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EVALUATION OF BIOGAS PRODUCTION POTENTIAL FROM CASSAVA MILL EFFLUENTS AND GUTTER SLUDGE UNDER ANAEROBIC CONDITIONS

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ABSTRACT: The increasing demand for sustainable energy and improved waste management strategies has intensified interest in biogas production from organic wastes. This study evaluated the biogas production potential of cassava mill effluents (CME) co-digested with gutter sludge under anaerobic conditions. Samples were collected from Nise community in Awka South Local Government Area, Anambra State, Nigeria, and subjected to microbiological, physicochemical, and nutritional analyses before and after a 32-day anaerobic digestion period. Microbial enumeration revealed the presence of Staphylococcus aureus, Bacillus cereus, Aspergillus spp., and Candida spp., indicating active biodegradation potential.

Significant reductions in cyanide concentration (75.0 to 40.0 mg/L) and selected heavy metals, namely, chromium and cadmium, were observed. Nutritional parameters such as crude protein, fatty acids, nitrogen, phosphorus, and potassium decreased, reflecting microbial utilization, while carbohydrate content increased due to hydrolysis of complex polymers. Biogas production was confirmed by flammability tests from Day 16. The findings demonstrate that co-digestion of CME and gutter sludge is a feasible and environmentally beneficial approach for renewable energy generation and waste remediation, with significant implications for sustainable waste management and energy security in developing regions.

Keywords: *Biogas, Cassava Mill Effluents, Gutter, Sludge, Anaerobic Conditions.*

Introduction

The rapid increase in global energy demand, coupled with the environmental consequences of fossil-fuel dependence, has intensified the search for sustainable, low-cost, and environmentally friendly renewable energy sources. Biogas technology has emerged as a viable solution, particularly in developing countries where organic waste is abundant and waste management challenges persist (Khan et al., 2018). Biogas, which is primarily composed of methane (CH_4) and carbon dioxide (CO_2), is produced through the anaerobic digestion of organic materials such as agricultural residues, industrial effluents, and municipal wastes. Its production not only generates clean energy but also reduces greenhouse-gas emissions, mitigates environmental pollution, and provides nutrient-rich bio-slurry for agricultural use (Mbuligwe, 2020). Numerous studies have demonstrated the role of microorganisms in biodegradation, waste stabilization, and environmental sustainability, highlighting their importance in pollution control and renewable-energy systems (Awari et al., 2023; Agu et al., 2015a).

Among the numerous feedstocks available for biogas production, cassava mill effluents (CME) and gutter sludge present promising substrates, especially in regions where cassava processing activities and inadequate drainage systems contribute significantly to environmental degradation. Cassava remains a major staple crop in West Africa, and Nigeria is the world's largest producer, generating large volumes of

effluents during processing (FAO, 2022). These effluents are rich in organic matter, cyanogenic compounds, starch residues, and suspended solids, which are often discharged untreated into surrounding soils and water bodies, leading to eutrophication, toxicity, and ecological disturbances (Aro, 2019). Similar waste discharges have been reported to significantly alter microbial ecology and increase bacteriological and heavy-metal contamination of groundwater and surface water systems in urban and peri-urban environments (Agu et al., 2014a; Ezeokoli et al., 2023).

The high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of cassava mill effluents make them suitable substrates for anaerobic digestion, enabling the transformation of environmental pollutants into productive bioenergy resources (Okudoh et al., 2014). Previous studies have demonstrated the capacity of indigenous bacteria, fungi, and cyanobacteria isolated from oil-polluted and agro-industrial waste–impacted soils to degrade hydrocarbons and other complex organic pollutants, underscoring their relevance in waste-to-energy and bioremediation processes (Agu et al., 2013; Agu et al., 2015a; Mbachu et al., 2014; Agu et al., 2022; Orji et al., 2022).

Similarly, gutter sludge collected from urban drainage systems is composed of decomposing organic matter, domestic waste, fecal residues, sediments, and microbial biomass. In many developing regions, blocked drainage channels contribute to flooding, groundwater contamination, and the spread of waterborne diseases. Studies have shown that wastes from abattoirs, refuse dumps, and urban drainage systems harbor diverse microbial populations capable of organic matter degradation but also pose public-health risks if poorly managed (Agu et al., 2014b; Ezeokoli et al., 2023). Nevertheless, the organic fraction of gutter sludge exhibits considerable biodegradability, making it a suitable co-substrate for anaerobic digestion (Odey et al., 2021).

