock restrictions [3].

Looking ahead, second- and third-generation bioethanol technologies including cellulosic ethanol and algae-derived fuels are essential for long-term sustainability and deeper decarbonization [9].

Agro-industrial residues, particularly those from cassava-based industries such as garri processing, offer untapped potential for conversion into biofuels, chemicals, and other useful materials.

Cassava peels, starch residues, and garri processing effluents are commonly underutilized or improperly disposed of, leading to environmental hazards such as groundwater contamination, air pollution from open burning, and greenhouse gas emissions from uncontrolled decomposition.

Rather than being discarded, these wastes can serve as critical feedstocks in bioethanol production systems, contributing to waste reduction, energy generation, and economic development [10, 11].

II. MATERIALS AND METHODS

A. SSF Unit Fabrication

A pilot-scale SSF bioreactor was custom-fabricated from stainless steel (50 L capacity) with an integrated 3 phase gear motor for agitation, temperature control (heating/cooling system maintaining 30-95°C), pH and temperature probe for real-time monitoring, and sampling ports for time-course analysis.

This setup allowed simultaneous enzyme action and fermentation under controlled anaerobic conditions, minimizing contamination and enabling efficient integration of hydrolysis and ethanol production steps.

Simultaneous saccharification and fermentation (SSF) was initiated post-liquefaction by adding activated S. cerevisiae (10^6-10^8 CFU/mL inoculum, pre-cultured in YPD at 30°C for 24 h) along with glucoamylase. The mixture was supplemented with yeast extract (2 g/L) and incubated at 30-35°C, pH 4.5-5.5, under anaerobic conditions (sealed with parafilm) for 42 h with agitation at 150 rpm.

Ethanol production was monitored, with final effluent collected for comprehensive characterization. This integrated approach mitigated glucose inhibition on enzymes by concurrent sugar consumption.

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Figure 01: *Fabricated Simultaneous Saccharification and fermentation Skid Unit*

B. Analytical Methods

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

a. 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.
b. 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.
c. Effluent Physicochemical Properties: pH (meter), temperature (thermometer), turbidity (nephelometer, NTU), colour (Pt-Co scale), odour (sensory), solids (TS, TSS, TDS, VSS, SS via gravimetry), ash (muffle furnace), COD (dichromate reflux), BOD_5 (respirometric), TOC (analyser), 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, fibres by detergent methods, starch by iodine).

III. RESULTS AND DISCUSSION

A. Fermentation Performance

Table 4.5: *Physicochemical and Microbiological Properties of SSF Effluent from Cassava Peel Bioethanol Production*

Parameter	Value	Unit

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Physical Parameters		
Temperature	26.7	°C
pH	6.68	-
Colour	25.4 (Gray)	Pt-Co
Odor	Musty-Alcoholic	-
Turbidity	53.84	NTU
Solid Fraction		
Total Solids (TS)	2,510.63	mg/L
Total Suspended Solids (TSS)	350.52	mg/L
Total Dissolved Solids (TDS)	2,160.11	mg/L
Volatile Suspended Solids (VSS)	284.91	mg/L
Settleable Solids (SS)	68.74	mL/L
Ash Content	0.84	%
Organic Load & Nutrients		
Chemical Oxygen Demand (COD)	860.82	mg/L
Biochemical Oxygen Demand (BOD_5)	270.14	mg/L
Total Organic Carbon (TOC)	158.46	mg/L
Total Nitrogen (T.N)	75.96	mg/L
Ammonia Nitrogen (NH_3-N)	58.45	mg/L
Nitrate (NO_3)	36.17	mg/L
Nitrite (NO_2)	3.42	mg/L
Total Phosphorus (TP)	18.94	mg/L
Sulphite (SO_3)	1.63	mg/L
Hydrogen Sulphide (H_2S)	0.45	mg/L
Phosphate (PO_4^{3-})	27.31	mg/L
Protein Content	0.48	%
Fats, Oils, and Greases (FOG)	0.07	%
Neutral Detergent Fiber (NDF)	2.45	%
Acid Detergent Fiber (ADF)	1.76	%
Residual Starch	35.82	%
Hemicellulose	19.53	%
Major Ions & Elements		
Potassium (K)	42.39183	mg/L
Sulphate (SO_4^{2-})	420.13	mg/L
Chloride (Cl^-)	170.98	mg/L
Sodium (Na)	2.17462	mg/L
Calcium (Ca)	10.9847	mg/L

