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Author Affiliation:
[1]Department of Animal Production, Faculty of Agriculture, University of Jos, Plateau State, Nigeria
[2]Department of Civil Engineering, Faculty of Engineering, University of Jos, Plateau State, Nigeria

[*]**Corresponding Author**
Department of Animal Production, University of Jos, Jos North LGA, Plateau State, Nigeria. Phone: +2349068628463. E-mail: hakolahwohle34@gmail.com

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Assessment of the methane yield and efficiency of anaerobic co-digestion process in microbial stabilization of layer and broiler birds droppings (30:70)

Abdullateef AI[1*], Hope BN[1], Alfa IM[2], Okpanachi U[1]

ABSTRACT

This study assessed the anaerobic co-digestion of layer and grill droppings (30:70) for methane output and microbial stabilisation efficiency. Comparing microbial load decrease, evaluating procedure effectiveness in stabilising particular infections, and estimating methane yield were the objectives of the study. pH, temperature, electrical conductivity, salinity, total dissolved solids, and gas generation were all measured daily for 30 days. Analysis of the data showed that both the total coliform count (1.20±0.20 to 0.00±0.00) and the total viable count (1.32±0.02 to 0.85±0.03) significantly decreased after digestion. Following digestion, the initially elevated levels of Shigella, Salmonella, E. coli, and Klebsiella were lowered to undetectable levels. After the initial acidity, the pH stabilised at about neutral (6.28). Temperatures varied between 21.0°C and 34.5°C, which is the mesophilic range. When measured in dm^3, gas production followed a typical pattern of anaerobic digestion: low starting volumes (0.00481-0.00641 dm^3), a steady increase that peaked at 0.05911 dm^3, and a fall (0.05078-0.02419 dm^3) as organic matter depleted. According to the study's findings, anaerobic co-digestion produced a significant amount of methane—up to 0.05911 dm3—significantly decreased microbial loads, and efficiently removed targeted pathogens. These results point to the possibility of using anaerobic digestion to produce energy from chicken droppings.

Keywords: Poultry Manure, Anaerobic Digestion, Anaerobic Co-Digestion, Microbial Stabilization, Methane Yield

1. INTRODUCTION

Poultry manure (PM) is an organic waste produced by the poultry industry that has a number of environmental consequences if not properly disposed off as a result of soil, air and water contamination occasioned by released odours and gases, as well as its

nitrogen and pathogen content (Yetilmezsoy and Sakar, 2018; Roeckel et al., 2017; Pizarro et al., 2019). The efficient treatment or disposal of PM is a significant challenge. PM's high organic matter content makes it an appropriate substrate for anaerobic digestion (AD) processes, which produce methane and help to stabilise pathogens and valorise waste. Chicken faeces contain significant amounts of biodegradable components that, if improperly handled, might cause unwanted environmental problems.

Improper application of manure with high levels of phosphorus and nitrogen to fields can affect surface water supplies, groundwater, and soil. Anaerobic digestion, which breaks down different organic components to produce energy-rich biomethane, is one of the technologies used to process poultry manure (Wu et al., 2016; Güngör-Demirci and Demirer, 2004; Manyi-Loh et al., 2013). Numerous factors impact the efficiency of the complex microbial process known as anaerobic digestion, which consists of several stages: Hydrolysis, acidogenesis, acetogenesis (by bacteria), and methanogenesis (by methanogenic archaea) (Manyi-Loh et al., 2013; Westerholm et al., 2016). Adding various organic or inorganic ingredients to the reactors is one method of boosting biogas production. Microbial cultures, green biomass, and other carbon-based supplements are examples of organic additions.

As inorganic additions, a variety of mineral elements—both macro and micro—as well as minerals—such as magnetite and zeolites—are employed (Romero-Guiza et al., 2016; Angelidaki et al., 2018; Ziganshina et al., 2020; Ziganshina et al., 2022). In addition, interest in methane production from animal waste has increased due to the need for biobased and renewable energy sources as well as the issue of managing livestock waste. Poultry droppings are regarded as a possible substrate for biogas production by AD because of their high organic matter content (Manyi-Loh et al., 2013). One substrate or a combination of two or more substrates (co-digestion) can be used to feed AD units (Ganzoury and Allam, 2015).

