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Production of Biogas from Poultry Droppings using Anaerobic Digestion

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Abstract

The increasing demand for renewable energy sources highlights the need for optimizing biogas production. This study investigates the potential of poultry droppings as a substrate for biogas generation, focusing on the influence of temperature and pH variations on yield as key bacterial species involved were characterized. Anaerobic digestion was conducted using poultry droppings over seven weeks retention periods using the water displacement method, while temperature and pH variations were monitored throughout the study.. The highest biogas yield was recorded at week 2 (14 days) with 535.00±70.50 mL, while the lowest yield was observed at weeks 3 and 4 (21-28 days) with 0.00 mL. The highest temperature was recorded at week 6 (42 days) with 41.71±0.64°C, and the lowest at week 4 (28 days) with 30.20±2.78°C. The lowest pH was observed at week 2 (6.15±0.462) after digestion, indicating acidogenesis, while the pH stabilized in the later weeks. Microbial analysis revealed Bacillus subtilis (35.71%) as the most dominant bacterium, followed by Staphylococcus aureus and Escherichia coli (21.42%) each, while Shigella species had the lowest occurrence with 7.14%. The results indicate anaerobic digestion of poultry droppings can yield significant amounts of biogas, particularly during the second week of retention.

Keywords: Biogas, Temperature, pH, microbes, Poultry droppings

INTRODUCTION

Biogas is a flammable, colorless gas produced through the anaerobic breakdown of organic matter, including waste from animals, plants, humans, industries, and municipalities. This biological fermentation process primarily generates methane (50–70%) and carbon dioxide (30–50%), along with trace amounts of gases such as nitrogen, hydrogen, ammonia, hydrogen sulfide, and water vapor (each contributing less than 1%) (Ngumah *et al.*, 2013). Biogas can be derived from any biodegradable material suitable for anaerobic digestion (Adeleke *et al.*, 2023).

The production of biogas via anaerobic digestion (AD) of animal manure, slurries, and various organic wastes transforms these substances into a renewable energy source. Anaerobic digestion is a well-established technology, making biogas a recognized 'first-generation' biofuel. Initially developed as a waste treatment method, it has evolved into a process focused on methane generation for energy use (Sher *et al.*, 2024; Orhorhoro and Oghoghorie, 2019).

Renewable natural gas (RNG) is a sustainable, carbon-neutral substitute for conventional fossil-based natural gas, providing a clean and reliable energy source derived from organic waste. Among the available production techniques, anaerobic digestion is the most commonly used and technologically advanced. This process occurs in a low-oxygen environment, allowing naturally occurring bacteria to break down organic material efficiently (Rasapoor et al., 2019).

Biogas generated from poultry droppings typically contains 55–70% methane, making it a valuable energy source due to its high calorific value (Hagos *et al.*, 2016). The methane concentration is influenced by factors such as feedstock composition, digestion temperature, and microbial efficiency (Subbarao *et al.*, 2023). The presence of CO_2 (25–45%) affects the methane content and overall energy potential of the biogas (Subbarao *et al.*, 2023; Wainaina *et al.*, 2020).

Hydrogen sulfide (H_2S: 0–1%) is another component, primarily determined by the sulfur content in the feedstock and the activity of sulfate-reducing bacteria (Paramaguru *et al.*, 2017; Ibrahim *et al.*, 2019). High levels of H_2S can lead to corrosion issues, but its

concentration can be controlled through treatment methods such as chemical scrubbing or biological processes (Paramaguru *et al.*, 2017).

Ammonia (NH_3: 0–1%) results from the high nitrogen content in poultry waste, largely due to the protein-rich poultry diet (Subbarao *et al.*, 2023; Anukam *et al.*, 2019). Excess ammonia can inhibit microbial activity if not properly controlled (Hagos *et al.*, 2016; Anukam *et al.*, 2019). Similarly, nitrogen (N_2: 0–2%) originates from protein degradation and does not directly contribute to methane production. However, its derivatives, particularly ammonia, influence digestion stability (Hagos *et al.*, 2016; Anukam *et al.*, 2019).

Hydrogen (H_2: 0–1%) plays a role in methanogenesis, depending on microbial balance and digestion efficiency (Anukam *et al.*, 2019). Oxygen (O_2: <0.1%) is generally low, as higher levels may indicate potential disturbances in the anaerobic process. Volatile Organic Compounds (VOCs: <0.1%) are present in trace amounts and may have environmental and health implications (Ngumah *et al.*, 2013).

The biogas production process consists of four key stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Hagos *et al.*, 2016). During hydrolysis, complex macromolecules such as carbohydrates, fats, and proteins are enzymatically broken down into their monomeric components by microbial activity, primarily involving *Bacteroides*, *Clostridia*, and facultative bacteria such as *Streptococci*. In the subsequent acidogenesis phase, these monomers undergo further degradation into short-chain fatty acids, including acetic, propionic, butyric, and carbonic acids, along with alcohols, hydrogen, and carbon dioxide. Acetogenesis then facilitates the conversion of these short-chain acids into acetate, hydrogen, and carbon dioxide. Finally, during methanogenesis, methanogenic archaea metabolize these intermediates, producing methane and carbon dioxide. Notably, approximately one-third of methane generation results from the reduction of carbon dioxide by hydrogen (Subbarao *et al.*, 2023).

