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EVALUATION OF *Artocarpus altilis* (BREADFRUIT) HULLS, *Ipomoea asarifolia* (GINGER-LEAF MORNING GLORY) LEAVES AND COW DUNG FOR BIOGAS PRODUCTION

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ABSTRACT

The generation of biogas via the co-digestion of *Artocarpus altilis* (breadfruit) hulls and *Ipomoea asarifolia* (ginger-leaf morning glory) leaves was investigated using cow dung as inoculum. The aim to evaluate biogas production from the co-digestion of the feedstock combination, as these feedstocks have not been utilised in biogas production. The substrates were pre-treated and mixed with water using a 1:1 ratio. A locally constructed bioreactor with a capacity of 16 litres was used. A batch-feed system was employed with a hydraulic retention time of 4 weeks (30 days). An inoculum-to-substrate ratio (ISR) of 1:1 was used. The proximate compositions and physicochemical analyses were evaluated, while the microorganisms were isolated and characterised using microbiological standard methods. The results of proximate composition analysis indicated that the substrates were suitable for biogas production. The physicochemical analysis revealed that the total and volatile solids decreased throughout the digestion period, while a pH range of 5.3 to 7.5 and a temperature range of 30ºC to 34ºC were observed. A mean biogas yield of 2.0 mL, which was flammable, was obtained. The bacterial counts for total aerobic and anaerobic bacteria ranged from $2.5x10^5$cfu/g to $2.9x10^6$cfu/g and $9.0x10^4$cfu/g to $4.0x10^5$cfu/g, respectively. The bacterial isolates were identified as *Bacillus* sp., *Escherichia coli*, *Clostridium* sp., *Lactobacillus* sp., and *Streptococcus* sp. Biogas production was successfully obtained in this study, indicating that *Artocarpus altilis* (breadfruit) hulls and *Ipomoea asarifolia* leaves are good substrates for biogas production.

Keywords: Anaerobic co-digestion, Breadfruit hulls, Cow dung, *Ipomoea asarifolia*, Renewable energy.

1. Introduction

Processing of crops for food production and other agricultural activities can generate substantial amounts of waste. These waste types are composed largely of lignocellulosic materials, such as breadfruit hulls, bran and rice husk, groundnut husk, grasses, etc (Salihawa *et al*., 2024). The improper disposal of these generated

wastes is of public health concern (Ezenwa *et al.*, 2024). Therefore, a reliable and sustainable means of waste management in Nigeria is of paramount importance.

The demand for renewable fuel is increasing with growing concern about climate change, air quality, energy import dependence and the depletion of fossil fuels (Islam *et al.*, 2026). Biogas is among the versatile renewable fuels used for power and heat generation, or it can be upgraded to biomethane and used as vehicle fuel (Rilwanu *et al*., 2025). Biogas is produced by the anaerobic digestion of biological wastes (Kumar *et al*., 2024) to give mainly methane (50-70%), carbon dioxide (20-40%) and traces of other gases such as nitrogen, hydrogen, ammonia, hydrogen sulphide, water vapour, etc (Djimtoingar *et al.*, 2023). The composition of biogas depends on the source of feedstock and the management of the anaerobic digestion process (Ukaegbu-Obi *et al*., 2023). The anaerobic digestion of organic materials has long been used to generate valuable resources which have been harnessed for human benefit (Yusuf *et al*., 2026).

Nigeria has been reported to heavily rely on charcoal and firewood for domestic use (Obada *et al.*, 2024). This has reportedly led to massive deforestation in the country. From a One Health perspective, the health of people, our communities, and the environment in Nigeria needs a shift away from fossil fuels to an efficient, sustainable, and renewable-based energy system and should be considered a necessity (Obiorah *et al*., 2025). The energy in plant vegetation, animal manure, industrial and domestic waste can be released in terms of a useful gas when fermented anaerobically in the absence of oxygen. This can be used in a country like Nigeria that is richly blessed with agricultural residues (Salihawa *et al*., 2024).

