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Research Article

Anaerobic Co-Digestion of Cassava Mill Effluent with Pig Manure for Enhanced Biogas Yield, Detoxification, and Nutrient Enrichment

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Abstract

This study investigated the anaerobic co-digestion of cassava mill effluents (CME) and pig manure (PM) for biogas generation, with a focus on microbial dynamics, physicochemical characteristics, and nutrient composition. Cassava mill effluent is known to contain cyanogenic compounds, heavy metals, and organic matter that can cause environmental hazards if untreated. Pig manure provides buffering nutrients for microbial growth during anaerobic digestion. Samples were co-digested in a locally constructed digester for 32 days under controlled conditions. Microbial isolates included *Staphylococcus aureus, Bacillus cereus, Aspergillus* spp., and *Candida* spp. Biochemical and physiochemical parameters such as pH, cyanide content, heavy metals, total organic carbon (TOC), and macro-nutrient concentrations were analyzed at Day 0 and Day 32. Results showed significant reductions ($p < 0.05$) in heavy metals (Pb: 0.487-0.212 ppm; Cr: 1.929-0.034 ppm), cyanide (75-70 mg/L), and fatty acids (158.9-98.9 µg/mL). Nutritional values improved, with protein (5.60-6.30%), carbohydrate (94.2-100.6 mg/L), and nitrogen (0.896-1.008%) all increasing. The pH shifted from acidic (6.50) to alkaline (8.92), supporting methanogenesis, while cumulative flammability confirmed methane presence. The findings suggest that anaerobic co-digestion of CME and PM offers a sustainable solution for biowaste management, renewable energy generation, and mitigation of environmental hazards. .

Keywords: Co-digestion; Cassava; Effluent; Pig Manure; Biogas; Yield

Introduction

Cassava (*Manihot esculenta*) is a major staple crop in sub-Saharan Africa, contributing significantly to food security and rural livelihoods. However, cassava processing generates large volumes of effluent rich in organic matter, cyanogenic compounds, and heavy metals, which if untreated, pose serious environmental and health risks [1]. Traditional disposal practices such as open dumping and discharge into water bodies contribute to pollution, oxygen depletion, and ecological imbalance. The use of various microbes in bioremediation has been documented by researchers such as [2,3]. Furthermore, the capacity of various microorganisms to degrade different compounds, including lipase production by fungi from palm oil-contaminated soil [4], mannanase production from decaying palm press cake [5], hydrocarbon biodegradation by cyanobacteria [2,6] and bacteria [3], and the degradation of Chlorpyrifos by bacteria [7] and glyphosate by fungi [8] have been demonstrated. Recent studies have also shown the monoculture degradative potential of fungi like *Aspergillus niger* and *Aspergillus flavus* on Congo red [9] and the degradation of spent engine oil [10], as well as the degradation of polyvinyl chloride (PVC) and polyvinyl alcohol (PVA) by *Pseudomonas aeruginosa* [11].

Cassava (*Manihot esculenta*) is indeed a versatile and widely cultivated crop in tropical and subtropical regions, serving as a vital source of food, feed, and industrial products [12]. Cassava processing involves the transformation of cassava roots into various products such as starch, flour, and food items. This process typically includes: peeling and washing, which is to remove the outer skin, followed by thorough washing to eliminate dirt and other impurities [13]; grating or chopping, which is to facilitate the extraction of cassava starch and other components as it breaks down the cellular structure of cassava, making it easier to extract the desired materials [14]; starch extraction, which involves mechanical pressing, sieving, and traditional methods that involve squeezing the cassava pulp in bags to separate starch from fibrous materials [15]; fermentation, which involves reducing the cyanogenic compounds naturally present in cassava [16]; and drying, to reduce moisture content in the extracted starch resulting in the production of cassava flour or other cassava-based products that are essential in food and industrial applications [16]. The environmental risks of the resulting effluents are evident, as demonstrated by the potential of solid waste dumps leachate to contaminate groundwater with bacteria and heavy metals [17], and the microbial and physicochemical quality of wastewater [18]. Research has also explored the ecological effects of palm oil mill effluents [19] and the isolation of fungal species from pesticide-contaminated soil [20]. The general ubiquity and harmful effects of microorganisms are widely recognized [21].