To avoid some issues, such as excess or insufficient nitrogen, the possibility of acidification because of high biodegradability, the absence of microelements, and the presence of long-chain fatty acids (LCFAs), co-digestion is necessary. In addition, it has many advantages over mono-digestion, including dilution of harmful compounds, nutrient balance, increased activity of microorganisms, and subsequent intensification of methane yield (Bose et al., 2012). For instance, co-digesting poultry manure with agricultural waste reduced the toxicity typically found in avian manures alone and produced 32% higher methane yields along with a 43.7% reduction in ammonia accumulation.

A higher load of easily biodegradable organic matter can be valued, harmful substances can be diluted, the mixture's buffering capacity is increased, and the digested product's quality is improved—all of which are benefits of this economically viable optimisation technique (Salvador et al., 2017; Li et al., 2007; König et al., 2022). Because of their high nitrogen content, poultry droppings can present certain inhibition issues when digested alone (Güngör-Demirci and Demirer, 2004). The latter can inhibit the AD process when it surpasses a threshold (between 1500 and 7000 mg_L-1) (Romero-Guiza et al., 2016). Therefore, co-digestion is recommended (Salvador et al., 2017); nevertheless, the co-substrate should be chosen with care when taking into account its composition and influence on the primary substrate and optimize the mixing ratio as well (Liu et al., 2012).

Whey, fruit and vegetable waste, rice straw, digested sludge, as well as other kinds of manure, such as bovine slurry, buffalo dung, and sheep manure, can all be used to effectively cure poultry droppings (Li et al., 2007; Liu et al., 2012). In a previous study, the physicochemical sludge (PCS), the final product of primary wastewater treatment after slaughter, produced more methane than the droppings. Additionally, it turned shown to be easily recovered and prepared, and it decomposes more easily than other poultry abattoir wastes. After preparation, a fairly homogenous sludge can be obtained (Romero-Guiza et al., 2016). PCS was chosen as a co-substrate as a result of these considerations.

2. MATERIALS AND METHODS

Study Area

The study was carried out at the Faculty of Agriculture, Department of Animal Production Teaching and Research Farm, located at the Naraguta Campus Jos North, Plateau State. The district's yearly temperature is 28.410C (83.140F), which is -1.05% lower than Nigeria's average. Jos North typically receives about 155.51mm (6.12 inches) of precipitation and has 181.54 rainy days (49.74% of the time) annually (https://weatherandclimate.com/amp/nigeria/plateau/jos).