Ipomoea asarifolia, commonly called “ginger-leaf morning-glory” or “the railway creeper”, is a species in the genus *Ipomoea*, which is the largest genus in the family Convolvulaceae. This genus comprises approximately 500-700 species worldwide (Bolarinwa *et al*., 2018). The distribution of this genus covers the tropical and subtropical regions of the world. Only two species occur in Europe, while 37 species occur in West Africa and of these, 30 were found in Nigeria (Mukhtar *et al*., 2022). The plant comprises annual and perennial herbaceous plants, lianas, shrubs, and small trees (Bolarinwa *et al*., 2018). It is a common weed (not edible) of hydromorphic soils, low-lying and long-trailing along the ground in sandy, waste places, inland valleys, streams and river banks, found throughout West Africa (Shehu *et al*., 2019).

Its common names include ‘Duman kada’ in Hausa, ‘Gboro ayaba’ in Yoruba and ‘Ndukwu ohia’ in Igbo languages (Bello *et al.*, 2025).

Breadfruit is a fruit-producing plant which is native to Polynesia. The plant belongs to the *Moraceae* family of about 50 genera and over 1000 species (Mehta *et al.*, 2023). The processing of breadfruit produces hulls and edible seeds. The hulls, which are unpalatable, can be used as an absorbent for toxic dyes (Mehta *et al.*, 2023). The hulls are usually indiscriminately discharged into the environment and amassed as waste dumps on sites where breadfruits are processed. The increased production of hulls during breadfruit harvest season poses a significant environmental risk due to pollution. Different agro-wastes and grasses have been evaluated as substrates for biogas production. Akinola *et al.* (2025) worked on the co-digestion of cow dung with yam peels, maize husk, and cassava peels. Kuleve *et al.* (2025) investigated the co-digestion of cattle dung and maize cob waste to enhance biogas production. Muwarure *et al.* (2024) assessed the potential of carpet grass to produce biogas. They reported that the purified biogas powered a 2.5 kW generator for 30 minutes. Souvannasouk *et al.* (2021) co-digested Napier grass and cow farm slurry to produce biogas.

There is, therefore, a paucity of information on the co-digestion of breadfruit hulls and ginger-leaf morning glory leaves for biogas production. This research aimed to evaluate the potential of breadfruit hulls and ginger-leaf morning glory leaves to produce biogas. This study aligns with SDG 7, affordable and clean energy.

2. Materials and Methods

2.1. Construction of Anaerobic Digester

The anaerobic digester used in this study was a 16L capacity plastic-type digester (Figure 1). It was locally constructed using a water dispenser container connected to a one-litre drip bag for gas collection.



Figure 1: The experimental set-up.

2.2. Collection of Substrates

The substrates used in this study were *Artocarpus altilis* (breadfruit) hulls, *Ipomoea asarifolia* (Ginger-leaf morning glory) leaves and cow dung, which served as the inoculum. The *Artocarpus altilis* (breadfruit) hulls were obtained from a local breadfruit processor in Orie-Ugba market in Umuahia, Abia State, in sterile nylon bags. The breadfruit hulls were immediately taken to the Microbiology laboratory, Michael Okpara University of Agriculture, Umudike, Abia State, for analysis. *Ipomoea asarifolia* were collected from the premises of the College of Natural Sciences in the Michael Okpara University of Agriculture, Umudike. Fresh cow dung was collected from the cow farm at Michael Okpara University of Agriculture, Umudike, in a sterile container and immediately taken to the laboratory for analysis.

2.3 Pre-treatment of Substrates

The *Artocarpus altilis* (breadfruit) hulls were coarsely milled using a sterile electric blender, Silver Crest 2-in-1 8500w. The *Ipomoea asarifolia* (Ginger-leaf morning glory) leaves were chopped with a sterile knife into smaller sizes. It has been found that smaller particles increase the rate of biogas production and affect hydraulic retention time (Olatunji *et al.*, 2022).