Anaerobic digestion AD) has been widely recognized as a sustainable biotechnological process for managing organic wastes while producing renewable bioenergy in the form of biogas [22]. Nonetheless, cassava mill effluent (CME) alone is often unsuitable for efficient digestion due to its high acidity, cyanide content, and poor nutrient balance. Co-digestion with livestock manure has been shown to improve microbial activity, optimize the carbon-to-nitrogen (C/N) ratio, and enhance methane yield [23]. Pig manure (PM), in particular, is nutrient-dense and serves as a buffering substrate that can neutralize the inhibitory compounds in cassava waste. Previous studies have highlighted the influence of different surfactants on hydrocarbon-utilizing bacteria [24] and the effect of biosurfactants on crude oil degradation [25], indicating the role of biological and chemical enhancers in degradation processes. Furthermore, enzyme activity, such as peroxidase production by *Aspergillus* species, has been studied in petroleum hydrocarbon-spilled soil [26], and protease activity has been optimized in a local *Aspergillus niger* strain [27]. The hydrocarbon-degrading potentials of indigenous bacteria [3] and the utilization of unique materials like used GSM recharge cards for cellulase production [28], as well as the potential of local raw materials for mycological media production [29] and the characterization of proteolytic enzymes from fungi [30] have been documented. Research into the biosorption of heavy metals like hexavalent chromium by *Pseudomonas aeruginosa* [31] is also relevant to detoxification. The application of biological products extends to harnessing the antifungal properties of *Ocimum gratissimum* for paint formulations [32].

Despite the promising potential of co-digestion, studies in Nigeria remain limited, especially those integrating microbial dynamics, heavy metal detoxification, nutrient enrichment, and biogas quality assessment. Previous works have focused mainly on energy yield without fully addressing the detoxification capacity of AD or the agronomic value of the digestate [33].

This study, therefore, investigates the anaerobic co-digestion of cassava mill effluent and pig manure, for biogas generation which will mitigate the adverse environmental impacts associated with untreated cassava mill effluents and enhance the renewable energy resource that will help mitigation against climate change.

Materials and Methods

Study area

The study was carried out in microbiology laboratory, Nnamdi Azikiwe University in Awka South LGA of Anambra State, Nigeria. Awka is in the tropical rainforest region and is located between latitude 6° 12'N and longitude 7° 06'E. The climate is humid with average daily relative humidity of 79.4%, annual rainfall of 2000-3000mm and average daily maximum and minimum air temperatures of 32.2˚C and 23.3˚C respectively. Awka area consists of low-lying plains of agricultural land. It has derived savanna vegetation resulting from human activities. The people are mainly farmers, itinerant traders, craftsmen, and civil servants. Agricultural crops include yam, cocoyam, cassava, maize, fruits, and vegetables. Palm produce, coconut and kola nuts are their main cash crop. They also combine crop cultivation with animal rearing especially goat, sheep, and cattle. Awka community is an urban town in south-eastern Nigeria. It has an estimated population of about 176,858. Awka community has many satellite villages that are linked by a poor road network. For the most part, villages have poorly developed infrastructures, though often with access to electricity but no pipe-borne water. Awka communit has two Local Government Areas: Awka North and Awka South. Nise is in Awka South LGA, and it consists of four villages namely: Ngodo, Arah, Umuazu, and Isiakpu. Arah village is situated in Nise, with geographical coordinates 6.1618° North, 7.0525° East and postal code 420106.

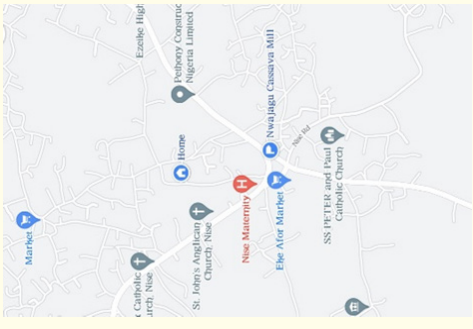

Figure 1: Google map showing Nwajagu cassava mill, Arah village Nise.
(Source: Google Map, 2022).