Several factors influence biogas production, including digester conditions, pH, nutrient availability, temperature, the carbon-to-nitrogen (C/N) ratio, and the presence of a starter culture (Sher *et al.*, 2024). Maintaining a stable and dynamic equilibrium within the anaerobic digester is essential for optimal microbial activity. The pH should be maintained within the range of 6.6 to 7.6, as methanogenic bacteria function most efficiently within this interval (Wainaina *et al.*, 2020). Adequate concentrations of essential nutrients, such as nitrogen and phosphorus, must be supplied to support bacterial growth (Adeleke *et al.*, 2023). Temperature is another critical factor, with mesophilic bacteria requiring an optimal range of 30–38°C, while thermophilic bacteria thrive at higher temperatures between 49–57°C. The ideal C/N ratio for efficient biogas production falls between 25 and 30.

Additionally, the use of a starter culture significantly enhances the degradation of organic matter, with activated sludge and rumen fluid being among the most commonly employed inoculants (Rasapoor *et al.*, 2019).

The primary challenge in the modern world is to harness an energy source that is both environmentally friendly and economically viable. This need has forced researchers to explore alternative energy sources. Unfortunately, alternative sources such as solar, hydro, and wind energy require significant financial investment and technical expertise, making them difficult to implement in developing countries like Nigeria (Paramaguru *et al.*, 2017). Energy consumption in Nigeria has been increasing at a high rate. On a global scale, the Nigerian energy industry is considered one of the most inefficient in meeting consumer needs. This inefficiency is most evident in the persistent disequilibrium in the markets for electricity and petroleum products (Ibrahim *et al.*, 2019). The poor energy service provision has adversely affected living standards and worsened both income and energy poverty, particularly in an economy where the majority of the population lives on less than $2 a day (Anukam *et al.*, 2019).

The development of biogas technology presents a viable alternative energy source that is both affordable and environmentally sustainable. It can help preserve forests and contribute to achieving the 7th mandate of the Millennium Development Goals on environmental sustainability (Sher *et al.*, 2024). In addition to addressing the urgent need for waste treatment to maintain a clean environment, anaerobic digestion offers potential value recovery from organic waste (i.e., "waste to wealth") through biogas production (Khanal *et al.*, 2021). Furthermore, millions of tons of waste released daily emit significant amounts of methane when

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exposed to the atmosphere, a gas 320 times more harmful to human health than carbon dioxide (Iglesias *et al.*, 2021).

This study aims to investigate biogas production from poultry droppings, assess the pH changes, and isolate bacteria involved in anaerobic digestion.

MATERIALS AND METHODS

2.1 Study area

Sokoto is geographically positioned between latitudes 12°45′N and 13°35′N, and longitudes 4°55′E and 6°00′E (Tsoho and Salau, 2012).

Sampling was conducted at Dankure Market situated within the Sokoto North Local Government Area (LGA) of Sokoto State, Nigeria. Kara Market is centrally located at approximately latitude 13.0625°N and longitude 5.2339°E. The strategic locations of these markets within an urbanized zone characterized by dense commercial and residential activities contribute significantly to the generation of substantial amounts of poultry waste (Ifabiyi and Ojoye, 2013).

2.2 Sample Collection and Preparation

Fresh poultry droppings samples were collected from Kara Market located at Latitude 13.0625°N and longitude 5.2339°E and placed in a clean polyethylene bag. The samples were processed at the Energy Research Centre, Usmanu Danfodiyo University, Sokoto, for laboratory analysis within 24 hours of collection. The freshly collected poultry droppings samples were first air-dried under the sun, then further dried in an oven at 105°C. After oven drying, the samples were left to dry at room temperature for two weeks before being ground into a fine powder using a pestle and mortar (Ibrahim *et al.*, 2019).