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Magnesium (Mg)	14.31613	mg/L
Heavy Metals		
Lead (Pb)	0.93872	mg/L
Cadmium (Cd)	0.01461	mg/L
Chromium (Cr)	19.80583	mg/L
Nickel (Ni)	0.13514	mg/L
Manganese (Mn)	3.1547	mg/L
Iron (Fe)	7.98037	mg/L
Copper (Cu)	5.21658	mg/L
Zinc (Zn)	8.65101	mg/L
Specific Process Analytes		
Ethanol Concentration	62.3	g/L
Residual Glucose	8.2	g/L
Glycerol	9.8	g/L
Lactic Acid	0.52	g/L
Acetic Acid	0.18	g/L
Phenolics	0.36	mg/L
Biological Load		
Viable Yeast Count	4.46x102	CFU/mL
Total Heterotrophic Bacteria Count (THBC)	3.78x103	CFU/mL
Total Heterotrophic Fungi Count (THFC)	9.31x103	CFU/mL
Residual Enzyme Activity		
Residual Glucoamylase Activity	185.2	U/g
Residual α-Amylase Activity	312.4	U/g

The fermentation performance in the simultaneous saccharification and fermentation (SSF) of garri processing waste, a starch-laden effluent from cassava-based garri production, resulted in an ethanol concentration of 62.3 g/L, representing a substantial yield that underscores the substrate's viability for bioethanol valorisation. This concentration, achieved with residual glucose at 8.2 g/L, indicates efficient carbohydrate utilization by Saccharomyces cerevisiae or similar yeasts, as the low residual sugars suggest minimal substrate inhibition and near-complete fermentation within the process timeframe. Comparable studies on cassava peel wastes have reported ethanol yields ranging from 16.42 g/L in optimized SSF without pre-hydrolysis to higher values like 103.74 g/L when combining stem and peel hydrolysates, highlighting how substrate composition and pretreatment influence outcomes [12, 13]. The presence of byproducts such as glycerol (9.8 g/L), lactic acid (0.52 g/L), and acetic acid (0.18 g/L) reflects typical yeast metabolic diversions under anaerobic conditions, where glycerol serves as an osmo-protectant and organic acids arise from minor heterofermentative pathways or contamination; these levels are lower than those in less controlled fermentations, where acetic acid can exceed 1

g/L and inhibit yeast [14]. Phenolics at 0.36 mg/L are notably low, minimizing toxicity, as cassava wastes often contain higher inhibitory compounds post-pretreatment, which can reduce yields by 20–30% if not mitigated [15].

Physicochemical properties of the SSF effluent, including a pH of 6.68 and temperature of 26.7°C, align with near-optimal ranges for yeast activity (pH 4–6, 25–35°C), facilitating sustained fermentation without pH adjustments that could increase costs. The effluent's colour (25.4 Pt-Co, grey) and musty-alcoholic odour are characteristic of fermented cassava residues, stemming from Maillard reactions and volatile compounds, while turbidity (53.84 NTU) and total solids (2,510.63 mg/L) comprising suspended (350.52 mg/L), dissolved (2,160.11 mg/L), and volatile suspended solids (284.91 mg/L) indicate a slurry-like consistency amenable to downstream separation. These values compare favourably to cassava pulp effluents, where total solids often exceed 5,000 mg/L, complicating handling [16]. Organic load metrics, such as COD (860.82 mg/L), BOD_5 (270.14 mg/L), and TOC (158.46 mg/L), suggest moderate pollution potential, with the BOD/COD ratio (~0.31) implying good biodegradability for wastewater treatment; this is consistent with bioethanol effluents from lignocellulosic sources, where COD reductions of 50–70% are achievable via anaerobic digestion [17]. Nutrient contents, including total nitrogen (75.96 mg/L), ammonia (58.45 mg/L), nitrate (36.17 mg/L), nitrite (3.42 mg/L), and phosphorus (18.94 mg/L), along with phosphate (27.31 mg/L), position the effluent as a potential biofertilizer, echoing valorisation strategies where cassava waste nutrients enhance soil fertility without eutrophication risks at these concentrations [18].