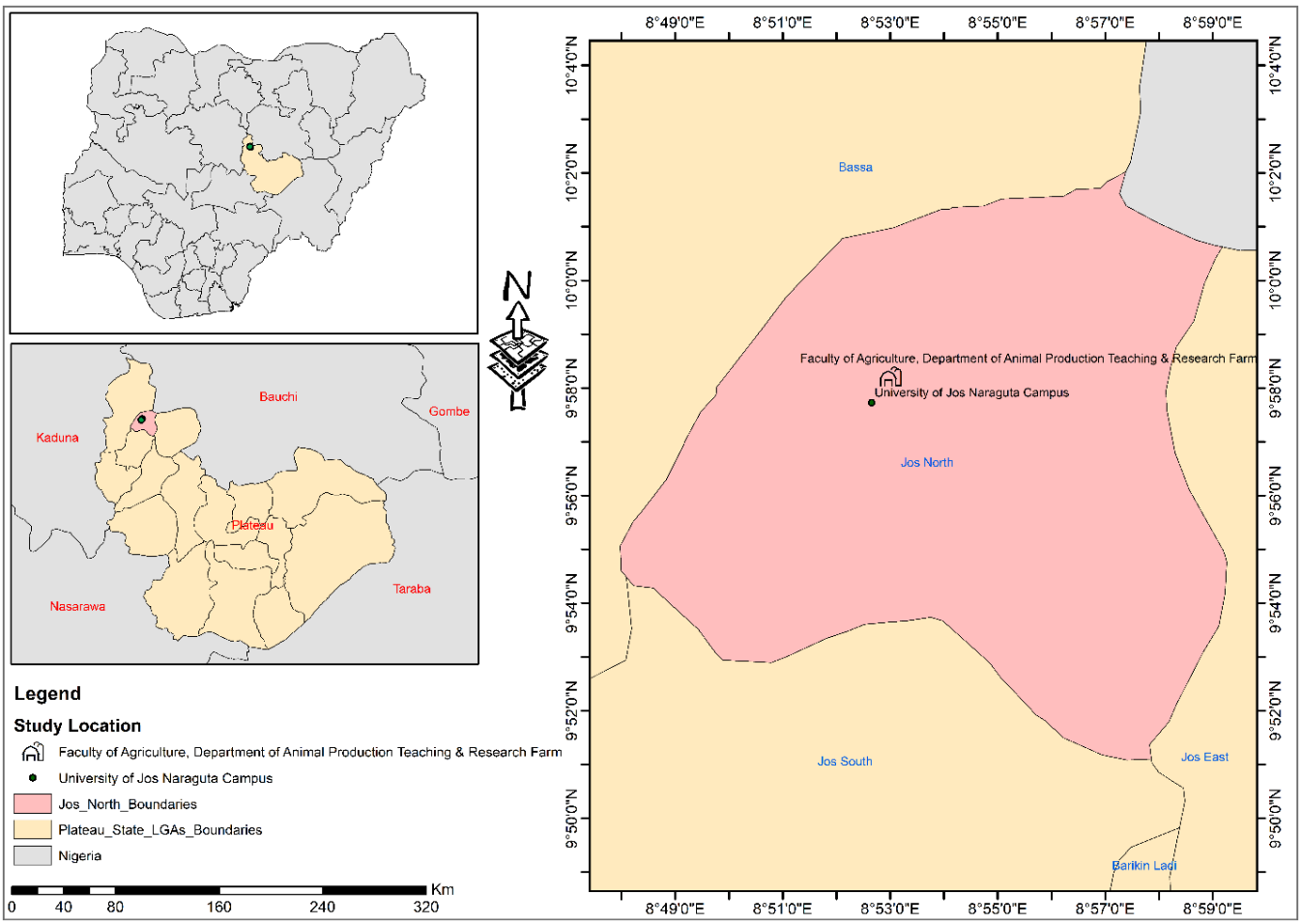

Figure 1 Map of Nigeria Showing Plateau State and the Study Area

Materials

The materials used in this research are listed below:

Broiler droppings is used as biomass

Layer droppings is used as biomass

Fresh cow dung is used as inoculum

An 80-liter bio-digester with accessories

A multifunctional water quality tester (Model Number EZ-9909SP)

Weighing balance for weight measurement

Thermometer

Sacks for storing fecal

Buckets for measuring

Sample collection bottles

A sieve

A meter rule for methane yield measurement

Biomass Collection

Layer and broiler bird's droppings were collected from the pens of the research and Teaching farm, Faculty of Agriculture, University of Jos. They were sun-dried, sieved to remove feed materials, and stored in new clean sacks. New and sterile sacks were used to store the droppings to prevent contamination.

Design and Development of Biodigester

The 80-liter bio-digester was designed at the University of Jos's Department of Civil Engineering, and was fabricated at Dilimi Fabrication Market, Jos North, Plateau State (Plate 1). The digester was fabricated with a galvanized iron sheet (this is because of its corrosion resistance).

Plate 1 An 80-liter biodigester with its accessories

Slurry Preparation

A slurry combining biomass (manure), and water was prepared. One part of the slurry was biomass, while the other was water in a 1:1 combination. The one part of the biomass was a combination of layer and broiler (30% and 70%) already dried droppings. 20-litre bucket was used for the slurry preparation, 3-litre of layers and 7-litre of broilers droppings were measured and poured into the bucket. Then, 10-litre of water were measured and added to the same bucket, making a total of 20-litre. Wooden spatula was used to mix the combination to get an even mixture. The pH and temperature of the slurry was taken with a multifunctional water quality Tester.

Digester Loading, Sample Collection and Laboratory Analysis

The prepared slurry was loaded into the 80-liter bio-digester through the inlet pipe with the aid of a funnel. A fresh cow dung of 1.6-litre was added into the digester to act as inoculum to seed the digester and kickstart the process of anaerobic co-digestion process. The

biodigester was kept under direct sunlight to enhance bacterial activity, as the warm temperatures from sunlight exposure can increase the metabolic rate of the bacteria in the biodigester and accelerate the breakdown of organic matter at a higher rate. The experiment was carried out for 30 days. The samples were later collected into already sterilized sample collection bottles. The bottles were also sterilized with hot water, both inside and outside were sterilized to kill any pathogenic organisms that can cause contaminations to the samples.