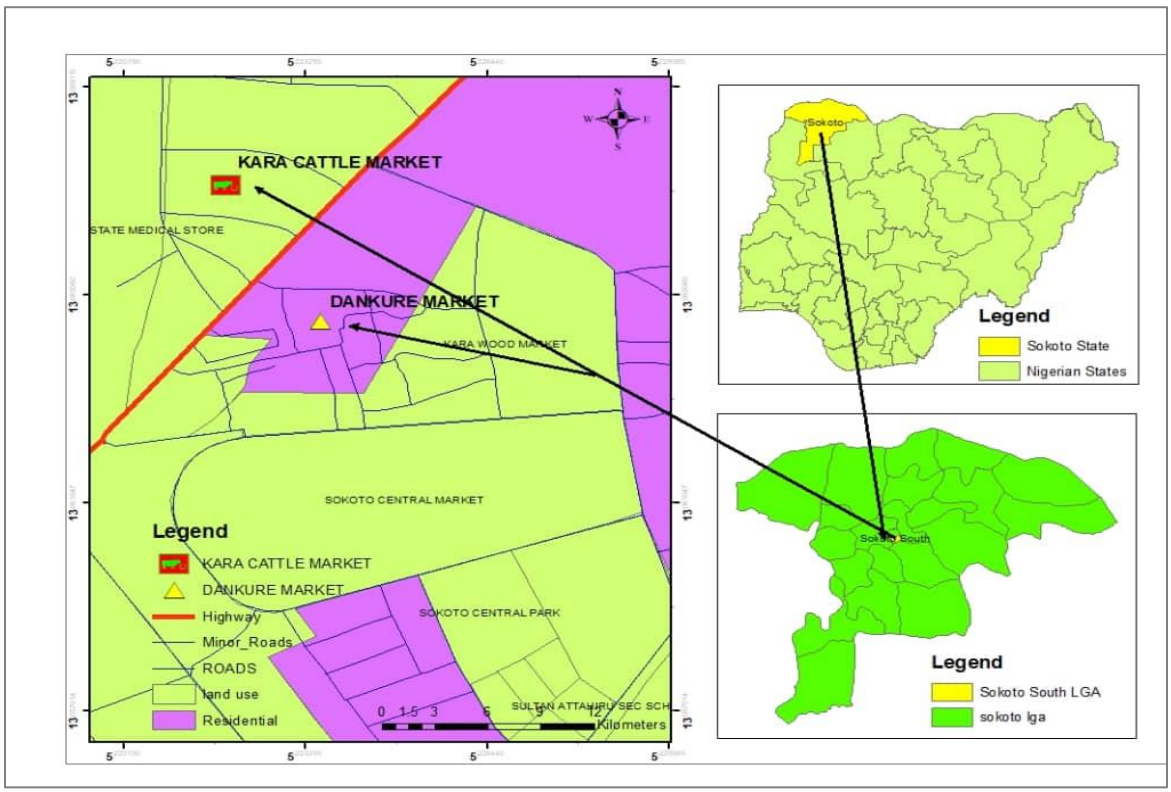

Figure 1: Map of Sokoto showing the study areas (Department of Geography, Sokoto State University, Sokoto, 2024)

2.4 Experimental Design

Biogas plant was set up using three milk tins, each with a capacity of 400g, as biogas digesters. A hole was drilled at the center of each tin's lid, and a 1-inch hose pipe was connected to the hole in each digester, sealed with epoxy steel gum to prevent gas leakage. The gas produced in the digesters was directed through the hose pipe into a 1000 cm^3 measuring cylinder filled with water, which was placed upside down in a basin of water (water displacement method). The cylinder was securely held in place by a retort stand. As the gas was produced from the digesters, it displaced the water in the measuring cylinder.

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

The volume of the produced gas was measured by the amount of water displaced (Onwuliri *et al.*, 2014). Daily temperature readings were recorded at 12:00 noon throughout the seven-week retention period (Anukam *et al.*,2019).

2.5 Slurry Preparation

A total of 100 grams of poultry droppings was measured using a digital weighing scale and transferred into three 400g-capacity tins designated as digesters. To each tin, 600 ml of water was added, maintaining a 1:6 substrate-to-water ratio. The mixtures were stirred using a rod for five minutes to ensure thorough homogenization (Onwuliri *et al.*, 2014). To prevent leakage and maintain anaerobic conditions, each digester was sealed with a combination of candle wax and epoxy gum for four minutes (Anukam *et al.*,2019).

Plate 1: Sample of substrate (a) Sample of poultry droppings (b) Grinded poultry dropping (d)An experimental design of biogas plant setup

2.6 Determination of pH

The pH values of the substrates were measured both before and after digestion on a weekly basis using a digital pH meter (Rabah *et al.*, 2010). A small sample of slurry was prepared by mixing the poultry droppings with water, which was then placed in a clean beaker (Agrawal,

Chaudhari, and Ghosh, 2023). The pH meter was calibrated with buffer solutions, and the pH of the slurry was measured by immersing the electrode in the digester. After each measurement, the electrode was thoroughly rinsed with distilled water before proceeding to the next substrate (Barua, Rathore, and Kalamdhad, 2018).

2.7 Determination of Temperature

To monitor the temperature, a wall-mounted thermometer (Taylor Precision Products 5329 Indoor/Outdoor Thermometer) was installed near the biogas digester. The thermometer was calibrated to ensure precise temperature readings (Anukam *et al.*,2019).

At 12:00 PM daily, the ambient temperature was recorded throughout the retention period. After each daily reading, the thermometer was reset to measure the next 24-hour cycle of temperature variation (Masinde, Nyaanga, Njue, & Matofari, 2020). The data collected were then analyzed to assess the relationship between the ambient temperature and biogas production yield (Babaei & Shayegan, 2019)..

2.8 Bacteriological Analysis

After seven weeks of anaerobic digestion, the digested samples (post-biogas production) were prepared for bacteriological analysis. The digester, equipped with a hose pipe for gas release, was securely sealed once digestion was complete. The sealed sample was then transported under refrigerated conditions (4°C) to preserve the sample's integrity for accurate microbial analysis. All samples were delivered within 24 hours to the Biology Laboratory at Sokoto State University for further analysis (Khanal *et al.*, 2021).