2.4 Preparation of Samples

Milled *Artocarpus altilis* (breadfruit) hulls (2kg), chopped *Ipomoea asarifolia* (Ginger-leaf morning glory) leaves (2kg), and cow dung (2kg) were weighed out respectively and mixed with water in the ratio of 1:1 to form a homogenised slurry as described by Yin *et al.* (2023).

2.5 Charging of the Digester

The slurry was charged to three-quarters of the digester volume, leaving a quarter-head space for gas collection. The digester was appropriately sealed to ensure an anaerobic environment. The content was manually shaken periodically during the retention period to ensure contact with the substrate and microbes. A hydraulic retention time (HRT) of 4 weeks (30 days) was employed using the batch-feed system.

2.6 Determination of Proximate Composition

The following proximate components, Moisture content, Ash content, Crude Protein, Crude Fibre, Fat and Carbohydrate were determined according to the methods of A.O.A.C. (2005).

2.7 Determination of Physicochemical Parameters

The pH, temperature, total solids (TS), and volatile solids (VS) were determined. The pH and temperature of the slurry were monitored using a pH meter (HANNA, HI98107) and a mercury-in-glass thermometer (0-100°C), respectively. Total solids (TS) and volatile solids (VS) were determined according to *Standard Methods for the Examination of Water and Wastewater* (APHA, 2012). Samples were collected weekly.

2.8 Microbiological Analysis

2.8.1 Isolation and enumeration of culturable bacterial isolates

Ten-fold serial dilutions of the slurry samples were prepared using 0.85% sterile physiological saline. From each dilution of 10^{-3} to 10^{-6}, aliquots (0.1ml) were aseptically plated on sterile Nutrient agar and Reinforced Clostridium agar in duplicates using the spread plate method. Nutrient agar plates were incubated aerobically at 37°C for 24 hours, while Reinforced Clostridium agar plates were incubated anaerobically using an anaerobic jar at 37°C for 48 hours as described by Mukhtar *et al*., 2018. Colonies were counted and expressed as colony-forming units per gram (CFU/g).

2.8.2 Characterisation of Bacterial Isolates

Pure stock cultures of the bacterial isolates were examined for colonial appearance and biochemically characterised using the following tests: Gram staining, Motility test, Catalase test, Citrate Utilisation test, Indole test, Hydrogen Sulphide Production test, Methyl Red – Voges Proskauer test, Oxidase test, Sugar fermentation test. Confirmatory identities of the bacteria were made using Bergey's Manual of Determinative Bacteriology (Boone and Castenholz, 2012).

2.9. Flammability test

A flame test was used to determine the rate of burning of gas produced from the biodigester. The quality of the biogas produced was tested by its flame colour.

3. Results and Discussion

3.1 Results of the proximate composition analysis

The results of the analysis of the proximate composition of the untreated slurry before anaerobic digestion are presented in Table 1. All values are expressed on a percentage wet weight basis.

Table 1: Proximate Composition of Untreated Slurry Before Digestion (Wet Weight Basis)

Parameter	**Value (%)**
Moisture	60.88
Crude Protein	6.62
Crude Fat (Ether Extract)	1.26
Crude Fibre	12.66
Ash	3.82
Carbohydrate (by difference)	14.76
Total	**100.00**