The substrate and the digestate were collected into a sterilized bottles and taken to the microbiological laboratory for analysis. Daily data collection is to measure methane yield (gas production) from the digester with a meter rule for the study duration (30 days). Samples of the slurry, both the digestate and substrate, were taken to the laboratory for analysis to determine the presence of Escherichia coli, *Klebsiella spp, Shigella spp,* and *Salmonella spp,* results were presented in tables. The Laboratory analysis was conducted at Optima Kings Research Center and Laboratories located at No.4 Miango Road, Kuffang, Jos, Plateau State, Nigeria.

Procedures for Microbial Examination

Isolation and Quantification of Bacteria and Fungi Present in Poultry Droppings

Aliquots (0.1 ml) were plated from various dilutions of a single millilitre of poultry dung that had been diluted up to 10 -2. The pour plate method was employed to enumerate the bacteria with nutrient agar, while fungi were counted on Potato Dextrose Agar supplemented with 30 mg/l streptomycin.

Isolation and Quantification of Total and Fecal Coliforms

Using a spread plate technique, the samples were plated from 10-1 and 10-2 dilutions. The faecal and total coliforms were isolated using eosin methylene blue agar and MacConkey agar, respectively. Typical colonies were counted at the conclusion of the incubation period.

Isolation and Enumeration of Specific Pathogenic Bacteria

Salmonella-Shigella agar, Mannitol salt agar, and Simon's citrate agar were used to isolate pathogenic bacteria like *S. aureus, Salmonella spp.,* and *Klebsiella spp*. The spread plate method was applied. The quantity of typical colonies was counted following the incubation period. Biochemical tests were then used to confirm these colonies.

Isolation of Pure Culture and Preservation of Isolates

Subculturing was used in the process until a pure culture was obtained. According to the pure isolate was stored in a refrigerator until it was required.

Characterization and identification of bacterial isolates

After identifying and characterising the bacteria based on their colony, cellular, and biochemical characteristics, standard textbooks were examined.

Procedure for Determining Methane Yield

Using a meter rule to measure the gas displacement in the gasholder, the methane yield was measured and recorded every day for 30 days.

Statistical/Data Analysis

The independent t-test from the Statistical Package for Social Sciences (SPSS) for Windows version 20.0 was used for statistical analysis.

3 RESULTS AND DISCUSSION

Tables 1-2 and Figure 1 display the results of laboratory analyses on the decrease in pathogenic bacteria both before and after digestion, as well as the effectiveness of the anaerobic co-digestion process in the microbiological stabilization of layers and grill droppings. Also, the experimental results obtained during the observation period for methane yield in the study are presented in (Table 3 and Figures 2-4).

Table 1 Microbial Load of Layer and Broiler Waste Matter (Substrate and Digestate) 30:70

Category	Undigested Mean±SEM	Digested Mean±SEM	P. value (≤0.05)
Total Viable Count(x103)	1.32±0.02a	0.85±0.03b	0.010
Total Coliform Count(x102)	1.20±0.20	0.00±0.00	0.105
E. coli count (cfu/ml) (x102)	3.60±0.30a	0.00±0.00b	0.053
Fungal Count (102)	0.63±0.03b	3.80±0.03a	0.000