Bacterial samples were transferred to the prepared media using a sterilized inoculating wire loop or sterile swab. The loop was heated until it glowed red, then allowed to cool briefly. After dipping the loop in the sample, it was streaked in a zig-zag pattern on the nutrient agar surface to ensure even bacterial distribution (Onwuliri *et al.*, 2014; Rabah *et al.*, 2010). The inoculated petri dishes were sealed with parafilm to minimize contamination and placed in an incubation chamber at 37°C for 24 hours. Plates were placed upside down to prevent condensation from forming on the agar surface. After incubation, the bacterial growth was observed (Rabah *et al.*, 2010).

After the 24-hour incubation period, the bacterial colonies were carefully examined for distinct characteristics such as size, shape, and color. Well-isolated colonies with unique morphological traits were selected. A single colony was picked using a sterile loop, and further sub-culturing was done if necessary to ensure purity. The colony was analyzed, and a pure culture was obtained through sub-culturing (Agrawal *et al.*, 2023). The isolated colony was transferred to a new sterile agar plate and streaked in a zig-zag pattern to promote growth. The plate was incubated at 37°C for 24 hours. After this incubation, plates were examined again, confirming the growth of a single colony type, which indicated a pure culture (Barua *et al.*, 2018).

3.9.7 Isolates Characterizations

Smear was prepared on a clean slide for each isolate, and Gram staining was conducted. The slide was examined under a microscope using an oil immersion objective lens (Madigan *et al.*, 2018).

2.9 Biochemical Characterization

2.9.1 Catalase Test

The catalase test identifies the production of the catalase enzyme by bacteria, indicated by bubble formation upon the addition of hydrogen peroxide (Madigan *et al.*, 2018). A sterilized wire loop was used to transfer bacterial colonies onto a clean glass slide containing a few drops of hydrogen peroxide. Bubble formation signals a positive result, while no bubbles indicate a negative result, suggesting the absence of the catalase enzyme (Amha *et al.*, 2017).

2.9.2 Oxidase Test

Bacterial colonies grown on agar plates are smeared onto a filter paper soaked with the oxidase reagent. A color change within 10–30 seconds indicates oxidase activity. A positive result is marked by a dark blue or purple coloration, while no color change indicates a negative result (Amha *et al.*, 2017).

2.9.3 Citrate Test

A citrate medium containing sodium citrate, ammonium dihydrogen phosphate, dipotassium phosphate, magnesium sulfate, sodium chloride, and bromthymol blue is prepared. After inoculating bacterial colonies onto the solidified medium, color changes are observed after

incubation at 37°C for 24-48 hours. A shift from green to blue indicates positive citrate utilization, while no color change confirms negative results (Madigan *et al.*, 2018).

2.9.4 Indole Test

Tryptone broth is prepared, inoculated with bacterial cultures, and incubated at 37°C for 24 hours. After adding Kovac's reagent, a red or pink color in the organic layer indicates a positive result, while a yellow or unchanged layer indicates a negative result (Madigan *et al.*, 2018)..

2.9.5 Triple Sugar Iron (TSI) Test

The medium Triple Sugar Iron agar contains glucose, lactose, sucrose, ferrous sulfate, sodium thiosulfate, and phenol red to differentiate bacteria based on sugar fermentation, gas production, and H_2S formation. After incubation at 37°C, color changes and gas production are observed:

Using a sterile needle, the medium is inoculated by stabbing the butt and streaking the slant, then incubated at 37°C for 18-24 hours. After incubation, a red slant/yellow butt indicates glucose fermentation only, yellow slant/yellow butt indicates fermentation of glucose and lactose and/or sucrose, and a red slant/red butt indicates no fermentation. Gas production appears as bubbles or cracks, while H_2S production is shown by a black precipitate in the medium. Slant growth reflects aerobic activity; butt growth indicates anaerobic metabolism (Madigan *et al.*, 2018).

2.9.7 MR-VP Test

The MR-VP test assesses the ability of bacteria to produce mixed acid or acetoin.

Methyl Red (MR) Test: After incubation, methyl red indicator is added. A red color indicates acid production (positive MR test), while yellow indicates a negative result.

Voges-Proskauer (VP) Test: Reagents A and B are added to the culture, and a red or pink color indicates acetoin production (positive VP test), while no color change indicates a negative result (Agrawal *et al.*, 2023; Madigan *et al.*, 2018)

2.10 Statistical Analysis

Data on biogas production and ambient temperature were summarized using means and standard deviations. A one-way Analysis of Variance (ANOVA) was performed to determine if significant differences existed in biogas production and temperature across the seven weeks. Post-hoc tests, such as Tukey's Honest Significant Difference (HSD), were used to identify specific weeks with significant differences.

RESULTS

The results of biogas yield across retention periods show notable differences, The highest biogas yield was recorded at 14 days (535.00 ± 70.50 mL) with a temperature of (37.60 ± 1.98°C), followed by 7 days (250.00 ± 10.00 mL), with a temperature of (34.20 ± 10.3°C), followed by 35 days (246.70 ± 32.10 mL), with a temperature of (37.32 ± 7.12°C) and the least biogas yield was recorded at 28 days (0.00 ± 0.00 mL) and 21 days (0.00 ± 0.00 mL), with temperatures of 30.20 ± 2.78°C and 33.40 ± 2.06°C, respectively (Table 1).

