FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 9 No. 12, December, 2025, pp 369 – 375
DOI: https://doi.org/10.33003/fjs-2025-0912-4173

DIVERSITY OF MICROORGANISMS IN BIOGAS PRODUCTION THROUGH CO-DIGESTION OF CATTLE DUNG, POULTRY WASTE, AND PALM OIL MILL EFFLUENT

[1]*Ojetokun, Oluwaponmile T., [2]Bada, Babatunde S., [3]Atayese, Adijat O. and [4]Ojetokun, Olumide J.

[1]Institute of Food Security, Environmental Resources and Agricultural Research, (IFSERAR) Federal University of Agriculture, Abeokuta, Nigeria.
[2]Department of Environmental Management and Toxicology, Federal University of Agriculture, Abeokuta, Nigeria.
[3]Centre of Excellence in Agricultural Development & Sustainable Environment, (CEADESE) Federal University of Agriculture, Abeokuta, Nigeria.
[4]Department of Community Medicine, Ahmadu Bello University, Zaria, Nigeria.

*Corresponding author's email: tosinojetokun@gmail.com

ABSTRACT

This study assessed microbial diversity in biogas production from co-digestion of cattle dung, poultry waste, and palm oil mill effluent (POME). The experiment used a batch feeding system with 28-day retention, examining six substrate treatments: (CD), (PW), (CD:PW 1:1), (CD: POME 1:1), (PW: POME 1:1), and (CD: PW: POME 1:1:2). pH and temperature were monitored using a pH/temperature meter, and biogas volume was measured by water displacement. The data underwent descriptive and inferential statistical analyses. The optimal co-digestion treatment of PW: POME (1:1) yielded the highest cumulative biogas volume (8.33 dm^3) and peak production of 3.80 dm^3 on day 14. Initial substrate analysis showed total viable bacterial counts of 6.35×10^5, 2.17×10^5, and 0.27×10^5 CFU/mL for CD, PW, and POME, respectively, while total viable fungal counts were 2.05×10^5, 3.25×10^5, and 3.05×10^5 CFU/mL. Microbial analysis identified four suspected bacterial species (*Bacillus aquimaris*, B. *cibi*, B. *altitudinis*, and B. *cereus*), five fungal species (*Curvularia lunata*, *Aspergillus flavus*, A. *nidulans*, A. *aculeatus*, and *Trichoderma viride*), and two yeast species (*Candida albicans* and C. *tropicalis*), with *Bacillus* and *Aspergillus* being dominant genera. The biogas slurry is rich in nutrients, particularly potassium, indicating potential as a biofertilizer. The co-digestion process promoted a diverse microbial community dominated by *Bacillus* and *Aspergillus* species, which enhance substrate breakdown and biogas production.

Keywords: Cattle dung, Microbial, Biogas, *Bacillus sp.*, Nutrient content

INTRODUCTION

The increasing global demand for renewable energy underscores the significance of anaerobic digestion (AD) in generating biogas (Ab Aziz *et al*., 2025). Biogas, produced by the microbial breakdown of organic waste, such as agricultural residues, industrial byproducts, and municipal solid waste, has significant energy potential (Nwoke *et al*., 2024). AD is a biological process that occurs in the absence of oxygen, where a group of microorganisms transforms biodegradable material into methane (50–80%) and carbon dioxide (30–50%). (Grando *et al*., 2017). Beyond energy generation, AD is applied in wastewater and solid waste treatment, offering environmental benefits such as reduced waste, renewable energy, and biofertilizer production at relatively lower costs than conventional energy sources (Sarvari *et al*., 2016).

Animal and plant waste serve as substrates for AD, with microbial communities, particularly Eubacteria and Archaea, coordinating degradation through food chain interactions influenced by factors such as pH, temperature, substrate type, and digester design (Wirth *et al*., 2012). However, organic waste can also harbor pathogens such as *Salmonella* spp., *Listeria* spp., and *Escherichia coli*, which pose risks to human and animal health (Nelson and Murray, 2008).

Cattle dung, which is rich in cellulose, hemicellulose, lignin, and minerals, is a major substrate for biogas production (Gupta *et al*., 2016). It harbors diverse fungi, including *Trichoderma reesei*, *Aspergillus niger*, and *Fusarium oxysporum*, which enhance fiber degradation and symbiotic digestion in ruminants (Azad *et al*., 2020). Poultry manure, which has high nitrogen and microbial diversity, improves biogas yield and stability (Ab Aziz *et al*., 2025). Although Palm Oil Mill Effluent (POME) is rich in energy, it may inhibit AD due to long-chain fatty acids and contribute to water pollution when improperly discharged (Nvene *et al*., 2024). AD is carried out in biodigesters, which have evolved from simple concrete structures to flexible plastic systems. Their efficiency largely depends on microbial community dynamics and their ecological stability (Westerholm *et al*., 2018). The main objective of this study is to assess diversity of microorganisms in biogas production through co-digestion of cattle dung, poultry waste, and palm oil mill effluent.