The moisture content of the untreated or undigested slurry was 60.88%. This indicates a high moisture content, though values above 80% are more typical for conventional wet digestion. The high moisture content is necessary for microbial activity and substrate solubilisation within the digester. Kumar *et al*. (2024) opined that high moisture levels uniquely facilitate the anaerobic digestion process. The crude protein content was measured as 6.62%. The low crude protein content could be due to the presence of cow dung (inoculum), as *Artocarpus altilis* (breadfruit hulls) and *Ipomoea asarifolia* (Ginger-leaf morning glory) leaves are high lignocellulosic feedstocks. Adequate nitrogen content in the substrate is important for maintaining an optimal carbon-to-nitrogen (C/N) ratio. This is critical for digester stability and biogas yield. This differs from the findings of Rilwanu *et al.* (2025), who reported a crude protein of 9.50, likely due to differences in feedstock composition. The crude fat content was low at 1.26% while the crude fibre content was 12.66%. This reflects the inherently lignocellulosic nature of the feedstock. In contrast, Rilwanu *et al*. (2025) reported a crude fibre content of 6.23%, which differs from the value obtained in the present study. This variation could be attributed to the type of feedstock materials used. Hulls and leaves are largely made of cellulose and lignin. The high fibre content is significant as it represents the primary organic substrate available for microbial degradation during anaerobic digestion. The ash content was 3.82%, representing the inorganic mineral fraction of the slurry. This indicates the presence of essential minerals such as potassium, calcium, and phosphorus derived from the feedstock materials. These minerals may serve as micronutrients that support the metabolic activities and stability of microbial consortia in the digester (Salazar-Batres *et al.*,2025). Carbohydrate content, determined by difference, was 14.76%, representing soluble and structural carbohydrates in the feedstock. The proximate composition analysis results of the untreated slurry indicate that the substrates are suitable for anaerobic digestion.

3.2 The findings of the physicochemical parameters analysed

The findings of the physicochemical parameters, pH, temperature, total and volatile solids analysed are shown in Figures 2 to 5.

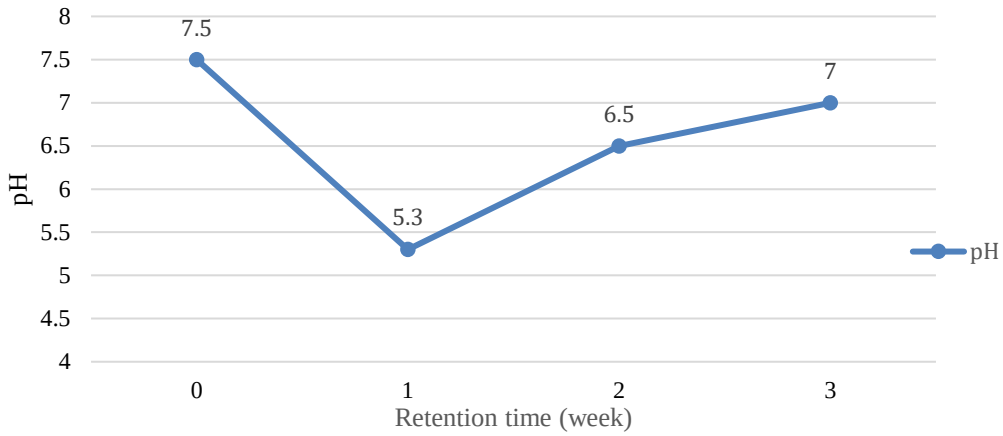


Fig. 2. pH values during the co-digestion of *Artocarpus altilis* hulls and *Ipomoea asarifolia*

The pH values recorded (Fig. 2) ranged from 5.3 to 7.5. This was in line with the findings of Djimtoingar *et al.* (2023). They recorded a pH range of 5.6 to 7.4 and ascribed the stabilisation of pH to the buffering effect of the cow dung. The observed fall in pH values within weeks 0 and 1 can be attributed to the activities of the hydrolytic and acidogenic bacteria which degrade organic matter to produce volatile fatty acids. The significant rise in pH as the retention time progressed (weeks 2 and 3) is consistent with the study of Yin *et al*. (2023), who reported a similar rise in pH during the co-digestion of rice straw and cow manure. Burg *et al.* (2018) opined that the conversion of acetogenesis by-product to methane, along with the ammonium compounds from protein degradation, raises the pH. On the contrary, Muwarure *et al*. (2024) reported the pH range of 4.1 to 5.2 over 30 days in their study, assessing the potential of cow dung and carpet grass as feedstock for biogas production to power a generator.