Co-digestion of substrates with complementary nutrient profiles—such as carbohydrate-rich cassava effluent and nitrogen-containing gutter sludge—enhances microbial activity, improves methane yield, and stabilizes the anaerobic digestion process (Zhang et al., 2019). The effectiveness of microbial consortia in degrading

diverse organic substrates has been well documented, including the influence of surfactants, enzymes, and environmental conditions on microbial growth and metabolic efficiency (Anaukwu et al., 2016a; Anaukwu et al., 2016b; Chidi-Onuorah et al., 2015). Enzyme-producing fungi and bacteria isolated from polluted environments have further demonstrated significant potential for biomass degradation and bioenergy-related applications (Agu et al., 2014c; Oparaji et al., 2024; Orji et al., 2014).

Biogas production from cassava mill effluents and gutter sludge therefore offers a dual benefit of effective waste management and renewable-energy generation, aligning with the Sustainable Development Goals (SDGs), particularly SDG 7 (affordable and clean energy), SDG 11 (sustainable cities and communities), and SDG 13 (climate action). Despite the availability of these substrates and their environmental impact, their combined potential for biogas production remains underexplored in many regions, especially at community and small-scale levels.

Therefore, this study investigates the anaerobic digestion of cassava mill effluents and gutter sludge, evaluating biogas yield, substrate interaction, and the feasibility of integrating these waste streams into sustainable, community-based renewable-energy systems.

MATERIALS AND METHODS

Study area. The study was carried out in the Microbiology Laboratory of Nnamdi Azikiwe University, Awka, situated in Awka South Local Government Area of Anambra State, Nigeria. Awka lies within the tropical rainforest belt between latitude 6°12'N and longitude 7°06'E. The area experiences a humid climate with an average relative humidity of 79.4%, annual rainfall of 2,000–3,000 mm, and mean daily maximum and minimum temperatures of 32.2°C and 23.3°C, respectively. The terrain is dominated by low-lying plains with derived savanna vegetation resulting from extensive anthropogenic activities. The population of Awka is estimated at 176,858 and is engaged primarily in farming, trading, craftsmanship, and civil service. Major cultivated crops include yam, cocoyam, cassava, maize, fruits, and vegetables, while palm produce, coconut, and kola nut serve as important cash crops.

Mixed farming with livestock such as goats, sheep, and cattle is also common. The cassava mill effluent used in this study was sourced from Nise, a community in Awka South LGA comprising the villages of Ngodo, Arah, Umuazu, and Isiakpu. Arah village is located at coordinates 6.1618°N and 7.0525°E (postal code 420106).

Sample collection. Cassava mill effluent (CME) was collected from Emeka Cassava Milling Site in Nise using a sterile 5-L container. Gutter sludge was collected from roadside drainage channels in the same community using sterile polyethylene bags. All samples were transported immediately to the Microbiology Laboratory under aseptic conditions for analysis.

Enumeration of total heterotrophic bacteria and fungi. A tenfold serial dilution was performed following Smith et al. (2020). Ninety milliliters of sterile nutrient broth were prepared, and 9 mL aliquots were dispensed into tubes labeled 10^{-1} and 10^{-2}. A 10 mL portion of CME was added to 90 mL nutrient broth and allowed to stand for 10–20 minutes. From this suspension, 1 mL was transferred into the 10^{-1} tube, then into the 10^{-2} tube. Subsequently, 0.1 mL aliquots were inoculated onto Nutrient Agar, Sabouraud Dextrose Agar, Cetrimide Agar, and Mannitol Egg Yolk Polymyxin Agar using the pour plate technique. Plates were incubated at 37°C for 24 hours (bacteria) and at room temperature for 72 hours (fungi). Colony-forming units (CFU/mL) were enumerated.

Characterization and Identification of Bacterial Isolates. Discrete colonies were repeatedly subcultured onto fresh Nutrient Agar and Sabouraud Dextrose Agar plates to obtain pure cultures. Identification was followed, involving observation of colony morphology, microscopic examination, and biochemical characterization. Bacterial and fungal colonies were purified through repeated streaking on Nutrient Agar (bacteria) and Sabouraud Dextrose Agar (fungi). Pure cultures were maintained on agar slants in sterile bottles for subsequent analyses. Bacterial isolates were characterized based on colony color, elevation, margin, texture, and moisture content.. Gram staining was used to determine reaction type, cell morphology, and arrangement. Biochemical tests conducted included Gram staining, motility, oxidase, catalase, citrate utilization, indole production, methyl red (MR), Voges–Proskauer (VP), and urease tests, following standard microbiological procedures.