Major ions and elements in the effluent, such as potassium (42.39 mg/L), sulphate (420.13 mg/L), chloride (170.98 mg/L), sodium (2.17 mg/L), calcium (10.98 mg/L), and magnesium (14.32 mg/L), reflect the mineral profile of cassava, with sulphate and chloride levels potentially originating from pretreatment chemicals or soil residues. These concentrations are within safe limits for discharge but require monitoring, as elevated ions can affect microbial ecosystems in treatment ponds [14]. Heavy metal profiles lead (0.94 mg/L), cadmium (0.01 mg/L), chromium (19.81 mg/L), nickel (0.14 mg/L), manganese (3.15 mg/L), iron (7.98 mg/L), copper (5.22 mg/L), and zinc (8.65 mg/L) raise environmental concerns, particularly chromium and zinc, which exceed typical thresholds for agricultural reuse (e.g., WHO limits <0.05 mg/L for chromium); however, these are comparable to untreated cassava effluents, where bioaccumulation from varietal differences or processing contributes, and bioremediation using yeasts like Pichia kudriavzevii can reduce them by 40–60% [18, 15].

Microbiological properties, with viable yeast counts at 4.46×10^2 CFU/mL, total heterotrophic bacteria at 3.78×10^3 CFU/mL, and fungi at 9.31×10^3 CFU/mL, demonstrate a balanced microbial community dominated by fermentative organisms, minimizing spoilage risks. These counts are lower than in open fermentations of cassava peels, where bacterial loads can reach 10^6 CFU/mL and lead to acid buildup, but align with controlled SSF where yeast predominance ensures ethanol selectivity [14, 17]. Residual enzyme activities glucoamylase (185.2 U/g) and α-amylase (312.4 U/g) indicate incomplete deactivation, offering opportunities for enzyme recovery and cost reduction in scaled processes, as enzymes constitute 20–30% of bioethanol production expenses [16].

Organic components like protein (0.48%), fats/oils/greases (0.07%), neutral detergent fibre (2.45%), acid detergent fibre (1.76%), residual starch (35.82%), and hemicellulose (19.53%) reveal untapped potential in the effluent solids, where residual starch could support secondary fermentations or animal feed, similar to cassava pulp valorisation yielding 50–60% starch recovery [15], Sulphite (1.63 mg/L) and hydrogen sulphide (0.45 mg/L) are minimal, avoiding odour issues common in sulphur-rich wastes. Overall, these results affirm garri waste's efficacy for bioethanol, with efficiencies rivalling cassava peels (yields up to 57.2%) and stems (103.74 g/L), but emphasize integrated treatment for effluents to enhance sustainability in cassava-dependent regions [13, 12].

a. Ethanol Titre, Productivity & Yield on Sugar

Table 4.6: *Summary of Ethanol Production Kinetics and Process Performance Metrics from SSF*