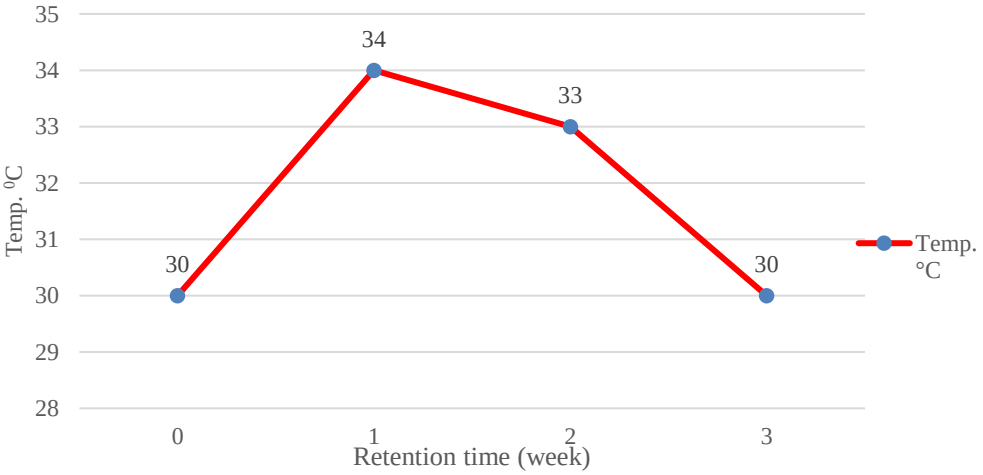


Fig. 3. Temp.^{0}C variations during the co-digestion of *Artocarpus altilis* hulls and *Ipomoea asarifolia*

The temperature readings in this study, as shown in Fig. 3, were within the mesophilic range. It fluctuated between 30°C and 34°C. Temperature remains a key driver in the anaerobic digestion process, as it has a correlation to the enzymatic actions of microbial communities present. The findings of the present study are in line with previous works of Salihawa *et al.* (2024), who reported temperature fluctuations ranging

from 32.5 °C to 37.7 °C in anaerobic digestion of maize husk in co-digestion with goat and cow dung; and Ukaegbu-Obi *et al* (2023), who reported a temperature range of 30 ^{o}C to 35 ^{o}C in anaerobic digestion of cassava peels. Muwarure *et al*. (2024) observed that warmer temperatures promote faster microbial activity and lead to higher biogas production.

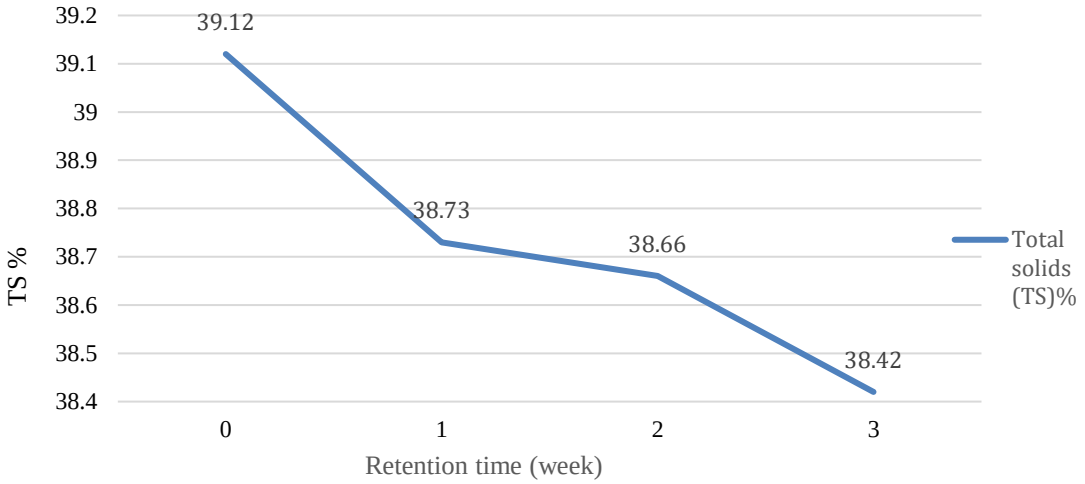


Fig. 4. Total solids (TS) during the co-digestion of *Artocarpus altilis* hulls and *Ipomoea asarifolia*