Identification of Fungal Isolates. Each of the fungal isolates was separately collected with a sterile wooden stick and teased out on a drop of Lactophenol cotton blue stain and potassium hydroxide solution on a clean glass slide. The wet mount preparation was then viewed under the microscope for branched and unbranched hyphae

Physicochemical composition of cassava mill effluents and gutter sludge

Determination of total organic carbon. The Walkley-black titration method as described by Poudel (2020) was used. Exactly 2g of the sample was measured into a beaker containing 5ml of 0.4M potassium dichromate solution ($K_2Cr_2O_7$) followed by addition of 10 ml of concentrated sulfuric acid. The mixture was gently swirled and left at room temperature in a fume cupboard for 16-18 hours and then, 100 ml of distilled water was added to the mixture. The excess of dichromate was back-titrated potentiometrically with the use of standard 0.5M ferrous ammonium sulfate ($Fe(NH_4)2(SO_4)2.6H_2O$) solution. Blank titration of the acidic dichromate with ferrous ammonium sulfate solution was performed also. Organic carbon content in the sample was calculated as:

$$\text{Organic carbon (\%)} = \frac{(B - S) \times M \times 0.003 \times f \times 100}{\text{Weight of sample}}$$

Where B= the volume of ferrous solution used in the blank titration

S= is the volume of ferrous solution used in the sample titration,

M= molarity of ferrous ammonium sulfate

F = correction factor, 1.3

Determination of heavy metals. Standard stock solutions with a concentration of 1000 ppm of each element in 2N nitric acid served as the basis for preparing standard solutions of lead (Pb), iron (Fe), chromium (Cr), nickel (Ni), and cadmium (Cd). Utilizing an Atomic Absorption Spectrophotometer (AAS), the presence and levels of these elements were determined. Calibration curves for each element were established through the least square method to accurately quantify the concentration

of heavy metals in the sample. To prepare intermediate standard solutions, 100 mg/l concentrations of each metal were derived from the stock solutions of Pb, Fe, Cd, Cr, and Ni. Subsequently, serial dilutions of the intermediate stock solution were executed through extraction, generating adequate working standards for each metal. The concentrations of these working standards were then determined using AAS by aspirating the solutions into the Spectrophotometer, and the corresponding absorbance values were recorded (Edori and Edori (2012)).

Determination of cyanide. Exactly 1g HCN powder was added to 0.1 M phosphoric acid and made up to 25 ml in a standard flask. Standard solutions of 1.00g HCN and acetone cyanohydrin were prepared and added to 0.1 M phosphoric acid. 2ml of the sample (cassava effluent) was added to a test tube containing 2.0 ml of 4M sulphuric acid and the mixture heated for 5 minutes in a stopper test tube in boiling water bath. The sample was cooled in ice cold water, with the stopper loosely in place. 5.0 ml of 3.6 M sodium hydroxide was added and after 5 min, 1 ml was added to 7 ml of 0.2M acetate buffer at pH 5.0. About 5 mins later 1.6 ml of barbituric acid. After one hour of color development, the absorbance was measured at 600nm using UV-Spectrophotometer

Determination of Nutritional Components

Carbohydrate content. Exactly 2.5g of sample was weighed and 50ml of cold water was added and allowed to stand for one hour then 20ml of concentrated HCL and 150ml of distilled water was introduced and refluxed for two hours in a 250ml round bottom flask. The solution was allowed to cool and neutralized with 5ml of NaOH to make up the mark. The carbohydrate content was determined using anthrone reagent, that is, 5ml of anthrone reagent was added to 1ml of the solution then covered and allowed to boil in a water bath for 20 minutes for colour development. The absorbance was read at 620nm using a UV spectrophotometer.