Sample collection

The cassava mill effluent and pig manure were collected from cassava processing mill and livestock farm established by the family of Ajagu Elele and Raymond Onyiuka from Umuenweze kindred Ara village Nise and are operated by Chimezie Elele and Mr. Raymond Onyiuka, member of the family and indigenes of the community. A sterile four (4) liter plastic container was used to collect the cassava mill effluents and a sterile plastic bucket was also used to collect the pig manure. The samples were immediately transported to the laboratory for microbiological and physiochemical analyses.

Enumeration of total heterotrophic bacterial and fungal counts

This was determined using 10-Fold serial dilution method Smith., *et al*. [34]. Exactly 90 ml nutrient broth was prepared and sterilized, then 9 ml of the nutrient broth was aliquoted into two test tubes (10^{-1} and 10^{-2}). Ten-fold serial dilution of 10 ml of cassava mill effluent sample was prepared with 90 ml of the nutrient broth and allowed to rest for 10-30 minutes. 1 ml from the 100 ml solution was added into 10^{-1} test tube and then transferred into 10^{-2} test tube. 0.1 ml of the sample was inoculated into Nutrient agar, Sabouraud dextrose agar, Cetrimide agar base and Mannitol egg yolk polymyxin agar base plates in duplicate labelled 10^{-1} and 10^{-2} using pour plate method for bacterial and fungal enumeration respectively. The inoculated plates were sealed up and incubated at room temperature of 37°C for 24-72 hours for the enumeration of the total heterotrophic bacterial and fungal counts respectively. The results were expressed in colony forming units per milliliter (cfu/ml) of the sample.

Characterization and identification of bacterial isolates

Discrete colonies of bacterial and fungal isolates were purified by subculturing thrice into freshly prepared nutrient and sabouraud dextrose agar plates respectively. Pure cultures of bacterial isolates were characterized and identified based on their cultural, morphological, and biochemical characteristics by standard methods described by Garcia., *et al*. [35] and Bauer., *et al*. [36]. Representative colonies of bacteria isolates were evaluated using morphological characteristics on media such as shape, color, margin, and elevation.

Biochemical characteristics

The Bergey's manual of determinative bacteriology was used as described by Nwakoby., *et al*. [37]. The bacterial isolates were also identified based on biochemical characteristics by carrying out the following biochemical test.

Gram staining

A drop of water was added on clean, non-greasy, dust free sterile slides. Thin smear of the isolate was made on the slides, air dried and heat fixed. The smear was flooded with crystal violet and washed off after 1 minute with gentle running water. The slide was flooded with 1% of Gram's iodine (mordant) and washed off after 1 minute. The slide was decolorized with ethanol and washed off immediately. The slide was counter-stained with safranin and washed off after 1 minute and allowed to dry. The slide was viewed under oil immersion lens microscope (x 100). Purple color indicated Gram positive organisms while red or pink color indicated Gram negative organisms.

Catalase test

The materials used include inoculating loop, dry clean grease free glass slide, 3% hydrogen peroxide (H_2O_2), Bunsen burner, 24-hour bacterial culture. The test is used to detect the presence of catalase, an enzyme that catalyze the release of oxygen from hydrogen peroxide solution when broken down. Exactly 3 ml of 3% solution of hydrogen peroxide was transferred into a sterile tube. Then 3 loopful of a 24-hour pure culture (B_1 and B_2) of the test bacteria were inoculated into the test tube. The tube was observed for immediate bubbling indicating positive and no bubbling indicating negative. In the presence of catalase (enzyme), hydrogen peroxide is reduced to water and oxygen.

$$2H_2O_{2(aq)\ catalase} \qquad 2H_2O_{(l)} + O_{2(g)}$$

Citrate utilization test

The materials used include Simmon citrate agar, 24-hour culture, inoculation loop, Bunsen burner, test tubes, measuring cylinder. This test is based on the ability of an organism to utilize citrate as its sole carbon and energy source then ammonium as sole source of nitrogen. Exactly 1.5 gram of Simmon citrate agar was prepared in 50 ml of water, mixed and boiled to thicken. Then the mixture was poured into test tube. The isolates (B_1 and B_2) were inoculated into the mixture and incubated for 24 hours. The tube was observed. A positive test was indicated by a change of color from green to blue on the surface of the medium. No color change indicated negative reaction.