Parameter	Symbol	Value	Unit	Calculation Basis & Remarks
Initial Total Sugar Concentration	S_0	143.6	g/L	Total reducing sugars from hydrolysis at t=0 h of SSF (Table 4.1).
Final Ethanol Titre	P	62.3	g/L	Measured concentration in SSF effluent. Equates to ~7.9% v/v.
Final Residual Glucose	S	8.2	g/L	Indicates incomplete sugar consumption.
Sugar Consumed	ΔS	135.4	g/L	S_0 - S = 143.6 - 8.2. Represents total fermentable sugars utilized.
Theoretical Ethanol Yield	Y_{theo}	78.9	g/L	Calculated as ΔS × 0.511 (g ethanol / g glucose consumed) + (Glycerol × 0.38).
Process Ethanol Yield	Y_p/s	0.46	g/g	P / ΔS = 62.3 / 135.4. Mass of ethanol produced per mass of sugar consumed.
Theoretical Yield Coefficient	-	90.2	%	(Y_p/s / 0.511) × 100 = (0.46 / 0.511) × 100.
Volumetric Productivity	Q_p	1.48	g/L·h	P / t = 62.3 g/L / 42 h. Based on total SSF time.
Maximum Productivity (0-24h)	$Q_{p,max}$	~2.35	g/L·h	Estimated average rate in the first 24 hours where most sugar consumption occurs.
Biochemical Oxygen Demand (BOD_5)	BOD_5	270.14	mg/L	Lower than expected due to consumption of organics (sugars) to produce ethanol.
BOD_5/COD Ratio	-	0.31	-	270.14 / 860.82. Suggests a portion of the residual COD is from less biodegradable compounds.

The simultaneous saccharification and fermentation (SSF) of garri processing waste hydrolysate, derived from cassava peels, achieved an ethanol titre of 62.3 g/L, corresponding to approximately 7.9% v/v, with a process yield of 0.46 g/g sugar consumed and a volumetric productivity of 1.48 g/L·h over 42 hours. This performance reflects

efficient conversion, as the sugar consumption reached 135.4 g/L from an initial 143.6 g/L total reducing sugars, leaving only 8.2 g/L residual glucose, which suggests minimal substrate inhibition but potential opportunities for further optimization to enhance completeness. The theoretical yield coefficient of 90.2% indicates high efficiency relative to the stoichiometric maximum of 0.511 g ethanol/g glucose, accounting for minor byproducts like glycerol, which was factored into the theoretical calculation at 0.38 g/g. Such results align with optimized SSF processes for cassava-based wastes, where high substrate loadings and enzyme-yeast synergy minimize energy inputs compared to separate hydrolysis and fermentation (SHF), though the maximum productivity of ~2.35 g/L·h in the initial 24 hours highlights a rapid phase followed by deceleration, likely due to ethanol accumulation or nutrient depletion [19]. In comparison, similar studies on cassava peel SSF have reported ethanol titres ranging from 16.42 g/L in small-scale flasks to over 80 g/L in scaled fermenters, underscoring the influence of scale and conditions on output [12, 13].

The BOD_5 of 270.14 mg/L and BOD_5/COD ratio of 0.31 in the effluent suggest that while much of the organic load was converted to ethanol, residual less-biodegradable compounds contribute to the remaining COD (860.82 mg/L), which is consistent with cassava waste fermentations where lignocellulosic residues and byproducts like acetic acid or phenolics persist post-SSF. This ratio implies potential for aerobic or anaerobic post-treatment to reduce environmental impact, as higher biodegradability (BOD/COD >0.4) is often targeted in bioethanol effluents to facilitate wastewater valorisation [17]. The process's volumetric productivity (1.48 g/L·h) surpasses many reported values for cassava peels, such as 0.53 g/L·h in co-culture SSF with fungal amylases, but falls short of high-loading systems achieving 2.21 g/L·h, possibly due to differences in pretreatment or yeast strains [20, 13]. For instance, in single-step SSF from raw cassava starch, productivities of 1.14 g/L·h were attained at laboratory scale with 75.29% efficiency, declining to 0.98 g/L·h at industrial scale due to mixing limitations, mirroring potential scalability challenges for garri waste [19]. The 90.2% theoretical yield here exceeds typical efficiencies of 67–75% in scaled processes, attributing to the high initial sugar concentration and effective glucoamylase activity, though incomplete sugar utilization (94.3%) indicates room for yeast adaptation or nutrient supplementation to mitigate osmotic stress [16].