Crude protein content. The indirect Kjedahl method as described by Hanne et al (2018) (ensure the reference is added to the reference section) was used. Exactly 1g of sample was weighed into a 300ml Kjehdahl flask (gently to prevent the sample from touching the walls of the side of each and then the flasks were stoppered and

shaken. Then 0.5g of the Kjedahl catalyst mixture was added. The mixture was heated cautiously in a digestion rack on electric hot plate until a clear solution appeared. The clear solution was then allowed to stand for 30 minutes and allowed to cool. After cooling about 100ml of distilled water was added to avoid caking and then 5ml of the filtrate and 5ml of 40% Na0H was transferred to the Kjedahl distillation apparatus. A 250ml receiver beaker containing 10ml of 10% boric acid and indicator mixture containing 5 drops of Bromocresol blue and 1 drop of methylene blue was placed under a condenser of the distillation apparatus so that the tap was about 20cm inside the solution. Then 5ml of 40% sodium hydroxide was added to the digested sample in the apparatus and distillation commenced immediately until 50 drops gets into the receiver beaker after which it was titrated to pink colour using 0.01N hydrochloric acid.

Fatty acids profile. Exactly 2ml of the sample was pipette into beaker containing 20ml of N-hexane, shake very well and poured into a separating funnel and allowed to stand for 30 minutes. The N-hexane layer was collected and stored in sample vial for fatty Acid Profile Analysis with GC-FID.

Determination of nitrogen, phosphorus, and potassium. One hundred (100mg) of the digestate were digested in 2ml of sulphuric acid in a 500ml digester at 3300C for 2hours. Four drops of hydrogen peroxide were dropped in the mixture and digested for another 1 hour. Digested solutions were filtered with Whatman filter paper No. 44 and the filtrate was made up to 50ml with distilled water in a volumetric flask. Then the nitrogen and phosphorus concentration in the solution were determined using an auto analyzer (QuickChem, 8000). The concentration of potassium was determined using Atomic Absorption Spectrophotometer (Edori and Edori, 2012).

Composition of biogas digester. A locally fabricated anaerobic digester was assembled using a drip set, glue, thermometer, airtight bowl, and gas collection bottles. The digester incorporated basic temperature control and agitation to support microbial activity. A mixture of 400 g gutter sludge and 500 mL CME was introduced into the digester and allowed to ferment for 32 days. To absorb carbon dioxide, 25 mL of calcium hydroxide solution (1.48 g/100 mL) was placed in the first container of the gas-collection assembly. Samples were collected on days 0 and

32 for analysis of heavy metals, cyanide, nutritional parameters, and microbial load. pH, temperature, and gas flammability were recorded at four-day intervals. Flammability was tested using a water displacement method.

RESULTS

Table 1: Count of colony forming units on a nutrient media from cassava mill effluents sample.

Microbes	Total microbial count (cfu/ml)
BACTERIA	$3.8X\ 10^4$
FUNGI	$4\ X\ 10^4$

The isolates found in cassava mill effluent include both bacteria and fungi. Two (2) bacteria and two (2) fungi were isolated from cassava mill effluent sample (namely; *Staphylococcus aureus, Bacillus cereus, Aspergillus spp* and *Candida spp.*

Table 2. Cultural morphological, macroscopy, microscopy, and biochemical properties of isolates

Colonies	**CM**	**GS**	**Mac**	**Mic**	**Cat**	**Cit**	**Coag**	**ID**	**MR**	**VP**	**MT**	**PO**
Colony 1	Yellowish, large, mucoid, flat, irregular, undulate colonies.	+	ND	ND	+	+	-	+	-	+	Motile	*Bacillus cereus*
Colony 2	Creamy white, small, smooth, raised, circular, entire colonies	+	ND	ND	+	+	+	-	+	+	Non motile	*Staphylococcus aureus*
Colony 3	ND	ND	Whitish brown-like cottony colonies with greenish center.	Septate hyphae with conidia bearing sterigmata.	ND	ND	ND	ND	ND	ND	ND	*Aspergillusspp*
Colony 4	ND	ND	Creamy raised non-mucoid colonies.	Budded yeast cells	ND	ND	ND	ND	ND	ND	ND	*Candida spp*

Key: CM (cultural morphology), GS (gram stain reaction), Mac & Mic (macroscopy and microscopy), Cat (catalase), Cit (citrate), Coag (coagulase), ID (indole), MR (methyl red), Mot (motility), VP (Voges and Proskauer), ND (Not determined), PO (presumptive organisms)

Table.3: Physicochemical analysis (heavy metals, total organic carbon) of the mixed samples of CME and gutter sludge