Coagulase test

The materials used include bacterial culture, blood serum, inoculation loop, glass slide. To determine the ability of a bacterial species to produce the enzyme coagulase. Using a sterile loop, a small amount of each bacterial culture (B_1 and B_2) was transferred to a glass slide containing blood serum. Swelling indicate a positive reaction.

Indole test

The materials used include micropipette, tips, bacterial culture, peptone, Kovac's reagent, inoculation loop. To detect the ability of bacteria to produce indole, a byproduct of tryptophan metabolism. Exactly 0.75 gram of peptone-TBL was prepared in 50 ml of water, mixed and sterilized. Then the mixture was poured into test tubes, isolates (B_1 and B_2) were inoculated and incubated for 24 hours after which 0.5 ml of Kovac's reagent was added and left for 10 minutes. The test tube was observed. A ring of red color in the surface layer within 10 minutes is indicative of a positive reaction and absence of red color is indicative of negative reaction.

Methyl red test

The materials used include sterile test tubes, methyl red reagent, MR-VP medium, inoculation loop. The test is to determine the ability of an organism to perform mixed acid fermentation, specifically the production of stable acid by-products from glucose metabolism. Exactly 1.5 gram of MR-VP medium was prepared in 50 ml of water, mixed and sterilized. Then poured into test tubes after which the isolates (B_1 and B_2) were inoculated and incubated for 24 hours. 5 drops of methyl red reagent were added to the solution. The production of a bright red color is indicative of a positive test and yellow color is indicative of negative test after vigorous shaking.

Motility test (hanging drop method)

The materials used include test tube, distilled water, inoculating needle, Bunsen burner, cotton wool, nutrient agar, and pipette. The test is used to check if the bacterial isolate is motile or non-motile. The agar stab technique was used. 1.4 gram of nutrient agar was prepared in 50 ml of water, mixed and sterilized for 15 minutes. The medium was poured in a test tube and allowed to gel. The isolates (B_1 and B_2) were picked using inoculating needle and stabbed into the gelled agar, incubated for 24 hours. The tube was observed, and the results were recorded as motile and non-motile.

Voges and Proskauer test

The materials used include potassium hydroxide (KOH), ethanol, alpha-naphthol, distilled water, bacterial culture, MR-VP medium,

test tubes, inoculation tube. The test is to detect the production of acetoin, a metabolic product of glucose fermentation in certain bacteria. Exactly 0.75 gram of MR-VP medium was prepared in 25 ml of water, mixed and sterilized. The mixture was poured into test tubes, the isolates (B_1 and B_2) were inoculated, and incubate for 24 hours. 0.2 ml of prepared KOH (1.2 gram of KOH in 3 ml of water) and 0.5 ml of prepared ethanol and alpha-naphthol (9.5 ml of ethanol, 0.5 ml of water, 0.5 gram of alpha-naphthol) were added, mixed, and observed after 15 minutes. The presence of red color after 15 minutes is indicative of positive test which shows the presence of diacetyl, the oxidation product of acetoin (test was always considered invalid after one hour because VP-negative cultures may produce copper-like color, which gives false positive result), lack of pink-red color denoted a negative reaction.

Identification of the fungal isolates

Lactophenol cotton blue staining technique

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.

Physiochemical composition of cassava mill effluents and pig manure

Determination of total organic carbon

The Walkley-black titration method as described by Poudel [38] was used. Exactly 2g of the sample was measured into a beaker containing 5 ml of 0.4M potassium dichromate solution ($K_2Cr_2O_7$) followed by addition of 10 ml of the concentrated sulfuric acid. The mixture was gently swirled and left at room temperature n 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.