Table 1: Biogas yield and temperature variations of anaerobic digestion of poultry droppings over seven weeks retention periods

Retention Period (Days)	Biogas Yield	Temperature (°C)
7	250.00±10.00^a	34.20±10.3^a
14	535.00±70.50^b	37.60±1.98^b
21	0.00±0.00^c	33.40±2.06^a
28	0.00±0.00^c	30.20±2.78^c
35	246.70±32.10^a	37.32±7.12^b
42	73.30±15.30^d	41.71±0.64^d
49	30.00±10.00^d	38.20±0.44^c

Results are expressed as mean ± standard deviation, means with the same letters are not significantly different (P≦0.05)

The figure showed biogas trends segmented into three distinct phases. The Lag Phase (day 7 to day 28), represented by yellow, showed moderate fluctuations in temperature. The exponential phase (day 28 to day 42), represented by green, showed a rapid increase in temperature and the decline phase (day 42 to day 49), represented by pink, showed a decrease in temperature (Figure 1a).

The figure represents an area map indicating the phases of temperature trends during the retention period. Lag Phase (day 7 to day 14), represented by yellow color, showed minimal temperature changes from 34.20°C to 37.60°C. Exponential Phase (day 14 to day 28), represented by green color showed an increase in temperature, reaching 30.20°C at day 28. Decline Phase (day 28 to day 49), represented by

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pink color showed a gradual decrease in temperature, dropping to 38.20°C by day 49 (Figure 1b).

The findings of pH before digestion showed that the highest pH value was observed at 21 days with (6.92±0.312), followed by 14 days with (6.85±0.672), followed by 7 days with (6.76±0.011), and the lowest value was at 42 days with (6.25±0.234). Similarly, the findings of pH after digestion showed that the highest pH value was at 28 days with (6.83±0.233), followed by 21 days with (6.56±0.312), followed by 42 days with (6.55±0.221), and the least was at 14 days with (6.15±0.462) (Table 2) (Figure 2).