Parameters	Day 0 Concentration	Day 32 Concentration
Lead (ppm)	0.120	0.212
Cadmium (ppm)	0.052	0.018
Iron (ppm)	0.058	0.112
Chromium (ppm)	0.943	0.012
Nickel (ppm)	0.122	0.210
TOC	0.379%	1.003%
Cyanide	75.0mg/l	40.0mg/l

The levels of heavy metals and organic composition in the mixed cassava mill effluent (CME) and gutter sludge at Day 0 and Day 32 are presented in Table 3. There were notable alterations in heavy metal concentrations during the 32-day anaerobic digestion. Lead (Pb) increased from 0.120 ppm at Day 0 to 0.212 ppm at Day 32, while cadmium (Cd) decreased from 0.052 ppm to 0.018 ppm. Iron (Fe) increased from 0.058 ppm to 0.112 ppm, whereas chromium (Cr) declined significantly from 0.943 ppm to 0.012 ppm. Nickel (Ni) also increased from 0.122 ppm to 0.210 ppm. Total organic carbon (TOC) rose from 0.379% at Day 0 to 1.003% at Day 32, indicating increased accumulation of organic intermediates during digestion. Cyanide concentration, initially high at 75 mg/L, declined to 40 mg/L by Day 32, suggesting progressive detoxification of cyanogenic compounds.

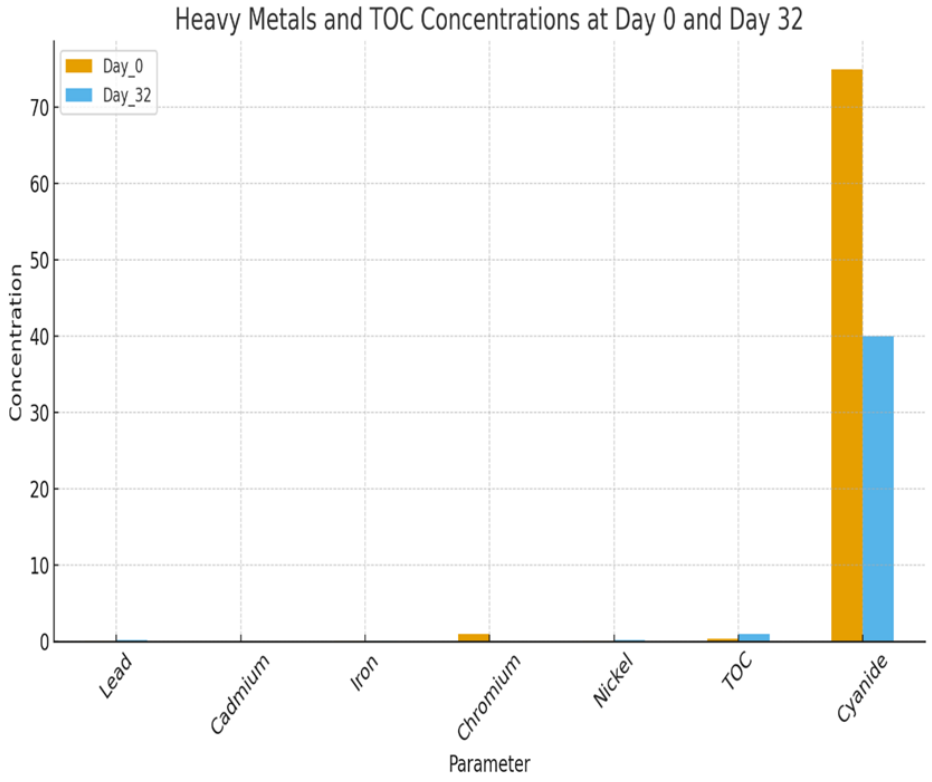


Table 4 Nutritional value of the mixed samples of CME and gutter sludge

Parameters	**Day 0 Concentration**	**Day 32 Concentration**
Crude protein	8.40%	5.950%
Carbohydrate	17.451mg/l	61.969mg/l
Fatty acids	138.9025 ug/ml.	92.3124ug/ml
Nitrogen	1.344%	0.952%
Phosphorus	4.663mg/l	2.700mg/l
Potassium	1.528mg/l	1.117mg/l

The nutritional parameters of the digesting substrate are summarized in Table 4. Crude protein decreased from 8.40% at Day 0 to 5.95% at Day 32, indicating microbial utilization of proteinaceous materials. Carbohydrate content increased markedly from 17.451 mg/L to 61.969 mg/L, implying hydrolysis of complex polysaccharides into simpler sugars during digestion. Fatty acids showed a reduction from 138.90 µg/mL to 92.31 µg/mL. Nitrogen and phosphorus concentrations also declined, from 1.344% to 0.952% and 4.663 mg/L to 2.700 mg/L, respectively. Similarly, potassium decreased from 1.528 mg/L to 1.117 mg/L. These reductions reflect nutrient assimilation and conversion by microorganisms during anaerobic digestion.