MATERIALS AND METHODS

Experimental Site

The study was conducted at the Teaching and Research Farm of the Federal University of Agriculture in Abeokuta, Ogun State. Fresh cattle dung and POME were obtained from the University Farms, while poultry waste was sourced from a nearby poultry farm at Alabata village, Abeokuta.

The Experimental Design and Setup

The fixed dome digesters were made of 25 L plastic kegs with a working volume of 18 L and 7 L headspace for biogas storage. The digesters were constructed with inlet and outlet valves. Gas delivery pipes were linked from the digesters to a water displacement unit which displaced water into an empty

container. This was measured using a measuring cylinder, and the water volume was recorded as the amount of gas produced. The experiment was performed in duplicate with 28 days retention period. The treatments are as follows: CD (Cattle dung), PW (Poultry Waste), CD:PW (1:1), CD: POME (1:1), PW: POME (1:1) and CD: PW: POME (1:1:2).

Feeding of Digesters

Batch feeding was employed, which involved loading the digester once and maintaining a closed environment throughout the retention period. The substrates were allowed to undergo anaerobic digestion for a retention period of 28 days.

Determination of Ph, Temperature and Biogas Volume

A calibrated pH/temperature meter (HACH Instruments) was used to measure the pH and temperature. A water displacement unit which displaces water into an empty container was connected to the digesters. The displaced water was measured using a measuring cylinder, and the volume was recorded as the gas produced.

Nutrient Analysis of the Biogas Slurry

After 28 days, slurry samples were gathered, filtered, and subjected to centrifugation at 10,000 rpm for 15 minutes. The supernatant obtained was then utilized for analyzing nutrients such as nitrogen (N), phosphorus (P), and potassium (K). The analysis of triplicate samples was conducted following the standard procedures outlined by AOAC (2015).

Sample Collection

Triplicate samples were collected from each digester in sterile bottles in the morning and transported immediately to the laboratory. When immediate analysis was not possible, the samples were refrigerated and processed within 24 h.

Microbial Isolation

Nutrient Agar (NA) and Potato Dextrose Agar (PDA) were prepared according to the manufacturer's instructions. To prevent bacterial growth, acetic acid was incorporated into the PDA. A dilution series was created by combining 1 g of slurry with 9 mL of sterile distilled water, followed by further dilutions to achieve a 10^{-4} concentration. Using the pour-plate technique, 1 mL samples from the appropriate dilutions were plated. NA plates were incubated at 37 ℃ for 24 hours, while PDA plates were kept at 25–27 ℃ for 3 to 5 days. The distinct colonies that developed were counted, isolated through subculturing, and stored for later analysis.

Colony counts were expressed as colony-forming units (cfu/mL) using the following formula:

$$\text{Colony count} = \frac{\text{Number of colonies}}{\text{Inoculant size} * \text{dilution factor}} \cdots \quad eqtn\ I$$

Morphological and Biochemical Characterization

Bacterial isolates were identified using standard morphological and biochemical methods (Cheesbrough, 2006). Gram staining was performed to distinguish Gram-positive and Gram-negative organisms. Biochemical tests included oxidase, catalase, indole, citrate utilization, and coagulase tests. Carbohydrate fermentation was assessed in media containing glucose, sucrose, fructose, and lactose, with acid production indicated by a color change and gas detected using Durham tubes.

Statistical Analysis

The data obtained were subjected to descriptive (mean and standard deviation) and inferential (ANOVA and Duncan Multiple Range Test) statistics using Statistical Analysis Software (SAS) version 21. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Temperature, Ph and Biogas Volume Variations

The temperature variation recorded in this study ranged from 27.9 to 34.4 ℃ (Table 1). Peak temperature was observed at the 14th day in all treatments except combination of PW with POME in the ratio 1:1 which had highest temperature reading at 21st day. The temperature range reported in this study falls closely within the values (27.2-33.8℃) as reported by Akintokun *et al*. (2017) during co-digestion of cow dung with rice husk, and (28-39℃) as reported by Asikong *et al*. (2016) in co-digestion of pig dung, banana peel and vegetable waste respectively. The optimum temperature obtained in this study for maximum biogas production was 34.1℃ in treatment PW: POME/1:1 ratio. Furthermore, Raja and Wazir (2017) stated that temperatures should be kept above 30 °C for effective anaerobic digestion. This is consistent with the temperature readings obtained in the present study. In anaerobic digestion, temperature plays a crucial role in determining the survival of microorganisms, affects the rates of biological reactions, and significantly impacts microbial growth, as each group of bacteria thrives best within a specific temperature range. (Bakraoui *et al*., 2020). Abubakar *et al.* (2022) identified four temperature categories where bacteria can thrive: psychrophilic (10-25 ℃), mesophilic (20-45 ℃, thermophilic (45-70 ℃, and hyperthermophilic (exceeding 70 ℃). The temperature regime observed throughout this study was in the mesophilic range. There was a significant ($p<0.05$) difference between the temperature value.