Determination of heavy metals

Standard methods described by Edori and Edori [39] was used. The standard stock solutions that contain 1000ppm of element in 2N nitric acid was used to prepare standard solutions of lead (Pb), iron (Fe), chromium (Cr), nickel (Ni) and cadmium (Cd). Atomic Absorption Spectrophotometer (AAS) was used to determine the presence and level of the elements. The calibration curve prepared for each of the elements by least square method for the determination of the heavy metals' concentration in the sample. 100mg/l intermediate standard solution of each metal was prepared from the stock solutions of Pb, Fe, Cd, Cr, and Ni. Serial dilution of the intermediate stock solution by extraction were used to prepare adequate working standards for each metal solution which was followed by the determination by the AAS by aspirating the solution into the Spectrophotometer and the absorbance was noted.

Determination of cyanides

Standard methods described by Edori and Edori [39] was used. HCN (1g) 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. 2 ml 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 stoppered test tube in boiling water bath. The sample was cooled in ice cold water, with the stopper loosely in place. Exactly 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 nutritional contents of cassava mill effluents

Determination of crude proteins

The indirect Kjedahl method as described by Hanne., *et al.* [40] was used. Exactly 1g of sample was weighed into a 300 ml Kjedahl 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 100 ml of distilled water was added to avoid caking and then 5 ml of the filtrate and 5 ml of 40% NaOH was transferred to the Kjedahl distillation apparatus. A 250 ml receiver beaker containing 10 ml of 10% boric acid and indicator mixture containing 5 drops of bro-

mocresol blue and 1 drop of the methylene blue was placed under a condenser of the distillation apparatus so that the tap was about 20 cm inside the solution. Then 5 ml of 40% sodium hydroxide was added to the digested sample in the apparatus and distillation commenced immediately until 50 drops get into the receiver beaker after which it was titrated to pink color using 0.01N hydrochloric acid.

Determination of carbohydrate

The colorimetric method was used. Exactly 2.5 g of sample was weighed into a flask, 50 ml of cold water was added and allowed to stand for 1 hour. 20 ml of concentrated HCl and 150 ml of distilled water was added and refluxed for 2 hours in a 250 ml round bottom flask, cooled, and neutralized with 5N NaOH and made up to mark. The carbohydrate content was determined using anthrone reagent. Exactly 5 ml of anthrone reagent was added to 1 ml of the solution, covered, and boiled in water bath for 20 minutes for color development. The absorbance was read at 620 nm using ultraviolet spectrophotometer.

Determination of nitrogen, phosphorus, and potassium

Standard methods described by Edori and Edori [39] was used. One hundred (100mg) of the digestate were digested in 2 ml of sulphuric acid in a 500 ml digester at 330^0C 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 50 ml 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.

Determination of fatty acid

Exactly 2g of the sample was measured into beaker containing 20 ml 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.

Composition of Biogas digester

The digestion was composed as described by Onuorah., *et al.* [41]. The materials used include one Drip set, Glue, thermometer, bowl with cover and 3 bottles. This digester is used to be able to detect methane gas from cassava mill effluent and pig manure. A locally made anaerobic digester system was employed. The digester was equipped with temperature control, agitation mechanisms to maintain optimal conditions for microbial activity during the digestion process. Exactly 400g of pig manure was mixed with 500 ml of cassava mill effluent, poured into local digester and allowed to stand for the period of 0-32 days. 25 ml of calcium hydroxide (CaOH) (1.48g in 100 ml of water) was added to the first container near the bowl to trap carbon dioxide (CO_2). Samples were collected from the digester and analyzed for heavy metals, cyanide, and nutritional value on the day 0 and on the day 32 of the digester composition. The pH reading, temperature reading, and flammability test was done every four days intervals for 32 days. Thermometer (comet borosilicate glass mercury thermometer), calibrated pH meter and gas lighter were used to check the temperature, pH, and flammability tests respectively. Microbial count was done on the day 0 and day 32 which is the last day of the setup.