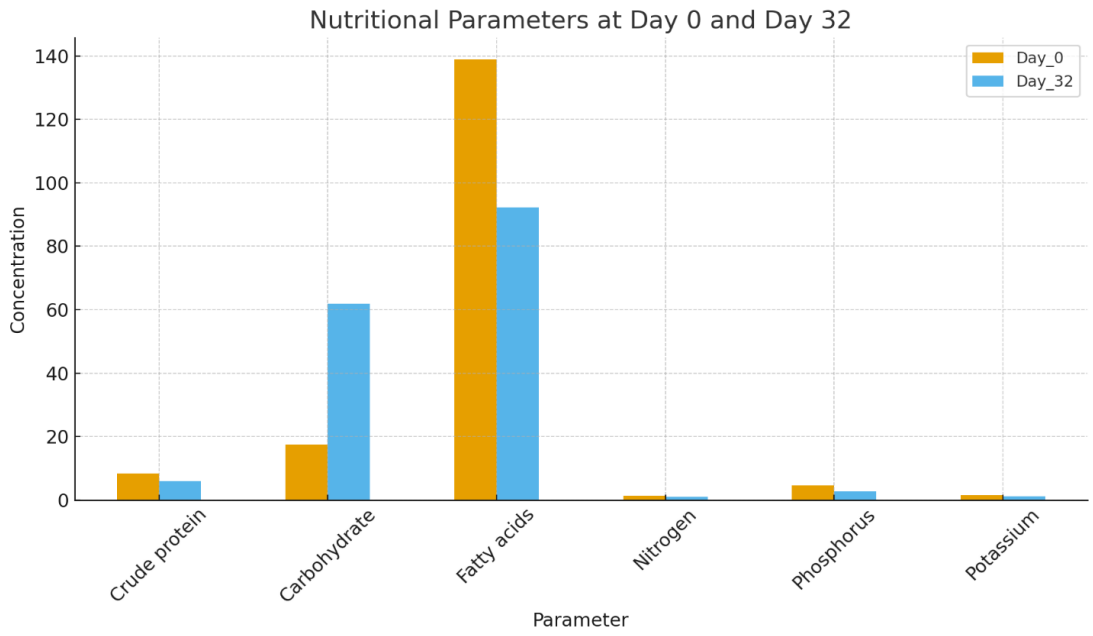
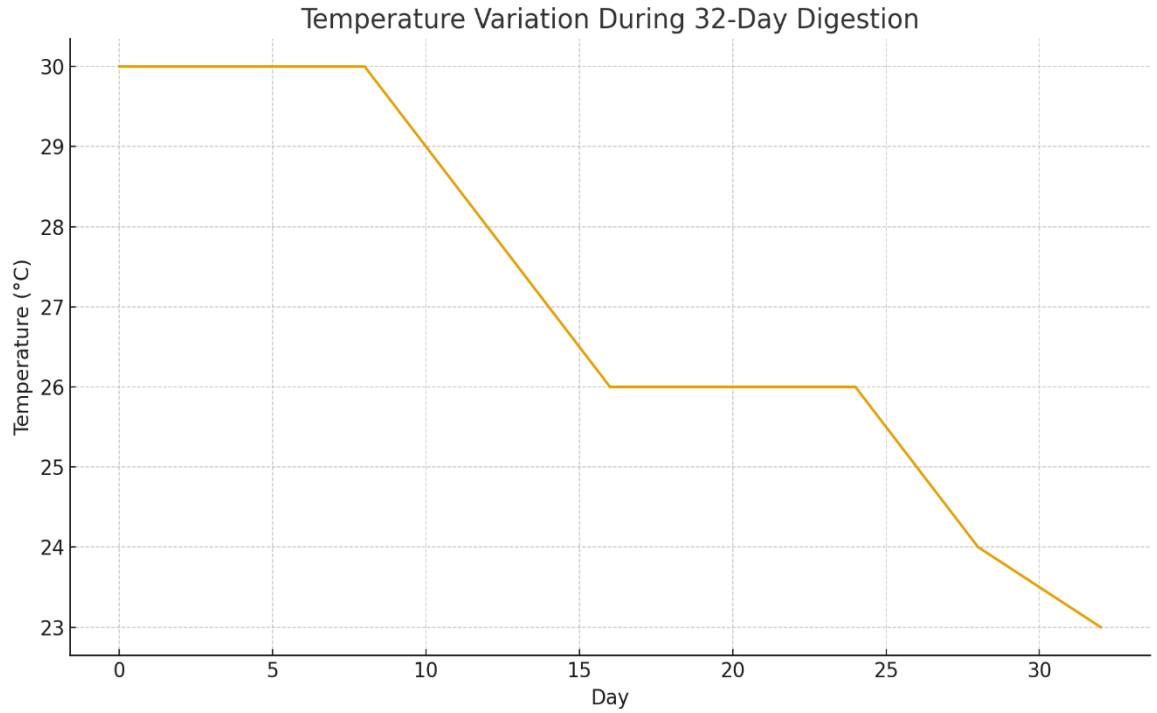