Table 1: Effect of Substrate Ratio and Retention Time On Temperature (°C)

Substrate	**7**	**14**	**21**	**28**
Retention Time (Days)				
CD (Cattle Dung)	6.53d ± 0.18	6.84a ± 0.31	7.14a ± 0.25	7.30a ± 0.02
PW (Poultry Waste)	6.98c ± 0.44	6.32b ± 0.13	6.18b ± 0.07	6.40b ± 0.14
CD: PW (1:1)	7.22bc ± 1.13	6.15b ± 0.03	6.41b ± 0.09	6.46b ± 0.01
CD: POME (1:1)	7.88a ± 0.33	5.48c ± 0.07	6.50b ± 0.69	5.88c ± 0.05
PW: POME (1:1)	7.13bc ± 0.45	5.49c ± 0.03	6.41b ± 0.09	5.94c ± 0.03
CD: PW: POME (1:1:2)	7.07c ± 1.08	5.46c ± 0.24	6.25b ± 0.63	6.05c ± 0.33

Means in the same column with different superscript are significantly different ($p < 0.05$)

The pH level is the most crucial factor influencing the breakdown of organic waste materials. As presented in Table 2, the pH values ranged between 5.48 and 7.88. This is closely to (5.78-7.95) obtained by Yengong *et al*. (2025) while co-digesting livestock manure with palm oil wastewater. The low pH recorded in the POME-based mixtures could be due to the acidic nature of POME and the rapid production of volatile fatty acids (VFAs). Raja and Wazir (2017) stated that low pH may inhibit bacterial growth and biogas production, leading to system failure or low buffering capacity. The optimum pH reading obtained in this study in treatment PW: POME (1:1) for maximum biogas production was 5.49. This result is consistent with the report of Yadvika *et al*. (2004), who stated that when the pH is greater than 5, methane production would be greater than 75%. Oyeleke *et al*. (2003) also stated that the volume of biogas produced at a pH of 5 is higher than that produced at a pH of 10. This may partly be responsible for maximum biogas production recorded while co-digesting PW with POME in the 1:1 ratio at pH of 5.49. However, various authors reported different pH values, Akintokun *et al*. (2017) obtained a pH of (6.1-7.8) in co-digestion of cow dung with rice husk; Abd and Othman, (2022) reported a pH variation of (6.0-8.0) during biogas production. These variations in pH readings may be attributed to the type of substrate used, retention period, and digester type. Significantly different ($p< 0.05$) pH values were recorded throughout the digestion period.

Table 2: Effects of Substrate Ratio and Retention Time On Ph

Substrate	7	14	21	28	Cumulative Biogas (dm3)
Retention Time (Days)					
CD (Cattle Dung)	1.38bc ± 0.40	2.44bc ± 1.59	1.42ab ± 0.13	2.25a ± 1.05	7.49
PW (Poultry Waste)	1.70b ± 0.81	2.67b ± 1.52	1.55a ± 0.51	2.38a ± 1.08	8.30
CD: PW (1:1)	1.33c ± 0.39	2.80b ± 1.65	1.47ab ± 0.37	2.13ab ± 1.71	7.73
CD: POME (1:1)	2.00b ± 0.46	2.00bc ± 1.25	0.98bc ± 0.60	1.88b ± 1.65	6.86
PW: POME (1:1)	1.83b ± 1.61	3.80a ± 1.80	0.98bc ±0.81	1.72b ±1.63	8.33
CD: PW: POME (1:1:2)	3.32a ± 1.98	2.92b ± 2.08	0.77c ± 0.39	0.88c ± 0.07	7.89

Means in the same column with different superscript are significantly different ($p < 0.05$)

As shown in Table 3, biogas production peaked on the 14th day of anaerobic digestion in most digesters, which was the period of highest microbial activity and temperature. Afterwards, there was a decline in biogas production by day 21, which was observed across all treatments, indicating substrate depletion or reduced microbial activity. The decline was mostly observed in treatments CD: POME (1:1), PW: POME (1:1) and CD: PW: POME (1:1:2) which plummeted from 2.00dm^3 to 0.98 dm^3, 3.80 dm^3 to 0.98 dm^3 and 2.92 dm^3 to 0.77 dm^3 at 14th to 21st day of retention respectively. Optimum biogas volume (3.80 dm^3) was recorded in digester PW: POME (1:1) on the 14th day of retention. Meanwhile, highest (8.33dm^3) and lowest (6.86dm^3) cumulative biogas yield was recorded in PW: POME (1:1) and CD: POME (1:1) respectively. All poultry waste combination treatments, outperformed cattle dung combination ones in terms of cumulative biogas volume. This may be attributed to high nitrogen content, sub-optimal C/N ratio requiring co-digestion with substrates of higher C contents, high biodegradability and low lignin content of poultry waste (Jurgutis *et al*., 2020).