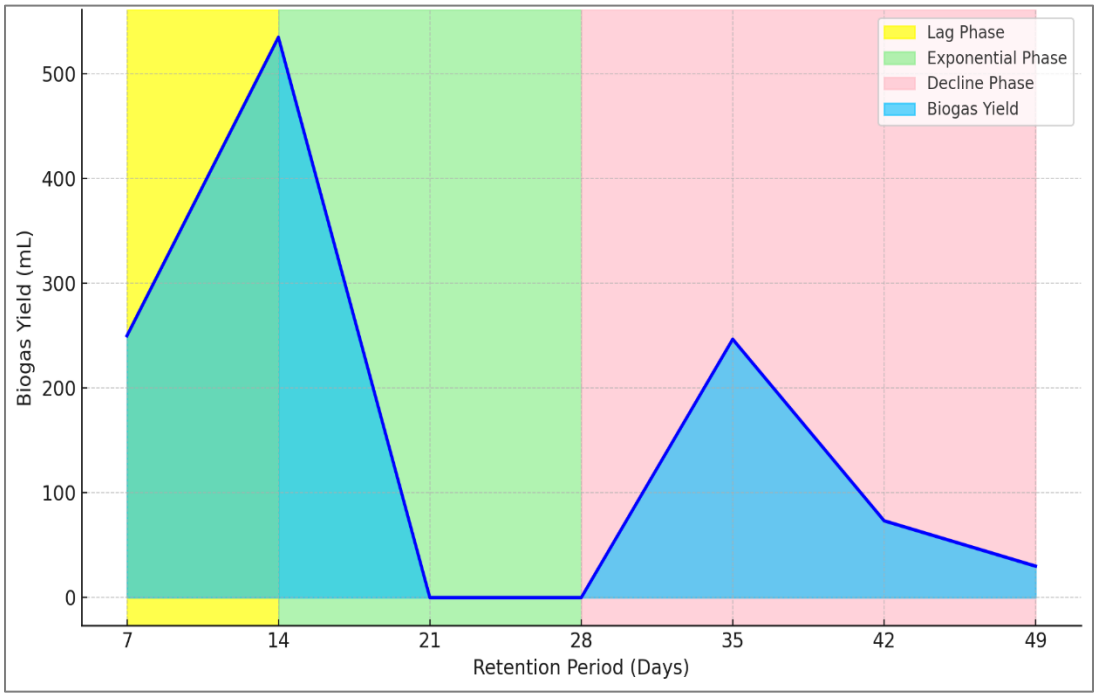

Figure 1a: Phases of biogas production from poultry droppings

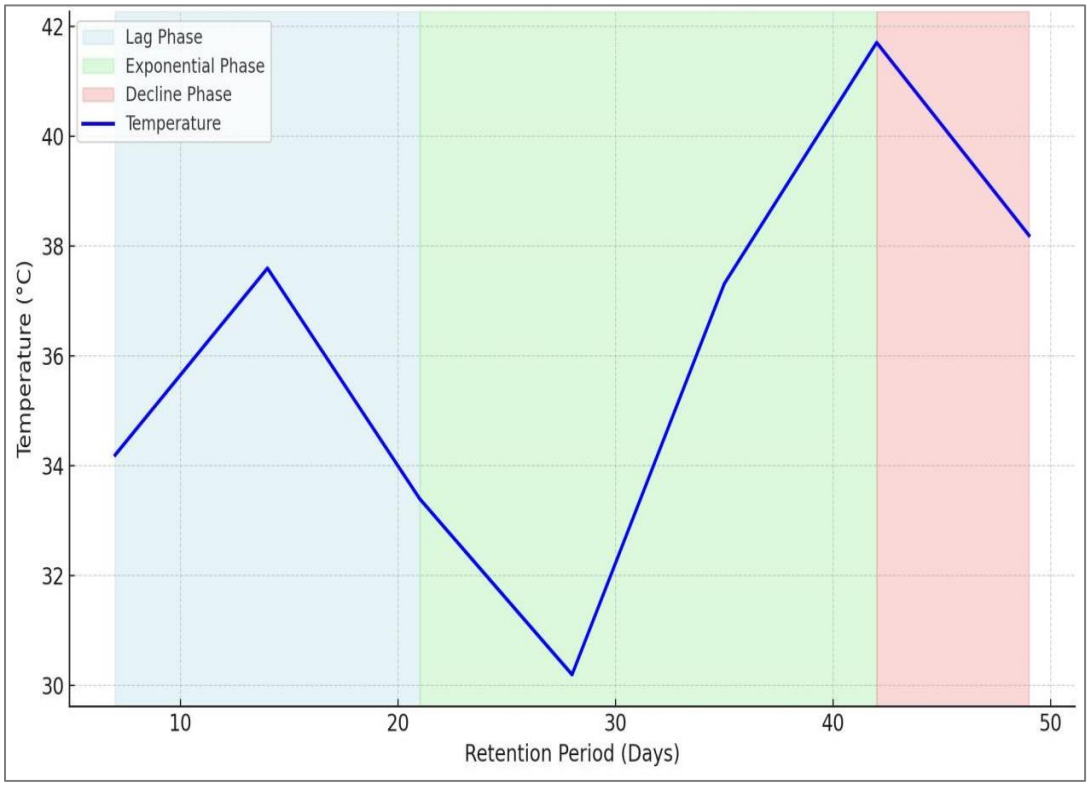

Figure 1b: Temperature Profile from poultry droppings

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

Table 2: pH of poultry droppings before and after anaerobic digestion over seven week retention period

Retention Period (Days)	Poultry Droppings (Before)	Poultry Droppings (After)
7	6.76±0.011 [a]	6.27±0.092 [a]
14	6.85±0.672 [a]	6.15±0.462 [a]
21	6.92±0.312 [a]	6.56±0.312 [a]
28	6.59±0.242 [a]	6.83±0.233 [a]
35	6.43±0.322 [a]	6.35±0.321 [a]
42	6.25±0.234 [a]	6.55±0.221 [a]
49	6.34±0.111 [a]	6.50±0.10 [a]

Results are expressed as mean ± standard deviation, means with the same letters are not significantly different (P≦0.05)

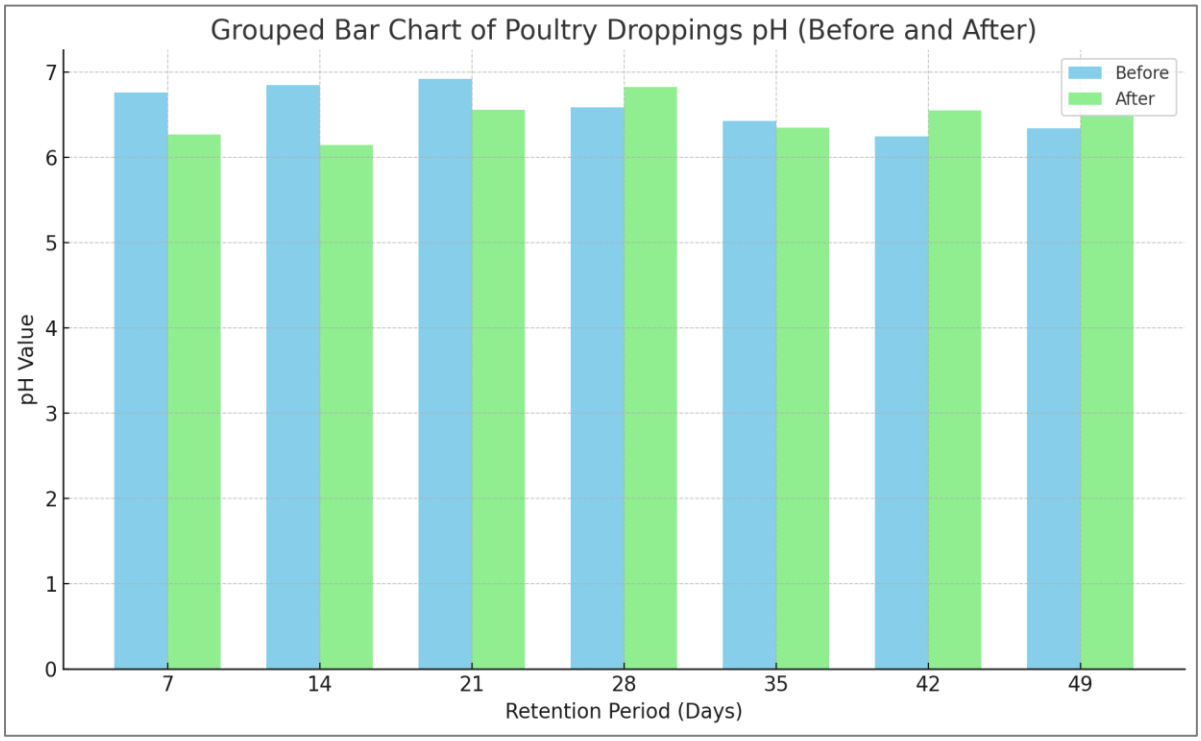

Figure 2: A bar chart (Grouped) showing mean pH of poultry droppings before and after anaerobic digestion over seven week retention period