Results

The isolates include both bacteria and fungi. Two (2) bacteria and two (2) fungi were isolated from cassava mill effluents. They include *Staphylococcus aureus, Bacillus cereus, Aspergillus* spp and *Candida* spp.

Discussions

The present study demonstrated that the anaerobic co-digestion of cassava mill effluent (CME) and pig manure (PM) significantly improved biogas production potential, reduced environmental toxicants, and enhanced nutrient quality of the digestate. The initial acidic condition (pH 6.5) shifted to alkaline (pH 8.9) by the end of digestion, which is consistent with the methanogenic phase of anaerobic digestion, where acidogenic products are converted into methane and carbon dioxide. This observation agrees with Adekunle and Okolie [1], who reported that an alkaline environment is crucial for methanogens to thrive, ensuring optimal methane yield. The reduction of toxicants, including cyanide (−6.7%) and fatty acids (−37.7%), indicates microbial detoxification and substrate stabilization during digestion. Cassava residues are known to contain cyanogenic glycosides, which limit their direct use in biogas production [33]. The significant reduction observed in this

Table 1: Physicochemical Parameters of Substrate before and after Anaerobic Digestion.

Parameters	Day 0 (Initial)	Day 32 (Final)	% Change	p-value
pH	6.50 ± 0.05	8.92 ± 0.07	37.2	<0.01
Cyanide mg/l	75.0 ± 0.8	70.0 ± 0.6	6.7	<0.05
TOC %	5.33 ± 0.12	5.47 ± 0.09	2.6	-
Fatty acids (µg/mL)	158.9 ± 1.2	98.9 ± 0.9	37.7	<0.01

Table 2: Heavy Metal Concentrations (ppm).

Metal	Day 0 (± SD)	Day 32 (± SD)	% Reduction	p-value
Lead	0.487 ± 0.02	0.212 ± 0.01	56.5	<0.01
Chromium	1.929 ± 0.05	0.034 ± 0.01	98.2	<0.001
Cadmium	0.014 ± 0.01	0.001 ± 0.00	92.9	<0.05
Iron	0.025 ± 0.01	0.019 ± 0.01	24.0	-

Table 3: Nutrient Composition of Digestate.

Nutrients	Day 0 (Initial)	Day 32 (Final)	% Change	p-value
Protein	5.60 ± 0.2	6.30 ± 0.3	12.5	<0.05
Carbohydrate	94.2 ± 2.1	100.6 ± 2.4	6.8	<0.05
Nitrogen (%)	0.896 ± 0.05	1.008 ± 0.04	12.5	<0.05
Phosphorus (%)	0.002 ± 0.00	0.005 ± 0.00	150	—
Potassium (%)	0.041 ± 0.00	0.048 ± 0.00	17.1	—