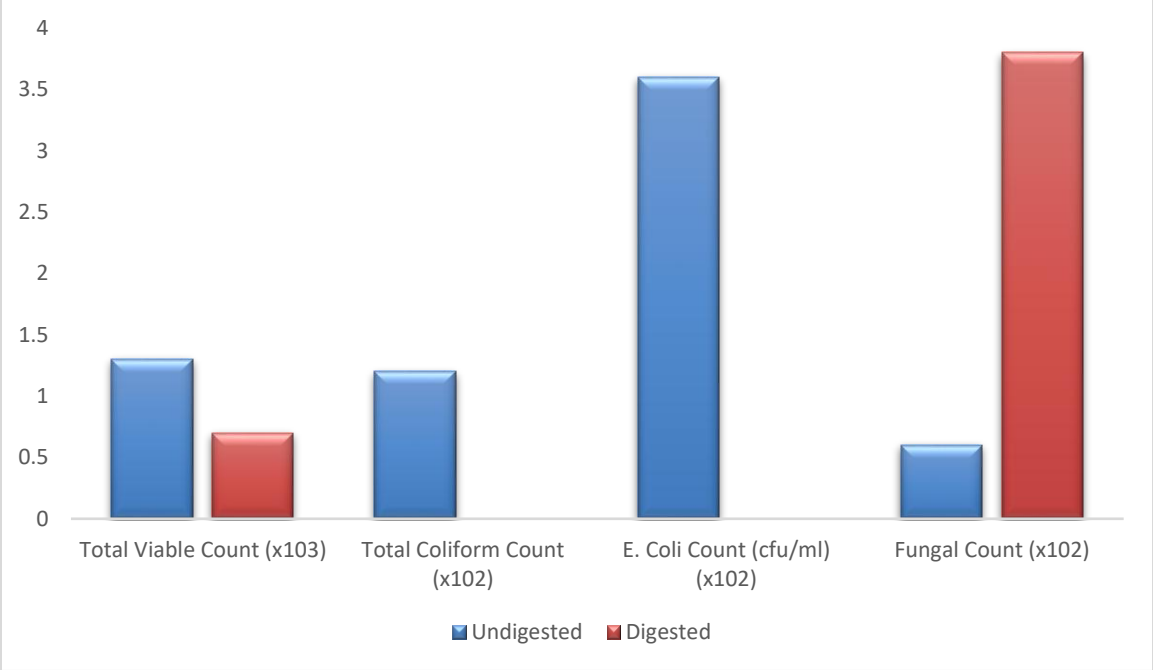

Figure 2 Comparison of Mean ± SEM for Undigested and Digested Samples

Table 1 and Figure 2 illustrate the reduction in pathogenic microorganisms before and after co-digestion of 30% layer and 70% broiler droppings. Significant decrease was observed in Total Viable Count (1.32±0.02 to 0.85±0.03), Total Coliform Count (1.20±0.20 to 0.00±0.00), and E. coli count (3.60±0.30 to 0.00±0.00) after digestion. These reductions align with previous studies by. Interestingly, the fungal count increased significantly post-digestion, suggesting better survival or favorable conditions for fungi, consistent with findings by. All things considered; the digesting process successfully decreased bacterial populations while maybe encouraging the growth of fungi.

Table 2 Efficiency of the Anaerobic Co-digestion Process in the Microbial (*Escherichia coli*, *Klebsiella spp*, *Salmonella spp,* and *Shigella spp*) Stabilization of Layers and Broilers Droppings (30:70)

Organism	Before Digestion	After Digestion	% Reduction	Recommended Standards	Conformity to Standard	References.
Salmonella spp.	+	-	100	-	100	*
E. coli	+	-	100	-	100	**
Shigella spp.	+	-	100	-	100	***
Klebsiella spp.	+	-	100	-	100	****

Key: (-) = Negative/Absent, (+) = Positive/Present, * FAO/WHO, (2022); *** WHO, (2006); *** EFSA, (2007)

Table 2 illustrates the efficiency of anaerobic co-digestion in microbial stabilization of layer and broiler droppings, comparing results with recommended standards. Before digestion, *Klebsiella spp., Salmonella spp., E. coli,* and *Shigella spp.* Showed high contamination levels. Post-digestion, all pathogens were reduced to undetectable levels, aligning with recommended standards: *Salmonella spp.* FAO/WHO, (2022), *E. coli*, *Shigella spp.* WHO, (2006), and *Klebsiella spp.* (EFSA, 2007). These findings corroborate studies by Kim et al., (2012), Berghaus et al., (2013), Ricke et al., (2013) demonstrating significant pathogen reduction or elimination through anaerobic co-digestion, ensuring safer reuse of digested material.

Table 3 Daily Methane Yield Record (30 days)