Kinetic assessment reveals that the rapid initial productivity aligns with exponential yeast growth phases, where sugar consumption rates peak before ethanol inhibition sets in around 50–60 g/L, a common threshold in Saccharomyces cerevisiae fermentations of starchy wastes. The calculated theoretical ethanol yield of 78.9 g/L, adjusted for byproducts, underscores the process's robustness, as actual output (62.3 g/L) represents 79% of this potential, higher than the 67.56% efficiency in large-scale cassava SSF where residual sugars climbed to 15 g/L [19]. Comparative literature on cassava peel valorisation shows yields of 0.46 g/g in enhanced SSF with co-products characterization, matching this study's Y_p/s, while co-culture approaches have yielded up to 0.441 g/g dry substrate, emphasizing the benefits of acid pretreatment for accessibility [21, 17]. The lower BOD_5 relative to initial organics reflects efficient carbon redirection to ethanol, but the presence of residual glucose suggests possible cyanide traces from cassava inhibiting yeast, a factor mitigated in optimized systems through detoxification [18]. Overall, these kinetics position garri waste SSF as economically viable for bioethanol in West

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Africa, with titres competitive to cassava stem-peel mixtures (103.74 g/L at 92.2% efficiency), though integrating prehydrolysis could boost productivity beyond 2 g/L·h by alleviating viscosity [12, 13].

The ethanol production kinetics from the SSF of garri processing waste hydrolysate demonstrate a balanced performance, with an ethanol titre of 62.3 g/L and a yield of 0.46 g/g on consumed sugars, achieving 90.2% of the theoretical maximum. These metrics suggest effective integration of saccharification and fermentation, minimizing intermediate glucose accumulation that could inhibit enzymes, as evidenced by the low residual glucose (8.2 g/L) from an initial 143.6 g/L. The volumetric productivity of 1.48 g/L·h, peaking at ~2.35 g/L·h early on, reflects rapid yeast metabolism in the nutrient-rich hydrolysate, though deceleration post-24 hours may stem from ethanol toxicity or pH shifts, common in high-gravity fermentations [19]. Comparatively, in valorisation of cassava peels via SSF, titres have varied widely; for example, optimized conditions without prehydrolysis yielded 16.42 g/L, while combined stem-peel hydrolysates reached 103.74 g/L with 92.2% efficiency, indicating that substrate blending or advanced pretreatments could elevate garri waste outputs [12, 13]. The BOD_5 (270.14 mg/L) and BOD_5/COD ratio (0.31) further imply that the process efficiently diverts organics to ethanol, leaving a moderately biodegradable effluent suitable for anaerobic digestion, aligning with sustainable practices in cassava waste management where higher ratios (>0.4) are ideal but rarely achieved without treatment [17]. Kinetic modelling in similar systems, such as co-culture SSF yielding 25.4 g/L at 0.53 g/L·h, underscores the potential of fungal-yeast synergies to boost productivity, though garri waste's 90.2% theoretical yield surpasses many at 67–75%, attributable to its starch-dominant composition [19, 20].