Table 3: Effects of Substrate Ratios and Retention Time On Biogas Production

Substrate	7	14	21	28
		Retention Time (Days)		
CD (Cattle Dung)	28.50a ± 0.29	34.07a ± 0.23	32.37b ± 0.40	32.53a ± 0.20
PW (Poultry Waste)	28.55a ± 0.43	34.12a ± 1.05	32.52b ± 0.42	32.23a ± 0.75
CD: PW (1:1)	28.42a ±0.29	33.78a ± 0.23	31.57c ± 0.40	32.43a ± 0.20
CD: POME (1:1)	27.97b ± 0.26	34.15a ± 0.19	33.33ab ± 1.22	31.90a ± 0.32
PW: POME (1:1)	28.08b ± 0.28	34.13a ± 0.54	34.40a ± 1.03	33.20a ± 0.53
CD: PW: POME (1:1:2)	27.88b ± 0.28	33.47a ± 0.54	33.27ab ± 1.03	32.52a ± 0.53

Means in the same column with different superscript are significantly different ($p < 0.05$)

Total Viable Counts (TVC)

Cattle dung, poultry waste, and POME are the sources of microbial inocula for anaerobic digestion in this study. Data presented in Table 4 shows that, the total viable bacterial count for raw cattle dung, poultry waste and POME were 6.35, 2.17 and 0.27 × 10^5 CFU/mL respectively. Meanwhile, total viable fungal count was 2.05, 3.25 and 3.05× 10^5 CFU/mL in cattle dung, poultry waste and POME respectively. Highest bacteria population was recorded in cattle dung while poultry waste and POME had highest fungal count. The total bacterial and fungal count was significantly higher ($p < 0.05$) in the cattle dung and poultry waste respectively. The total viable bacteria count obtained was within the range (6.6 × 10^6 CFU/mL) reported by Akpan *et al*., (2019) in cow dung, (17.4 × 10^5 CFU/mL) reported by Ofon *et al*., (2024) while co-digesting chicken manure with paper waste and coconut shell biochar, and 1× 10^7 CFU/mL obtained by Ezekoye *et al*., (2020) in the biodegradation of some plants and animal wastes. These values differ from the 2.68 × 10^8 CFU/mL reported by Ya'aba and Ramalan (2020) and 1.5 × 10^8 CFU/mL reported by Arekemase and Aweda (2021) for cow dung. The total viable fungal count was within the range (4.73× 10^5 CFU/g) obtained by Akintokun *et al*. (2017) in the co-digestion of cow dung with rice husk. Fungal strains are excellent degraders of the cellulose backbone; therefore, the co-digestion of multiple substrates is the best strategy for enhancing biogas production (Kazda *et al*., 2014). The presence and expansion of viable bacteria and fungi in POME are likely linked to its rich composition of carbohydrates, proteins, nitrogenous compounds, lipids, minerals, cellulose, hemicelluloses, and lignin. (Hii *et al*., 2012).

Table 4: Total Viable Bacteria and Fungi Count of Raw Substrates

Substrate	Total Bacteria Count (CFU/mL)	Total Fungi Count (CFU/mL)
Cattle Dung	$6.35^{a} \times 10^{5}$	$2.05^{b} \times 10^{5}$
Poultry Waste	$2.17^{b} \times 10^{5}$	$3.25^{a} \times 10^{5}$
POME	$0.27^{c} \times 10^{5}$	$3.05^{a} \times 10^{5}$

Means in the same column with different superscript are significantly different ($p < 0.05$)

Furthermore, after 28 days' retention period, treatment PW: POME (1:1) showed the highest bacterial (9.40×10^5 CFU/mL) and fungal (6.20×10^5 CFU/mL) counts making it the most microbiologically active biogas slurries and invariably the best performing treatment for fixed dome digester (Table 5). Generally, the bacterial and fungal loads in the slurries of single substrates reduced after 28 d of biogas production, while those of co-digested substrates had higher values. The increased total bacterial load observed in poultry waste only, after anaerobic digestion may be attributed to a hydrolysis process and release of nutrients, in which complex organic compounds (proteins, fats, carbohydrates, uric acid) are broken down into simpler, soluble molecules (sugars, amino acids, long-chain fatty acids). This process is carried out by hydrolytic and acidogenic bacteria. During this breakdown, a substantial amount of nutrients, previously confined within complex structures, is released, making them highly accessible. This nutrient source is significantly more available for microbial absorption than raw manure, providing an excellent environment for microbial growth (Langer *et al*., 2020; Jurgutis *et al*., 2020).