Table 3(a): Isolation and characterization of bacterial isolates from poultry dropping's digesters

Isolates	Gram Reaction	Bacterial Identified
P1(a)	Gram-positive cocci-shaped with cluster cells	*S. aureus*
P1(b)	Gram-negaive rod-shaped with single cells	*P. aeruginosa*
P1(c)	Gram-negative Rod-shaped with single and pair chains	*Shigella species*
P2(a)	Gram-positive Rod-shaped chains with and sub-terminal	*Bacillus subtilis*
P2(b)	Gram-negative rods-shaped with single and pair chain	*Escherichia coli*
P2(c)	Gram-positive rod-shaped chains with and sub-terminal	*Bacillus subtilis*
P3(a)	Gram-negative rods-shaped with single and pair chain	*Escherichia coli*
P3(b)	Gram-positive cocci-shaped with cluster cells	*S. aureus*
P3(c)	Gram-positive Rod-shaped chains with and sub-terminal	*Bacillus subtilis*

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Table 3(b): Isolation and characterization of bacterial isolates from poultry dropping's digesters

Isolates	Cat.	Oxi.	Cit.	Ure.	Ind.	Glu.	Suc.	Lac.	H_2S	Gas	MR	VP	Bacterial Identified
P1(a)	+	−	−	+	−	+	+	−	−	+	+	−	*S. aureus*
P1(b)	+	+	+	−	−	+	−	−	−	−	−	−	*P. aeruginosa*
P1(c)	+	−	−	−	+	+	−	−	−	−	+	−	*Shigella species*
P2(a)	+	+	+	−	−	+	+	+	−	+	−	+	*Bacillus subtilis*
P2(b)	+	−	−	−	+	+	+	+	−	+	+	−	*Escherichia coli*
P2(c)	+	+	+	−	−	+	+	+	−	+	−	+	*Bacillus subtilis*
P3(a)	+	−	−	−	+	+	+	+	−	+	+	−	*Escherichia coli*
P3(b)	+	−	−	+	−	+	+	−	−	+	+	−	*S. aureus*
P3(c)	+	+	+	−	−	+	+	+	−	+	−	+	*Bacillus subtilis*

KEYS: C(1,2,3) = Poultry droppings's digesters, C(abc) = replicates of poultry dropping's digester, Cat. = Catalase, Oxi. = Oxidase, Cit.= Citrate, Ure.= Urease, Ind. = Indole, Glu. = Glucose, Suc. = Sucrose, Lac.=LactoseH_2S =Hydrogen Sulphide, MR. = Methyl Red, VP. = Voges Proskauer

The bacteria isolated from poultry dropping's digesters were *Staphylococcus aureus, Pseudomonas aeruginosa, Shigella species, Bacillus subtilis, and Escherichia coli. as shown in (*Table 3a*).* *Staphylococcus aureus* was observed as Gram-positive cocci arranged in clusters, showing positive reactions for catalase, urease, glucose fermentation, hydrogen sulfide (H_2S), and gas production, and was found in samples P1(a) and P3(b). *Pseudomonas aeruginosa* appeared as Gram-negative rods, catalase-, oxidase-, and citrate-positive, but non-fermentative, and was isolated from P1(b). *Shigella* species, seen as Gram-negative rods, tested positive for catalase and indole, produced H_2S, but showed no sugar fermentation activity, and was identified in P1(c). *Bacillus subtilis* was the most frequently isolated bacterium, found in samples P2(a), P2(c), and P3(c); it appeared as Gram-positive rods with sub-terminal spores and was positive for catalase, oxidase, glucose and sucrose fermentation, and the Voges-Proskauer (VP) test. Lastly, *Escherichia coli* was detected in P2(b) and P3(a) as Gram-negative rods, positive for catalase, indole, glucose, and sucrose fermentation, H_2S, and gas production. The result showed that *Bacillus subtilis* had the highest percentage of occurrence with (35.71%), followed by *Staphylococcus aureus* and *Escherichia coli* with (21.42%) each, and the least was *Shigella species* with (7.14%) as shown in (Table 3b).

DISCUSSION

The observed biogas yield and temperature variations during the anaerobic digestion of poultry droppings over a seven-week retention period indicate an initial biogas yield of 250.00 ± 10.00 mL at 34.20 ± 10.3 °C on day 7. The biogas yield observed on day 7 in this study is lower than value reported by Buivydas *et al.* (2022). However, this discrepancy can be attributed to differences in substrate composition, digestion conditions, and experimental setup. The biogas yield of 250.00 mL aligns closely with the findings of Carlini *et al.* (2015), who reported 230–300 mL of biogas from poultry manure under mesophilic conditions. Similarly, Mahdy *et al.* (2019) observed initial biogas yields of 200–280 mL in untreated poultry litter digestion. These studies confirm that microbial adaptation in the lag phase leads to lower initial gas production.