Day/Date	pH	Temperature (oC)	Electrical Conductivity (EC)	Salinity	Total Dissolved Solid (TDS)	Vol. in dm3
1 (19th May)	6.90	29.20	11840	0.66	5953	0.00
2 (20th May)	4.34	30.40	10206	0.63	5845	0.00481
3 (21st May)	4.32	34.50	12791	0.57	6409	0.00641
4 (22nd May)	5.34	25.60	10363	0.58	5203	0.00801
5 (23rd May)	6.51	28.10	10269	0.62	6621	0.00961
6 (24th May)	6.91	21.00	3447	0.17	1759	0.00801
7 (25th May)	6.69	26.70	13065	0.73	6500	0.0016
8 (26th May)	6.28	28.60	15124	0.75	5842	0.00321
9 (27th May)	6.67	31.90	13947	0.81	6818	0.01121
10 (28th May)	6.32	30.80	12789	0.78	5401	0.01121
11 (29th May)	6.49	29.00	13643	0.75	6773	0.02003
12 (30th May)	6.49	25.40	7129	0.27	1955	0.02483
13 (31st May)	5.79	25.50	18082	0.41	5203	0.03556
14 (1st June)	5.36	24.00	9344	0.36	4734	0.04325
15 (2nd June)	6.01	28.80	12729	0.71	5422	0.04213
16 (3rd June)	5.85	27.00	12606	0.73	6485	0.04822
17 (4th June)	6.00	27.60	13175	0.19	6350	0.04918
18 (5th June)	6.21	26.90	10791	0.18	5411	0.03893
19 (6th June)	6.11	27.30	11983	0.19	5881	0.03316
20 (7th June)	5.97	24.00	10112	0.61	6143	0.03957
21 (8th June)	6.71	27.40	10781	0.17	5417	0.04709
22 (9th June)	6.04	32.20	16176	0.90	8032	0.05398
23 (10th June)	6.22	25.40	13430	0.76	6570	0.05911
24 (11th June)	6.64	23.40	14506	0.83	7167	0.05078
25 (12th June)	6.36	24.95	14028	0.80	6947	0.04068
26 (13th June)	6.30	24.95	13975	0.79	6720	0.02419
27 (14th June)	6.33	24.95	14001	0.80	6833	0.02195

28 (15th June)	6.43	24.20	10791	0.37	5181	0.01714
29 (16th June)	6.11	24.70	13708	0.64	6757	0.01602
30 (17th June)	6.80	32.70	16922	0.93	8436	0.01922

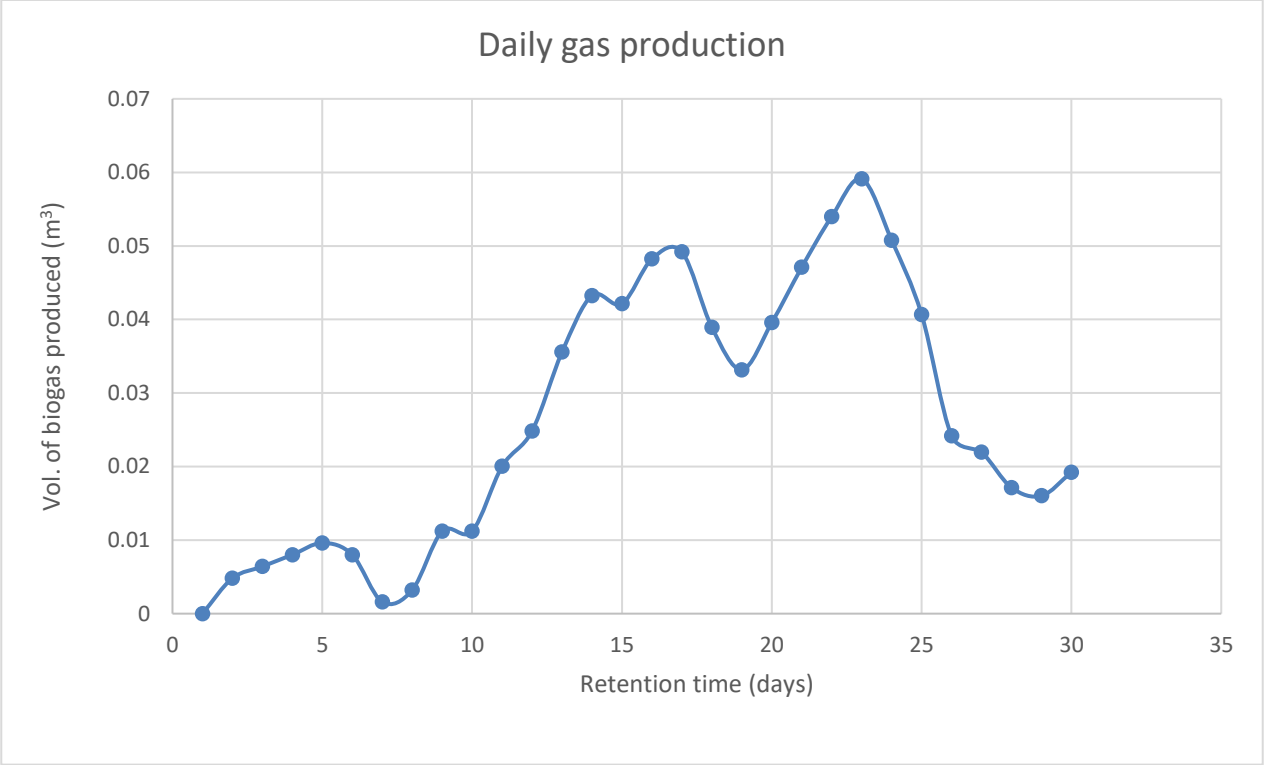

Figure 3 Daily Methane Production (dm3)