Digesters that contained only poultry waste (PW) and a 1:1 mixture of CD: POME exhibited minimal or no fungal growth after digestion. This reduction is likely due to the exhaustion of nutrients within the digesters, caused by the ongoing conversion of complex materials into simpler organic compounds (Langer *et al*., 2020). Fungi played a role in the anaerobic biogas process by adhering to and penetrating cell walls, which allows them to open the cells for various bacterial communities, thereby accelerating the overall decomposition process. (Leh-togi *et al*., 2024). Co-digestion of PW with POME resulted in significantly ($p < 0.05$) higher total viable bacterial counts.

However, results obtained showed that most bacteria survived the anaerobic digestion process, though with reduced counts. Although bacterial pathogens reduced significantly from manure to slurry samples, they were not completely eliminated. *Bacillus* and *Aspergillus* species were the most dominant species all through digestion period.

Table 5: Total Viable Microbial Count in Biogas Slurry

Biogas Slurry	Bacteria (CFU/mL) ($\times 10^5$)	Fungi (CFU/mL) ($\times 10^5$)
CD (Cattle Dung)	2.53^{cd}	0.17^{c}
PW (Poultry Waste)	2.66^{c}	0.00
CD: PW (1:1)	7.50^{b}	0.13^{c}
CD: POME (1:1)	2.57^{c}	0.00
PW: POME (1:1)	9.40^{a}	6.20^{a}
CD: PW: POME (1:1:2)	2.66^{c}	5.43^{b}

Means in the same column with different superscript are significantly different ($p < 0.05$)

Data presented in Tables 6, 7 and 8 shows the results of suspected bacterial, fungi and yeast isolates from the fixed dome digesters. A total of four morphologically and physiologically different bacteria species (*Bacillus aquimaris, B. cibi, B. altitudinis, and B. cereus*), five fungi species (*Curvularia lunata, Aspergillus flavus, A. nidulans, A. aculeatus, and Trichoderma viride*) and two yeast species (*Candida albicans,* and *C. tropicalis)* were obtained.

All the bacteria identified were rod-shaped (bacilli) and belonged to the spore-forming firmicutes phylum. The *Bacillus* species observed were consistent with those reported by Zainudin *et al.* (2014) during the breakdown of woody empty oil palm fruit bunches and Ofon *et al*., (2024) while co-digesting chicken manure with paper waste and coconut shell biochar. As noted by Horvath *et al*. (2016), the *Bacillus* genus is crucial in biogas syntrophic processes, where they break down VFAs, alcohols, and acetate to generate H_2, which is subsequently utilized by hydrogenotrophic methanogens to produce methane. These findings also align with Rabah *et al*. (2010), who documented various *Bacillus* species during biogas production using abattoir waste as inoculum. Researchers such as Ayedun *et al*. (2023), Cole *et al*. (2022), Adeyemo and Waleola, (2016), and Kartikey *et al.* (2016) have also isolated different *Bacillus spp*. from modified oil palm bunch wastes, cow dung, and cow intestinal exudates such as *Bacillus subtilis, Bacillus cereus, Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Streptomyces species, Pseudomonas species, Alcaligenes faecalis* and *Salmonella species.* Fungi species obtained compares with those earlier presented by Akintokun *et al*. (2017) who reported *Aspergillus sp*. *Mucor sp*. and *Penicillium sp*. while co-digesting cow dung with rice husk. Abdelhakim and Lamine (2023) opined that the genus *Aspergillus* accelerates degradation process of organic matter.

Table 6: Biochemical Test Results of Bacterial Isolates from The Fixed Dome Digester

Catalase	Coagulase	Citrate Utilization	Oxidase	Indole	Sucrose	Glucose	Lactose	Suspected organisms
+	-	+	+	-	AG	+	AG	*Bacillus aquimaris*
+	-	+	-	-	AG	+	AG	B. *cibi*
+	-	+	+	-	AG	+	AG	B. *altitudinis*
+	-	+	-	-	AG	+	AG	*B. cereus*

A: Acid, G: Gas, Acid + Gas

Table 7: Cultural and Morphological Characteristics of Fungi Isolated from Fixed Dome Digesters

Colour of mycelium	Type of spore	Septation	Colour of spore	Appearance of spore / Fruiting bodies	Reproduction	Probable organism
Yellow green	Conidia	Septate	Yellow	Smooth conidiospore	Asexual	*Aspergillus flavus*
Green	Conidia	Septate	Green	Branched Conidiospore	Asexual	*Trichoderma viride*
Green	Conidia	Septate	White	Smooth conidiospore	Sexual	*Aspergillus nidulans*
Black	Conidia	Septate	Black	Conidiospore	Sexual	*Curvularia lunata*
White	Conidia	Septate	Brown	Conidiospore	Asexual	*Aspergillus aculeatus*