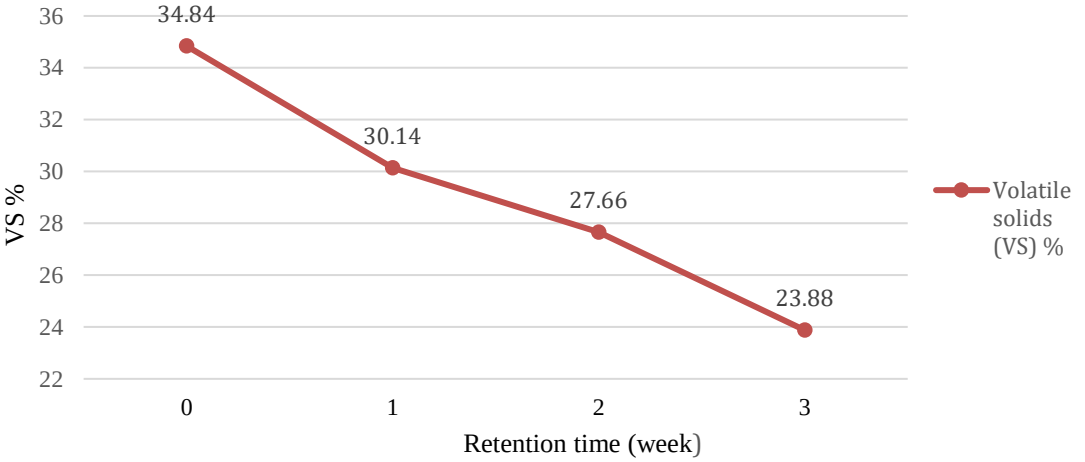


Fig. 5. Volatile solids (vS%) during the co-digestion of *Artocarpus altilis* hulls and *Ipomoea asarifolia*

The Total solids (TS) and Volatile solids (VS) contents, 39.12% to 38.42% and 34.88% to 23.88%, respectively, as depicted in Figures 2 and 3, decreased throughout the digestion retention time. This substantiates the studies of Ukaegbu-Obi *et al.* (2023) and Yusuf *et al.* (2026), who opined that the reduction in Total and Volatile Solids suggests the utilisation and conversion of organic matter by microbes. This leads to higher biogas production and efficiency of the AD process. This study, in line with the findings of Yusuf *et al.* (2026), who opined that a large proportion of biodegradable organic matter is bound in VS.

3.3 The Results and Interpretations of Microbiological Analysis.

The bacterial load (cfu/g) of the slurry is determined and shown in Fig. 6. The description of the colonial and biochemical characteristics of the bacterial isolates is shown in Table 2.

Table 2. Colonial Description and Biochemical Characteristics of the Bacterial Isolates

Colonial Description	Gram Reaction & Morphology	Catalase	Oxidase	Indole	MR	VP	Citrate	Glucose	Lactose	Sucrose	Mannitol	Likely Organism	Isolation Condition
Pink/red colonies on MCA, smooth, convex on NA	-ve Rod	+ve	-ve	+ve	+ve	-ve	-ve	AG	AG	A	A	*Escherichia coli*	Aerobic (NA)
Large, irregular, dry, opaque with rhizoid edges on NA	+ve Rod (spore-forming)	+ve	-ve	-ve	-ve	+ve	+ve	AG	-ve	A	A	Bacillus sp.	Aerobic (NA)
Small to medium, creamy white to greyish, raised colonies on RCA	+ve Rod (spore-forming)	-ve	-ve	-ve	+ve	-ve	-ve	AG	A	AG	A	Clostridium sp.	Anaerobic (RCA)
Small, pinpoint, smooth, white colonies on RCA/NA	+ve Rod	-ve	-ve	-ve	+ve	-ve	-ve	A	A	A	A	Lactobacillus sp.	Facultative / Anaerobic (RCA)
Small, round, translucent to white colonies, often in chains on NA/RCA	+ve Cocci in chains	-ve	-ve	-ve	+ve	-ve	-ve	A	A	A	A	Streptococcus sp.	Facultative / Anaerobic (RCA)