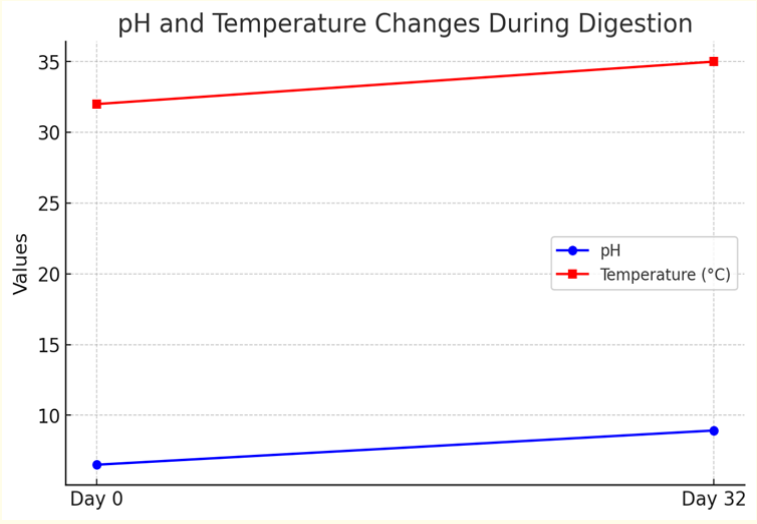

Figure 2

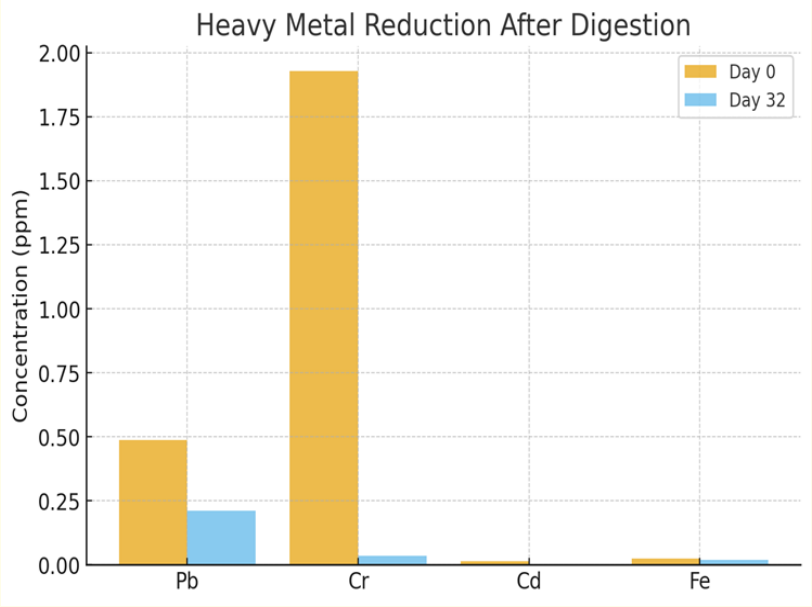

Figure 3

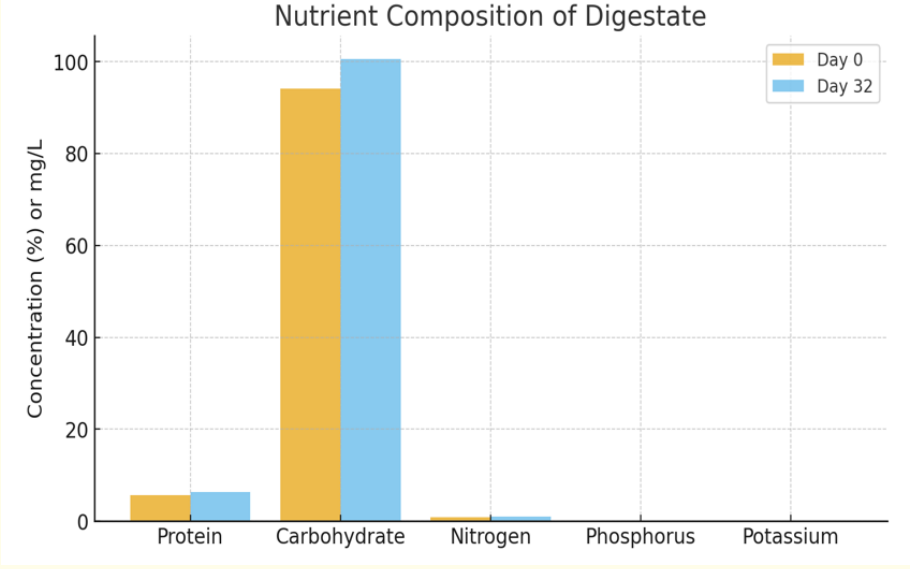

Figure 4

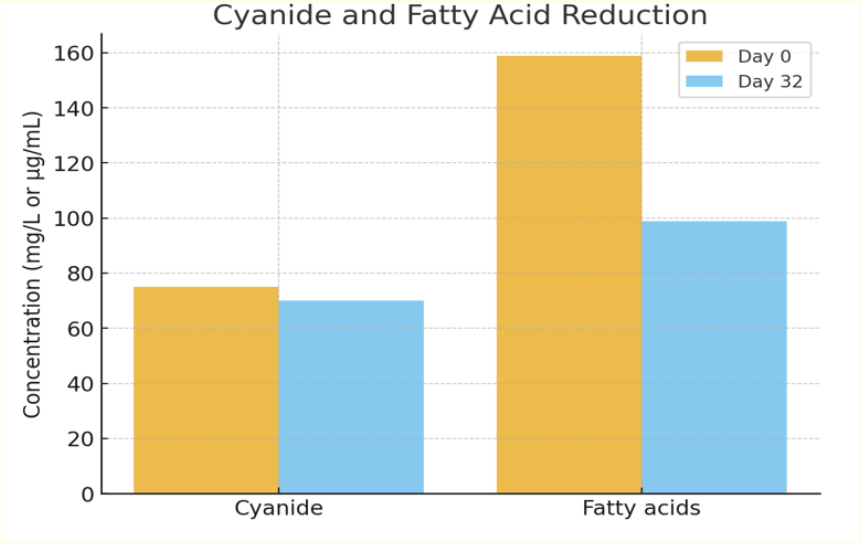

Figure 5

Table 4: Thirty-two-days digestion of CME and pig manure.

Duration	Cumulative concentration of the parameters of CME and pig manure digestion				
	Temperature (°C)	pH value	Flammability test	Bacteria	Fungi
Day 0	37	6.50	-	1.6×10^4	1.3×10^4
Day 4	37	6.59	-	ND	ND
Day 8	32	6.88	-	ND	ND
Day 12	34	7.61	-	ND	ND
Day 16	32	8.54	-	ND	ND
Day 20	29	8.92	-	ND	ND
Day 24	32	8.10	-	ND	ND
Day 28	33	7.88	-	ND	ND
Day 32	31	8.92	-	4×10^3	3.4×10^3

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

study suggests that pig manure provided buffering nutrients that enhanced microbial degradation of inhibitory compounds, thereby creating favorable conditions for methanogenesis.

Heavy metal concentrations decreased markedly, particularly chromium (−98.2%) and cadmium (−92.9%). This aligns with findings by Zhang., *et al*. [23], who showed that anaerobic digestion can immobilize or transform heavy metals through processes such as biosorption, precipitation, and chelation, thereby lowering their bioavailability. Such reductions are critical for the safe application of digestate as organic fertilizer. Nutrient enrichment increased in protein, carbohydrate, and nitrogen content, confirming the agronomic potential of the digestate. Similar improvements in nutrient recovery have been reported by Akinyele., *et al.* [22], who