Table 8: Morphological and Biochemical Characteristics of Yeast Isolates

Cultural Characteristics	Reproduction	Glucose	Lactose	Sucrose	Maltose	Fructose	Suspected Organisms
Whitish, circular, smooth, raised	Budding	AG	A	A	AG	A	*Candida albicans*
Creamy, circular, smooth, raised	Budding	A	AG	A	A	__	*Candida tropicalis*

A: Acid, G: Gas, Acid + Gas

Nutrient Composition in Slurry After Anaerobic Digestion

The Nitrogen content ranges from 1.62 to 2.46 % with CD having the highest and treatment CD: POME (1:1) reporting the lowest (Figure 1). High N content indicates a potential as a fertilizer especially for crops requiring significant N such as leafy vegetables. Highest P concentration (1.76%) was obtained in digester CD: PW: POME (1:1:2) while CD only had the lowest P (0.55%) concentration. Phosphorus is essential for root development and energy transfer in plants. The K content varies widely from 2.11% (lowest) in digester CD: POME (1:1) to 11.82% (highest) in treatment CD: PW: POME (1:1:2). This is significantly ($P<0.05$) higher than other treatments. Potassium is crucial for flowering, fruiting and overall plant health making this slurry particularly valuable for crops that demand high K levels. Nutrients which were previously locked up in substrates are known to be released during anaerobic digestion. Leh-togi *et al.* (2024) reported N, P, K values of 0.57%, 110ppm and 24.4µg in raw poultry dung, whereas Erraji *et al*. (2023) obtained N, P, K concentrations of 0.41, 0.11 and 0.98% in cow dung digestate.

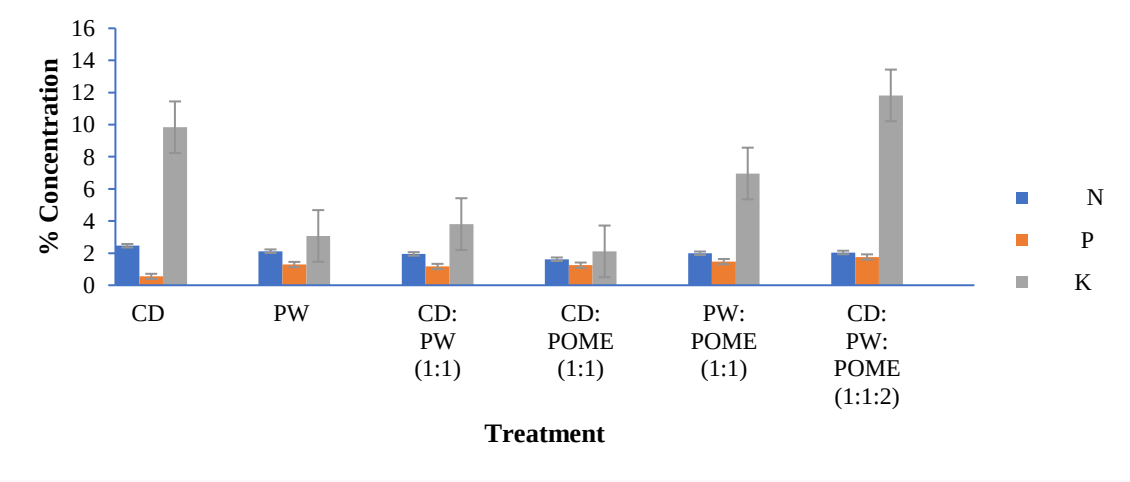

Figure 1: Percentage Concentrations of N, P, K in Each Treatment

CONCLUSION

This study investigated the diversity of microorganisms in biogas production through co-digestion of cattle dung, poultry waste, and palm oil mill effluent. The major findings demonstrated that substrate ratio significantly influences microbial activity and biogas yield. The PW: POME (1:1) co-digestion mixture was identified as the optimal treatment, achieving a peak biogas production of 3.80 dm^3 on day 14 under mesophilic conditions (34.1°C) and an acidic pH of 5.49. This treatment also yielded the highest cumulative biogas volume (8.33 dm^3), significantly outperforming all other substrates, including cattle dung mixtures, which produced the lowest cumulative volume (6.86 dm^3). Digesters co-digesting poultry waste consistently supported more diverse and microbiologically active slurries.

Microbial analysis revealed a diverse consortium of hydrolytic and fermentative microorganisms after a 28-day retention period. The community comprised four suspected bacterial species (*Bacillus aquimaris*, B. *cibi*, B. *altitudinis*, and B. *cereus*), five fungal species (*Curvularia lunata*, *Aspergillus flavus*, A. *nidulans*, A. *aculeatus*, and *Trichoderma viride*), and two yeast species (*Candida albicans* and C. *tropicalis*). Members of the genera *Bacillus* and *Aspergillus* were the most dominant throughout the digestion process.