Key: AG = Acid + Gas, A = Acid only (no gas), +ve = Positive, -ve = Negative, NA = Nutrient Agar (aerobic), RCA = Reinforced Clostridium Agar (anaerobic)

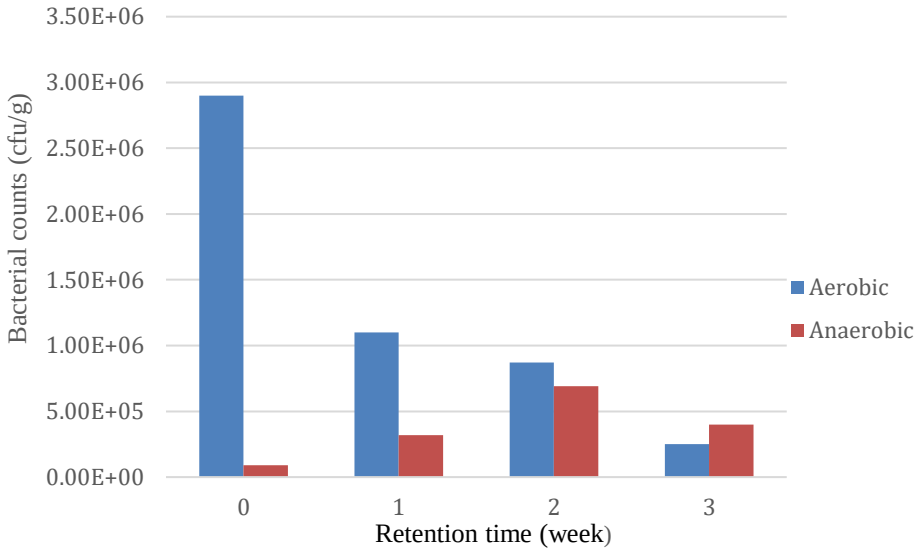


Fig. 6. Aerobic and anaerobic bacterial counts during co-digestion of *Artocarpus altilis* hulls and *Ipomoea asarifolia*

The results of the total heterotrophic and anaerobic bacterial counts during the digestion period showed a steady variation as fermentation progressed. The bacterial loads obtained for the total heterotrophic bacteria and anaerobic bacteria are shown in Figure 6. The aerobic bacterial counts decreased from $2.9x10^6$cfu/g in week 1 to 1.1 x 10^6 cfu/g in week 2. It further decreased to 8.7 x 10^5 cfu/g in week 2 and to 2.5 x 10^5 cfu/g in week 3. In contrast, the anaerobic bacterial counts increased from 9.0×10^4 CFU/g in Week 0 to 3.2×10^5 CFU/g in Week 1, and 6.9×10^5 CFU/g in Week 2 before decreasing to 4.0×10^5 CFU/g in Week 3. The observed decline in aerobic bacteria and corresponding increase in anaerobic bacteria indicate the gradual establishment of anaerobic conditions within the digester. The observed decline in anaerobic bacterial load during Week 3 may not necessarily reflect a reduction in methanogen abundance, but instead result from the inability of the culture medium used to isolate methanogens. These observations are consistent with the findings of Mukhtar *et al*. (2018), who reported a decrease in aerobic bacterial populations and an increase in anaerobic populations over a 28-day retention period.

The colonial morphology and biochemical characteristics of the isolates obtained during the digestion period are presented in Table 2. The isolates were identified as *Escherichia coli*, *Bacillus* spp., *Clostridium* spp., *Lactobacillus* spp., and *Streptococcus* spp. These findings are consistent with the findings of Mukhtar *et al*. (2018), who reported the presence of *Klebsiella oxytoca*, *Escherichia coli, Bacillus* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Clostridium* spp., confirming the microbial consortium associated with anaerobic digestion systems.

3.4 Biogas Yield and Flammability Test Results

Biogas yield was initially slow, with only 100 mL of gas recorded during the first week of digestion. It further increased progressively from week 1 to week 3, reaching a peak volume of 2000 mL. This conforms to the findings of Gana and Sa'id (2025).