Delving deeper, the sugar consumption (135.4 g/L) and process yield highlight the efficacy of Saccharomyces cerevisiae or equivalent strains in handling cassava-derived inhibitors like cyanide, which at low levels (post-processing) minimally impact fermentation, as supported by studies using stress-tolerant yeasts achieving 0.441 g/g dry substrate [17, 18]. The theoretical ethanol yield calculation (78.9 g/L), incorporating glycerol's minor contribution, reveals a 21% shortfall in actual output, potentially due to carbon flux toward biomass or byproducts, a phenomenon observed in cassava peel SSF where yields matched 0.46 g/g under enhanced conditions [21]. Scale-up considerations are critical, as laboratory productivities like 1.14 g/L·h drop to 0.98 g/L·h industrially due to hydrodynamic issues, suggesting garri waste SSF could benefit from stirred bioreactors to maintain the observed 1.48 g/L·h [19]. Moreover, the maximum early productivity echoes rapid phases in acid-pretreated peels, where co-cultures enhance enzyme-substrate interactions, yielding up to 0.491 g/g at small scale but emphasizing substrate concentration's role 15% w/v optimal in kinetics studies [16]. Environmental metrics like the low BOD_5 indicate reduced pollution potential compared to untreated cassava effluents, facilitating circular economy integration, though heavy metal traces from peels necessitate monitoring [17]. Ultimately, these results affirm garri processing waste's promise for bioethanol, with kinetics rivalling advanced cassava valorisation pathways and paving the way for economic assessments in resource-limited settings

b. Impact of Co-Fermentation & Inhibition Effects

Table 4.7.: *Analysis of Inhibition and Co-Fermentation Effects in SSF*

Parameter	Value	Unit	Inference on Inhibition & Co-Fermentation
Residual Glucose	8.2	g/L	Suggests inhibition of yeast metabolism prevented complete sugar uptake. This is not due to sugar depletion.
Glycerol Concentration	9.8	g/L	High glycerol production is a yeast stress response, often to osmotic stress or to re-oxidize NADH under imbalance, confirming metabolic inhibition.
Acetic Acid Concentration	0.18	g/L	Although below typically cited inhibitory thresholds (>2 g/L), it can act synergistically with other stressors.
Lactic Acid Concentration	0.52	g/L	Indicates minor bacterial contamination (e.g., Lactobacillus spp.). Its presence can contribute to stress and pH drop.
Phenolics Concentration	0.36	mg/L	Well below levels known to cause significant inhibition (< 1 g/L), ruling it out as a primary inhibitor.
Viable Yeast Count	4.46 x 10^2	CFU/mL	Critically low cell count at the end of fermentation. This is a strong indicator of severe inhibition or cell death, explaining the halted fermentation.
Total Heterotrophic Bacteria Count	3.78 x 10^3	CFU/mL	Bacterial population an order of magnitude higher than yeast. Suggests bacterial competition is a significant stressor.
Residual Starch Content	35.82	%	High residual starch signifies that enzymatic saccharification was also incomplete, potentially due to enzyme inhibition or accessibility issues.
Chromium (Cr) Concentration	19.81	mg/L	Extremely high concentration. Heavy metals, particularly Cr, are potent inhibitors of both yeast and enzymes. This is a primary candidate for the observed inhibition.
Other Heavy Metals (e.g., Cu, Zn, Fe)	5.22 - 8.65	mg/L	Elevated levels of these metals can contribute to cumulative microbial toxicity.

Table 4.8: *Comparison of SSF effluent properties against literature*

Parameter Category	Key Values from Study	Comparative Literature Values	Sources
Ethanol & Byproducts	Ethanol: 62.3 g/L; Residual Glucose: 8.2 g/L; Glycerol: 9.8 g/L; Lactic Acid: 0.52 g/L; Acetic Acid: 0.18 g/L	Ethanol: 16.42–103.74 g/L; Residual Sugars: 1.5–3.12 g/L; Acetic Acid: >1 g/L in some	[12, 13, 14]
Physicochemical	pH: 6.68; COD: 860.82 mg/L; BOD: 270.14 mg/L; TOC: 158.46 mg/L	pH: 3.8–5.5; COD: >1,000 mg/L in untreated	[16, 17]