Furthermore, the biogas slurry was characterized by high nutrient content, particularly potassium (K), which was significantly higher ($p<0.05$) than other elements. This high N, P, K value confirms the potential for the efficient valorization of the biogas slurry as a valuable bio-fertilizer, adding an important economic and agricultural benefit to the anaerobic digestion process.

REFERENCES

Ab Aziz, I. F., Man, H. C., Shukery, M. F. M., Jaffar, M. A. M., Shern, H. Y., Omar, R. and Jamali, N. S. (2025). A comparative study on the effects of co-digesting molasses with palm oil mill effluent (POME) or chicken manure on biomethane yield. *E3S Web of Conferences*, 636: 03002.

Abd, A. A. and Othman, M. R. (2022). Biogas upgrading to fuel grade methane using pressure swing adsorption: Parametric sensitivity analysis on an industrial scale. *Fuel*, 308:121986.

Abdelhakim, H. and Lamine, B. M. (2023). Study of the effect of some fungi on the yield of biogas. *Journal of Pollution and Effects on Community Health*, 2:1.

Abubakar, A. M., Silas, K. and Aji, M. M. (2022). An elaborate breakdown of the essentials of biogas production. *Journal of Engineering Research and Sciences*, 1(4): 93–118.

Adeyemo, I. A. and Waleola, O. A. (2016). Prevalence and incidence of *Escherichia coli* O157 in domestic goat dungs of Okitipupa township. *Nigerian Journal of Pure & Applied Sciences*, 29:2.

Akintokun, A. K., Abibu, W. A. and Oyatogun, M. O. (2017). Microbial dynamics and biogas production during single and co-digestion of cow dung and rice husk. *Applied Environmental Research*, 39(2): 6.

Akpan, J. F., Isong, I. A. and Asikong, E. B. (2019). Anaerobic digestion of organic waste for the production of biogas in Calabar, Cross River State, Nigeria. *World News of Natural Science*, 24: 9–21.

AOAC (2015). *Official Methods of Analysis*. 18th ed. Arlington: AOAC.

Arekemase, M. O. and Aweda, I. (2021). Production of biogas from mono and co-digestion of agricultural waste (cow dung, chicken dropping, and rice husk). *Iraqi Journal of Science*, 62(1): 45–60.

Asikong, B. E., Idire, S. O. and Tiku, D. R. (2016). Microorganisms associated with biogas production using vegetable (*Telfairia occidentalis*) wastes, banana peel and pig dung as substrates. *British Microbiology Research Journal*, 16(3): 1–12.

Ayedun, H., Adeyemo, A. I. and Ayadi, P. O. (2023). Biogas production from poultry waste modified with sawdust. *Science World Journal*, 18:2.

Azad, E., Fehr, K. H., Derakhshani, R., Forster, S., Acharya, E., Khafipour, E. and McGeough, T. M. (2020). Interrelationships of fiber-associated anaerobic fungi and bacterial communities in the rumen of bloated cattle grazing alfalfa. *Microorganisms*, 8(10):1543.

Bakraoui, M., Karouach, F., Ouhammou, B., Aggour, M., Essamri, A. and El Bari, H. (2020). Biogas production from recycled paper mill wastewater by UASB digester: Optimal and mesophilic conditions. *Biotechnology Reports*, 25: e00402.

Cheesbrough, M. (2006). *District Laboratory Practice in Tropical Countries, part 2*. Cambridge: Cambridge University Press. Pp 86-110.

Cole, S. D., Swiderski, M., Dietrich, J. and McGonigle, K. M. (2022). Comparison of a chromogenic urine culture plate system (UTid+) and conventional urine culture for canine and feline specimens. *Veterinary Science*, 16: 138–146.

Erraji, H., Asehraou, A., Tallou, A. and Rokni, Y. (2023). Assessment of biogas production and fertilizer properties of digestate from cow dung using household biogas digester. *Biomass Conversion and Biorefinery*, 14: 29001–29007.

Ezekoye, V., Offor, O. P., Agbogu, A. and Ezekoye, D. (2020). Methane production enhancement and comparative study of biodegradation of some plants and animal wastes. *Indian Journal of Biochemistry & Biophysics*, 57: 449–457.

Grando, R. L., de Souza Antune, A. M., da Fonseca, F. V., Sánchez, A., Barrena, R. and Font, X. (2017). Technology overview of biogas production in anaerobic digestion plants: A European evaluation of research and development. *Renewable and Sustainable Energy Reviews*, 80: 44–53.

Gupta, K., Kumar, K., Rai, A. and Deepanshu, R. (2016). Current status of cow dung as a bioresource for sustainable development. *Bioresources and Bioprocess*, 3: 28.

Hii, K. L., Yeap, S. P. and Mashitah, M. D. (2012). Cellulase production from palm oil mill effluent in Malaysia: Economical and technical perspectives. *Engineering in Life Sciences*, 12(1): 7–18.