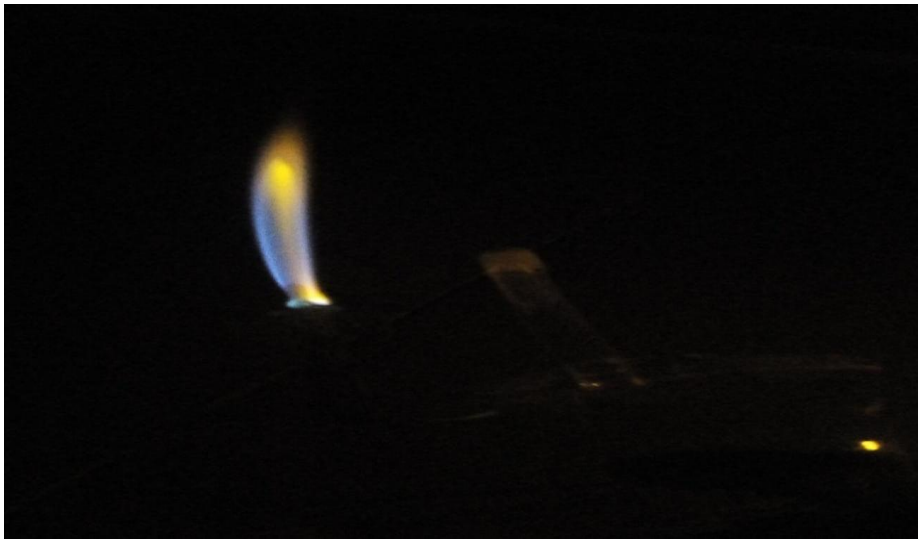


Plate 1: Flammability test of biogas produced from anaerobic co-digestion of *Artocarpus altilis* and *Ipomoea asarifolia* showing a blue-yellow flame

They opined that this increase may be attributed to enhanced methanogenic activity following the conversion of volatile fatty acids and intermediate products into methane and carbon dioxide. The rise in gas production coincided with the reduction in volatile solids observed during digestion, indicating active biodegradation of organic matter and efficient substrate utilization by anaerobic microorganisms.

The gas produced was tested using the flammability test. The inability of the gas produced in week 0 to sustain combustion at this stage suggests that there was no methane concentration and that carbon dioxide constituted a substantial proportion of the biogas produced. This lag phase is characteristic of anaerobic digestion. It depicts the period during which hydrolytic and acidogenic microorganisms establish themselves and degrade complex organic matter into simpler substrates suitable for methanogens. The gas produced in week 3 burned with a blue-yellow flame (Plate 1), indicating methane content as the major combustible component, together with minor impurities commonly associated with raw biogas.

Conclusion

The organisms isolated in this study depict the functional bacterial groups responsible for the progressive stages of anaerobic digestion. Facultative and fermentative bacteria such as *E. coli*, *Lactobacillus* spp., and *Streptococcus* spp. are primarily involved in hydrolysis and acidogenesis. These convert complex organic substrates into simpler compounds such as volatile fatty acids, carbon dioxide, and hydrogen. *Clostridium* spp., a strict anaerobe, plays a key role in the fermentation of carbohydrates and proteins, producing metabolites needed for methanogenic activity. *Bacillus* spp., spore-forming bacteria, aid substrate degradation and enhance system stability under fluctuating environmental conditions.

These microbial dynamics are consistent with the physicochemical trends observed over the 4-week retention period. The progressive reduction in total solids (TS) and volatile solids (VS) shows active biodegradation of feedstock in the digester. The variations in pH observed during the study are indicative of organic acid accumulation during early fermentation stages, followed by gradual stabilisation as digestion progressed.

In all, the physicochemical changes and microbial composition collectively reveal an active anaerobic digestion process. It also contributed to the knowledge that the feedstock materials, *Artocarpus altilis* (breadfruit) hulls and *Ipomoea asarifolia* leaves are good substrates for biogas production.

Conflicts of interest: The authors declare no conflicts of interest.

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