Nutrients & Ions	TN: 75.96 mg/L; TP: 18.94 mg/L; K: 42.39 mg/L; SO_4: 420.13 mg/L	TN: 50–100 mg/L; K: 20–50 mg/L	[15, 18]
Heavy Metals	Cr: 19.81 mg/L; Zn: 8.65 mg/L; Fe: 7.98 mg/L	Cr: <0.05 mg/L ideal; Zn: 5–10 mg/L typical	[14, 15]
Microbiological	Yeast: 4.46 × 10^2 CFU/mL; Bacteria: 3.78 × 10^3 CFU/mL; Fungi: 9.31 × 10^3 CFU/mL	Bacteria: up to 10^6 CFU/mL in open systems	[14, 17]
Residual Enzymes & Fibers	Glucoamylase: 185.2 U/g; Residual Starch: 35.82%; Hemicellulose: 19.53%	Enzyme Activity: 100–300 U/g residual; Starch: 20–40%	[15, 16]

Table 4.9.: *Comparison of SSF kinetics against peer studies*

Parameter	Study Value	Literature Comparison	Source
Ethanol Titre (g/L)	62.3	81.86 (lab scale); 70.74 (industrial); 103.74 (stem-peel mix); 34.53 (enhanced SSF); 25.4 (co-culture)	[13, 19, 20, 21]
Yield (g/g sugar)	0.46	0.43; 0.38; 0.46; 0.441 (g/g dry); 1.40 (reducing sugars, likely substrate basis)	[17, 19, 20, 21]
Productivity (g/L·h)	1.48 (overall); ~2.35 (max)	1.14; 0.98; 2.21; 0.53	[19, 13, 20]
Theoretical Yield (%)	90.2	75.29; 67.56; 92.2	[19, 13]
Residual Glucose (g/L)	8.2	7.90; 15.00; 1.21–1.32	[19, 20]
BOD_5 (mg/L)	270.14	Not commonly reported; aligns with low post-fermentation organics	[17]
BOD_5/COD Ratio	0.31	Indicates moderate biodegradability; similar to lignocellulosic effluents	[17]

The impact of co-fermentation and inhibition effects in the simultaneous saccharification and fermentation (SSF) of garri processing waste for bioethanol production reveals a complex interplay of microbial dynamics and chemical stressors that limited complete sugar utilization and ethanol yield. The residual glucose concentration of 8.2 g/L, despite ample initial sugars, indicates that fermentation halted not due to substrate exhaustion but likely from inhibitory factors impairing yeast metabolism, a phenomenon commonly observed in cassava waste processes where inhibitors accumulate during hydrolysis [12]. This incomplete uptake aligns with studies on cassava peels, where osmotic stress or toxic compounds lead to stalled fermentation, resulting in 5–10 g/L residual sugars even under optimized conditions [22]. The elevated glycerol level at 9.8 g/L further supports a stress response in Saccharomyces cerevisiae, as glycerol acts as an osmo-protectant and NADH re-oxidizer under adverse conditions like high gravity or inhibitor presence, diverting carbon away from ethanol and reducing overall productivity [23]. In cassava ethanol systems, glycerol production often increases by 10–20% under stress, with

titres reaching 9–11 g/L in viscous slurries, as cellulase addition has been shown to mitigate this by lowering viscosity and glycerol biosynthesis [23]. Similarly, salt-induced stress in thermotolerant yeasts like Pichia kudriavzevii enhances glycerol accumulation to protect against heat and oxidative damage, improving bioethanol yields at high temperatures but highlighting glycerol's dual role as both protector and yield reducer [24].

Acetic acid at 0.18 g/L and lactic acid at 0.52 g/L suggest minor bacterial contamination, possibly from Lactobacillus spp., which can contribute to pH drops and synergistic inhibition, though these levels are below typical thresholds (>2 g/L for acetic acid) that severely hamper yeast [25]. However, the bacterial count (3.78×10^3 CFU/mL) exceeding yeast viability (4.46×10^2 CFU/mL) points to competitive co-fermentation, where heterotrophic bacteria outcompete yeast for nutrients, leading to acid buildup and reduced ethanol selectivity, as seen in co-culture studies of cassava wastes where bacterial dominance lowered yields by 15–25% ([17] as referenced in broader co-fermentation literature). In lignocellulosic bioethanol from cassava-like substrates, co-fermentation with bacterial symbionts and yeast cocktails enhances sugar utilization but risks inhibition if bacterial growth isn't controlled, with efficiencies dropping from 88% to 60% due to acids and phenolics [26]. Phenolics at 0.36 mg/L are well below inhibitory levels (<1 g/L), ruling them out as primary culprits, consistent with delignified cassava mixtures where low phenolics (0.2–0.5 mg/L) had negligible effects on fermentation [25].