Horváth, I. S., Tabatabaei, M., Karimi, K. and Kumar, R. (2016). Recent updates on biogas production—a review. *Biofuel Research Journal*, 3(2): 394–402.

Jurgutis, L., Slepetiene, A., Volungevicius, J. and Amaleviciute-Volunge, K. (2020). Biogas production from chicken manure at different organic loading rates in a mesophilic full-scale anaerobic digestion plant. *Biomass and Bioenergy*, 141:105693.

Kazda, M., Langer, S. and Bengelsdorf, F. R. (2014). Fungi open new possibilities for anaerobic fermentation of organic residues. *Energy, Sustainability and Society*, 4: 6.

Langer, S. G., Ahmed, S. and Einfalt, D. (2020). Microbial population dynamics during anaerobic digestion of poultry manure: Impact of temperature and substrate. *Bioresource Technology Reports*, 11: 100491.

Leh-Togi, Z. S., Suanu, H. J., Mathew, I. O., Ogu, A. C., Tarimoboere, A., Bello, M., Ijah, U. J. and Abioye, O. P. (2024). The role of microbes in the production of biogas from poultry droppings. *International Journal of Modelling & Applied Science Research*, 3:9.

Nelson, K. L. and Murray, A. (2008). Sanitation for unserved populations: Technologies, implementation challenges, and opportunities. *Annual Review of Environment and Resources*, 33(1): 119–151.

Nvene, C., Suleiman, J. B., Ajayi, A. A. and Ebokaiwe, P. A. (2024). Assessment of biogas production potentials of palm oil mill effluent and cow dung substrates in anaerobic digester. *World Journal of Advanced Research and Reviews*, 22(2): 1279–1287.

Nwoke, O. A., Udealor, C. M., Uzoejinwa, B. B. and Ojike, O. (2024). Anaerobic co-digestion of brewery spent grain and cassava wastewater for the production of biogas using fixed dome biodigester. *Agricultural Engineering International: CIGR Journal*, 26(4): 129–141.

Ofon, U. A., Ndubuisi-Nnaji, U. U., Udofia, G. E., Fatunla, O. K. and Shaibu, S. E. (2024). Improving bioenergy production from anaerobic co-digestion of paper waste and chicken manure using coconut shell biochar. *FUDMA Journal of Sciences (FJS)*, 8(6): 107–113.

Oyeleke, S. B., Onigbajo, H. O. and Ibrahim, K. (2003). Degradation of animal waste (cattle dung) to produce methane (cooking gas). In: *Proceedings of the 5th Annual Conference on Animal Science of Nigeria (SAN)*, pp. 168–169.

Rabah, A. B., Baki, A. S., Hassan, L. G., Musa, M. and Ibrahim, A. D. (2010). Production of biogas using abattoir waste at different retention time. *Science World Journal*, 5(4): 23–26.

Raja, I. A. and Wazir, S. (2017). Biogas production: The fundamental processes. *Universal Journal of Engineering Science*, 5(2): 29–37.

Sárvári H. I., Tabatabaei, M., Karimi, K. and Kumar, R. (2016). Recent updates on biogas production—a review. *Biofuel Research Journal*, 3(2): 394–402.

Westerholm, M., Isaksson, S., Karlsson Lindsjö, O. and Schnürer, A. (2018). Microbial community adaptability to altered temperature conditions determines the potential for process optimisation in biogas production. *Applied Energy*, 226: 838–848.

Wirth, R., Kovacs, E., Maroti, G., Bagi, Z., Rakhely, G. and Kovacs, K. L. (2012). Characterisation of a biogas-producing microbial community by short-read next generation DNA sequencing. *Biotechnology for Biofuels*, 5: 41.

Ya'aba, Y. and Ramalan, A. S. (2020). Isolation, identification and characterization of some bacteria associated with biogas production from cow dung. *Equity Journal of Science and Technology*, 7(2): 91–99.

Yadvika, S., Santosh, S., Sreekrishnan, T. R., Kohli, S. and Rana, V. (2004). Enhancement of biogas production from solid substrates using different techniques. *Bioresource Technology*, 95: 1–10.

Yengong, F. L., Ngwabie, N. M., Tiku, D. T., Manga, V. E., Yuven, W. H., Ginyu, C. T. and Samson, R. H. (2025). Methane production from the digestion of different livestock manure and co-digestion with wastewater from palm oil production. *Science World Journal*, 19(1): 34–40.

Zainudin, M. H. M., Hassan, M. A., Shah, U. K. M., Abdullah, N., Tokura, M., Yasueda, H., Shirai, Y., Sakai, K. and Baharuddin, A. S. (2014). Bacterial community structure and biochemical changes associated with composting of lignocellulosic oil palm empty fruit bunch. *Bioresources*, 9(1): 316–335.