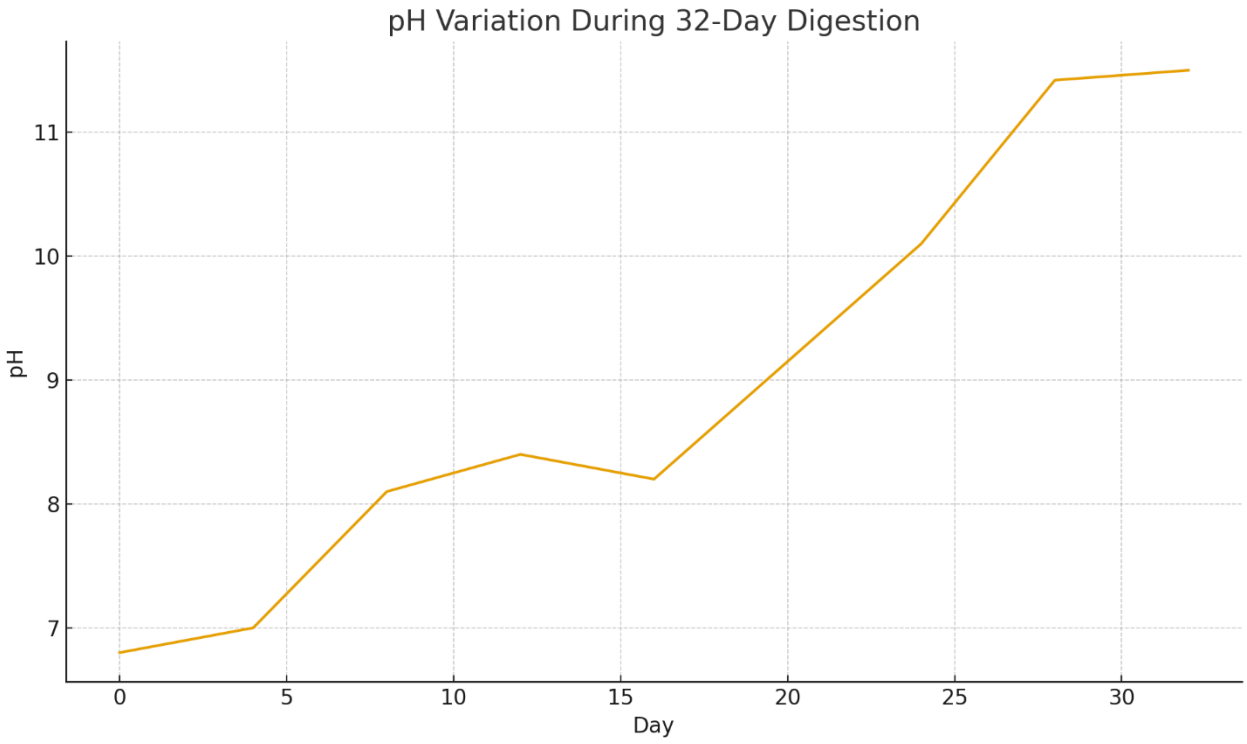
Table 5. Thirty-two-days digestion of CME and gutter sludge.