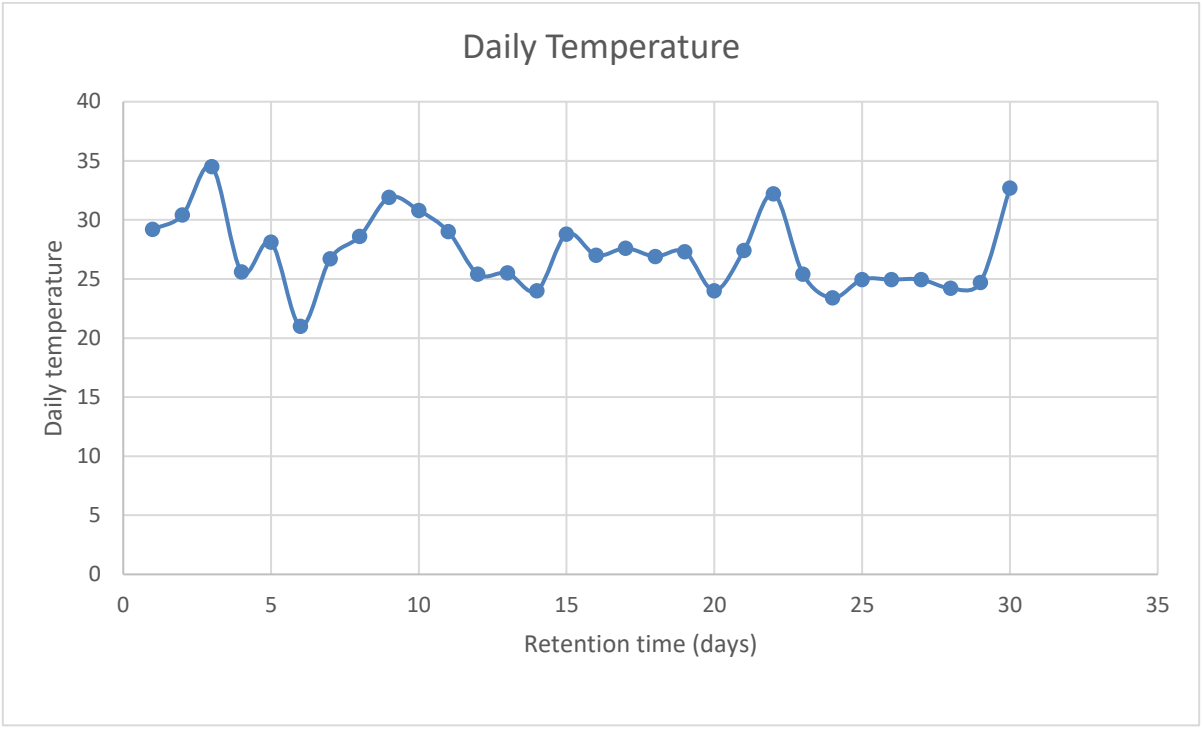

Figure 4 Daily Temperature Readings

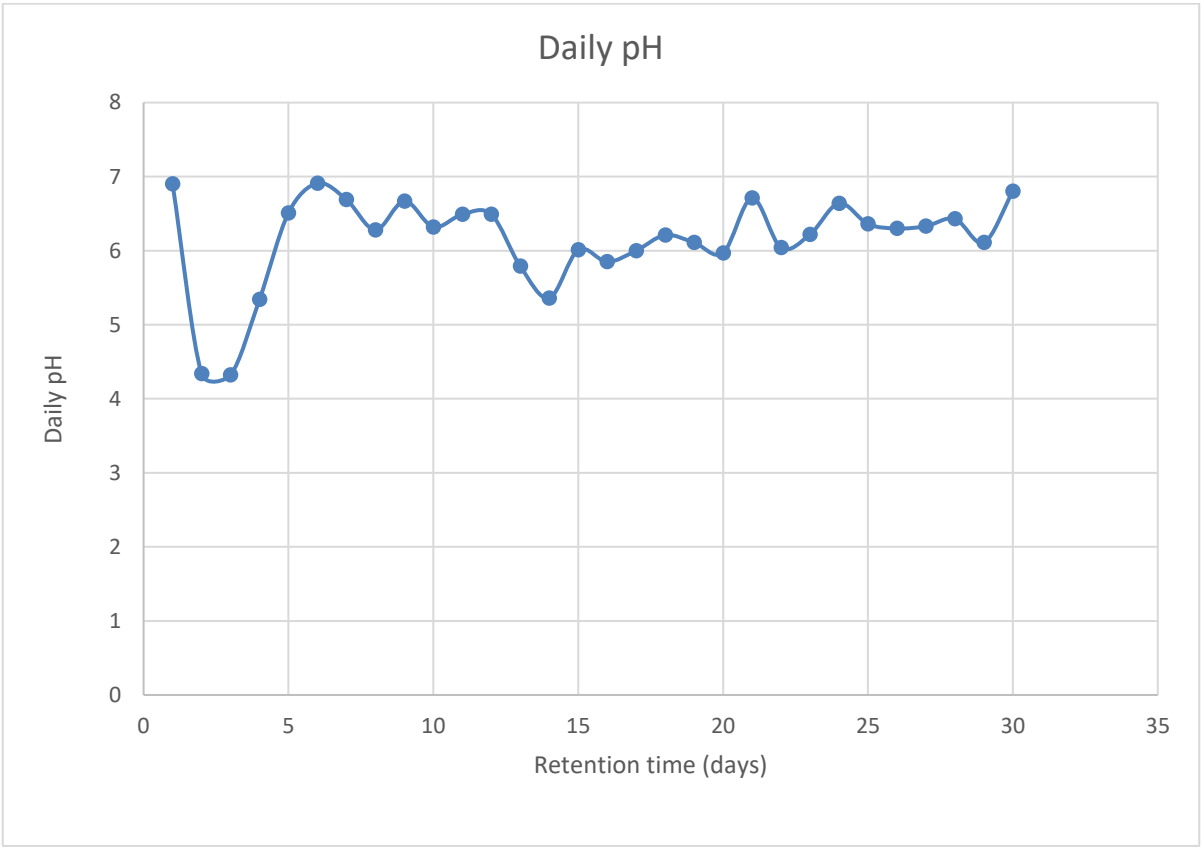

Figure 5 Daily pH Readings

Table 3 and Figures 3-5 present daily measurements of anaerobic co-digestion parameters over 30 days. pH fluctuated between 4.32 and 6.91, stabilizing around neutral (6.28) after day 7. Temperatures ranged from 21.0°C to 34.5°C, within the mesophilic range. Gas production, measured in dm^3, followed a typical pattern: Low initial volumes (0.00481-0.00641 dm^3) during the lag phase, steady increase peaking at 0.05911 dm^3 during exponential growth, followed by a decline (0.05078-0.02419 dm^3) as organic matter depleted. This result aligns with studies by Kafle and Chen, (2016), showing similar phases in anaerobic digestion of poultry manure. The peak production (0.05911 dm^3) falls within the typical range (0.04-0.06 dm^3) for poultry manure digestion.

4. CONCLUSIONS

This study demonstrated significant reductions in microbial loads and elimination of pathogens like *Salmonella sp., E. coli, Shigella sp.,* and *Klebsiella spp* in poultry litter (30:70 layer: broiler) after anaerobic co-digestion. The process produced substantial methane, indicating poultry droppings' potential as an alternative energy source. These findings align with established literature, suggesting efficient biogas production and sustainable waste management in the poultry sector. It is recommended that poultry droppings should be subjected to anaerobic co-digestion before usage by farmers as this will reduce the microbial loads present in the present and ensure its safety for reuse, also high-quality feed should be fed to both layer and broiler birds as this will increase the organic content of the droppings which directly influences methane yield.

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Authors Contribution

Idris AA: Gathered and organized data, conducted experiments and fieldwork. Created the manuscript's first draft, which included the main analysis and interpretation of the results.

Nathan HB: Gathered and organized data, conducted experiments and fieldwork.

Meshach IA: Oversaw the research process, provided guidance, and ensured the project stayed on track. Revised and edited the manuscript to improve clarity, quality, and accuracy.

Okpanachi U: Oversaw the research process, provided guidance, and ensured the project stayed on track. Revised and edited the manuscript to improve clarity, quality, and accuracy.

Conference presentation

This paper has not yet been presented at any conference or academic meeting.

Informed consent

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Ethical approval

Not applicable.

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Data and materials availability

All data associated with this study are present in the paper.

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