emphasized the role of co-digestion in balancing the carbon-to-nitrogen ratio, stimulating microbial activity, and enhancing digestate quality. This nutrient enhancement supports the integration of anaerobic digestion into circular bioeconomy models, where waste streams are converted into renewable energy and organic fertilizers. Microbial analysis revealed the persistence of species such as *Bacillus cereus*, *Staphylococcus aureus*, *Aspergillus* spp., and *Candida* spp. While some of these organisms are potential pathogens, their reduced counts by Day 32 suggest microbial succession and stabilization, consistent with earlier reports that anaerobic digestion lowers pathogen loads in livestock and agro-industrial wastes (Adekunle and Okolie, 2021). Overall, the findings underscore the dual benefits of anaerobic co-digestion of CME and PM: production of renewable energy in the form of biogas, and generation of nutrient-rich, less-toxic digestate suitable for agricultural application. Statistical analysis revealed that pH increase (6.50 → 8.92) was **statistically significant** ($t(2) = 9.12$, $p < 0.01$). Heavy metals decreased significantly, especially Cr ($p < 0.001$), confirming detoxification potential of anaerobic digestion. Nutrient enrichment (Protein, Carbohydrates, Nitrogen) was significant ($p < 0.05$), supporting fertilizer value of digestate. Cyanide decreased modestly (6.7%), but enough to reduce toxicity ($p < 0.05$). TOC showed no significant change, suggesting stable organic matter mineralization.

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