Duration	**Cumulative concentration of the parameters of CME and gutter sludge digestion**				
	Temperature (°C)	**pH value**	**Flammability test**	**Bacteria**	**Fungi**
Day 0	30	6.80	-	$1.4x\ 10^4$	$1.3\ x\ 10^4$
Day 4	30	7.00	-	ND	ND
Day 8	30	8.10	-	ND	ND
Day 12	28	8.40	-	ND	ND
Day 16	26	8.20	+	ND	ND
Day 20	26	9.15	+	ND	ND
Day 24	26	10.10	+	ND	ND
Day 28	24	11.42	+	ND	ND
Day 32	23	11.50	+	$3.8x\ 10^3$	$3.4\ x\ 10^3$

Key: CME (Cassava mill effluent), ND (Not determined)

Temperature and pH variations over the 32-day digestion period are shown in Table 4.5. The digester temperature decreased gradually from 30°C at Day 0 to 23°C at Day 32. Fluctuations in temperature correspond with ambient environmental conditions since a locally fabricated digester was used without active thermal regulation. The pH increased progressively from 6.80 to 11.50, showing a shift from slightly acidic to strongly alkaline conditions. The upward trend in pH reflects the breakdown of volatile fatty acids and increased formation of ammonia and other alkaline intermediates. Flammability tests showed the first appearance of a combustible flame at Day 16, confirming the presence of methane. Flammability remained positive at Day 32, indicating sustained gas production.





The bacterial and fungal counts at Day 0 and Day 32 are listed in Table 5. Initial bacterial load was 1.4 × 10^4 CFU/mL, reducing to 3.8 × 10^3 CFU/mL by Day 32.

Fungal counts exhibited a similar pattern, decreasing from 1.3×10^4 CFU/mL at Day 0 to 3.4×10^3 CFU/mL at Day 32. The decline in microbial counts toward Day 32 corresponds with substrate depletion and increased methane concentration, both of which inhibit further microbial proliferation.

DISCUSSIONS

In table 1, the total fungi count is 3.4 x 10^4cfu/ml, while the total bacterial count is 3.8 x 10^4cfu/ml. These findings indicate robust microbial community in our CME sample. The isolated microorganisms were *Staphylococcuss aureus*, *Bacillus cereus, Aspergillus spp* and *Candida spp.* In comparison with another study, The fluctuations in heavy metal concentrations observed in this study align with the chemical transformations commonly associated with anaerobic digestion (Adelekan & Bamgboye, 2015). The significant reduction in chromium and cadmium suggests possible metal precipitation, microbial uptake, or conversion into insoluble complexes under anaerobic conditions. The rise in TOC during digestion is consistent with the release of soluble organics following hydrolysis of complex biomass (Maina et al., 2022). Conversely, the drop in cyanide concentration demonstrates that cyanogenic compounds present in cassava waste are progressively detoxified through microbial degradation. A notable decline in crude protein and fatty acids indicates microbial utilization of these macromolecules. This agrees with earlier findings that proteins and lipids serve as key substrates for methane-producing consortia (Nwodo et al., 2020). The significant rise in carbohydrate content suggests increased breakdown of structural plant materials such as cellulose and starch. Reduced nitrogen, phosphorus, and potassium levels by Day 32 further confirm microbial assimilation during fermentation. Such nutrient depletion is typical of active anaerobic digestion where microbial populations rely on available substrates for growth and metabolic activities. The gradual increase in pH towards alkalinity corresponds with ammonia production and consumption of volatile fatty acids are typical indicators of methanogenesis. Methane ignition beginning at Day 16 supports earlier reports that cassava waste substrates require a maturation period before entering the methanogenic phase (Oparaku & Oparaku, 2010). The decline in temperature mirrors ambient environmental conditions and may have caused slight

delays in peak methane formation since mesophilic digestion typically performs best at 30–37°C (Chen et al., 2019). The reduction in bacterial and fungal counts by Day 32 is typical of anaerobic systems entering methanogenesis. Early hydrolytic and fermentative bacteria rapidly decline after the available nutrients decrease and methane concentrations rise, exerting inhibitory effects on other microbial groups. These results are consistent with patterns described by Leite et al. (2021), where microbial succession stabilizes following maximal methane generation.

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