The critically low yeast count signals severe inhibition or cell death, likely exacerbated by heavy metals, with chromium at 19.81 mg/L emerging as a key toxin, far exceeding safe thresholds (typically <0.05 mg/L for yeast viability) and disrupting enzyme and cellular functions [27]. Other metals like copper (5.22 mg/L), zinc (8.65 mg/L), and iron contribute to cumulative toxicity, as heavy metals above 100 µM inhibit yeast growth by damaging proteins and DNA, a common issue in agricultural wastes like cassava where soil-derived metals accumulate [27]. In Pichia kudriavzevii applications for cassava bioethanol, heavy metal bioaccumulation (e.g., Cr, Cd, Pb) under low pH enhances tolerance, but excessive levels like those here would inhibit fermentation, reducing yields by 20–40% [28]. This metal-induced stress mirrors findings in sugarcane molasses bioethanol, where reducing heavy metals improved fermentation by alleviating toxicity [29].

High residual starch (35.82%) underscores incomplete saccharification, potentially from enzyme inhibition by metals or substrate accessibility issues in fibrous garri waste, similar to cassava starch processes where viscosity and inhibitors leave 20–40% unhydrolyzed starch [19]. In consolidated bioprocessing of raw starch, residual levels of 15–20% occur due to crystalline regions resisting hydrolysis, necessitating enzyme optimization or pretreatments to boost conversion to 80–90% [30]. For sago hampas, a similar starchy waste, multi-cycle hydrolysis recovered up to 138 g/L glucose from residuals, achieving 93% ethanol yield, suggesting similar strategies could address garri waste's 35.82% residual [31]. Co-fermentation challenges in garri waste are amplified by these inhibitors, but using multi-stress-tolerant yeasts like P. kudriavzevii, which withstand acids (up to 18 g/L acetic) and metals, could mitigate effects, as demonstrated in cassava effluent fermentation yielding 5–7% ethanol [28]. Overall, these results highlight the need for detoxification or tolerant strains to overcome inhibition in garri waste valorisation, aligning with broader cassava bioethanol literature where metal chelation or bacterial-yeast synergies improve outcomes [25, 26].

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IV. CONCLUSION

The SSF of garri processing waste demonstrates considerable promise for bioethanol production, achieving ethanol titres comparable to other cassava-based feedstocks. However, inhibition from heavy metals – especially chromium – and incomplete saccharification due to residual starch limit overall efficiency. To enhance yield and process viability, future work should focus on detoxification strategies, the use of metal-tolerant yeast strains, and improved pretreatment methods to reduce inhibitor concentrations and increase sugar accessibility. Integrating effluent treatment for nutrient and metal recovery could further enhance the environmental and economic sustainability of bioethanol production from agro-industrial wastes.

APPENDIX

The appendix sits at the junction of the small intestine and large intestine. It's a thin tube about four inches long. Normally, the appendix sits in the lower right abdomen.

The function of the appendix is unknown. One theory is that the appendix acts as a storehouse for good bacteria, "rebooting" the digestive system after diarrheal illnesses. Other experts believe the appendix is just a useless remnant from our evolutionary past. Surgical removal of the appendix causes no observable health problems.

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Acknowledgement letter is very short business letter, and is intended to communicate brief and clear message. It is quite common to use this letter if you are not aware at the time of future developments in regard to someone's query. It is quite common to use this letter if you are not aware at the time of future developments in regard to someone's query. It is quite common to use this letter if you are not aware at the time of future developments in regard to someone's query.

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