The increased production at day 14 aligns with Fuchs *et al.* (2018), who reported 520–600 mL of biogas at similar temperatures (37–38 °C) when digesting poultry manure, marking the transition from the lag phase to the exponential phase. Rubežius et al. (2020) also found that mesophilic digestion at 37 °C resulted in significant methane production, supporting the observed trend.

The complete halt in biogas production at day 21 contrasts with Angelidaki *et al.* (2011), who observed continued biogas yield at this stage. Unlike previous studies that show continued gas production in the exponential phase, biogas yield dropped to 0.00 mL at Day 21. This contradicts the work of Angelidaki *et al.* (2011), who observed sustained production at this stage. However, the drop aligns with Massé *et al.* (2014), who reported ammonia inhibition in poultry manure digestion due to high nitrogen concentrations. The inhibition could be explained by high ammonia levels, as Massé *et*

al. (2014) found that poultry manure digestion sometimes experiences temporary inhibition due to nitrogen content and microbial imbalances. Møller *et al.* (2004) noted that a drop to 33 °C can indicate inhibited methanogenesis, aligning with the 33.40 °C recorded in this study. The continued inhibition aligns with Møller *et al.* (2004), who found that volatile fatty acid (VFA) accumulation and ammonia toxicity can completely halt gas production. However, Ofon *et al.* (2024) found stable gas production at 35–38 °C, suggesting that temperature fluctuations might have contributed to the deviations in this study.

The biogas production at day 35 aligns with Ofon *et al.* (2024), who found that ammonia-adapted microbial communities can recover after temporary inhibition. Similarly, Liu *et al.* (2009) reported biogas yields rebounding after adjustments in microbial consortia, especially under stable mesophilic temperatures (37 °C).

The declining yield is consistent with Buivydas *et al.* (2022), who observed that biogas yield drops significantly after 40 days due to substrate depletion. Similarly, Ezekoye *et al.* (2011) found that after six weeks, most biodegradable material is exhausted, explaining the reduced yield. This finding is consistent with Habib *et al.* (2024), who observed that biogas yield declines significantly beyond 40 days due to substrate depletion. Ezekoye *et al.* (2011) also found that after six weeks, most biodegradable material has been consumed, reducing gas production.

The final decline in biogas production aligns with Zhao *et al.* (2020), who found that residual gas production beyond day 49 is minimal due to the dominance of non-methanogenic microbes. Weiland (2009) also reported that biogas output approaches near-zero levels after 50 days, confirming that extended retention offers diminishing returns. The 38.20 °C temperature aligns with Massé *et al.* (2014), who observed that residual digestion continues at temperatures above 37 °C but with very low efficiency.

The temperature variations observed during the retention period follow a characteristic pattern. In the lag phase (Day 7–14), the temperature remained relatively stable between 34.20 °C and 37.60 °C, indicating the early microbial adaptation phase. This minimal fluctuation aligns with Wang *et al.* (2014) and Carlini *et al.* (2015), who reported that mesophilic digestion typically starts with steady temperatures between 34–38 °C as microbial communities establish themselves.

Contrary to expectations, the temperature declined to 30.20 °C by Day 28 rather than increasing. This trend deviates from Montecchio *et al.* (2017), who found that exponential biogas production is usually accompanied by a rise in temperature due to metabolic heat from microbial activity. However, this decline aligns with Buhlmann *et al.* (2019), who noted that poultry manure digestion can experience temperature drops due to ammonia inhibition, which disrupts microbial metabolism.

The slight final decline at Day 49 aligns with Weiland (2009), who reported that biogas systems experience reduced microbial heat generation due to substrate depletion in the final stages of digestion.

In poultry manure, the initial pH decrease from 6.76 to 6.27 is consistent with findings from Ezekoye *et al.* (2011), who reported a pH drop from 6.8 to 6.3 due to the accumulation of volatile fatty acids (VFAs) during early anaerobic digestion. This trend was also observed by Wang et al. (2014), where poultry waste digestion showed a decrease in pH to around 6.2 during the first week, attributed to acidogenesis.

The continued pH decrease from 6.85 to 6.15 aligns with the work of Nwokolo *et al.* (2020), who observed a similar decline from 6.8 to 6.2 during the second week due to VFA buildup. Adekunle and Okolie (2015) also reported a final pH of 6.4, which is in line with this finding.

The increase in pH from 6.92 to 6.56 in week three aligns with Nwankwo *et al.* (2021), who reported similar values ranging from 6.2 to 6.5 due to methanogenic activity, indicating the beginning of a stabilization phase.

The pH increase to 6.83 in week four supporting the findings of Alfa *et al.* (2013), who reported that pH typically ranges from 6.7 to 6.9 as digestion stabilizes. This is also confirmed by Massé *et al.* (2014), who found that increased methanogenic activity elevates pH levels to between 6.8 and 7.0.

The slight pH drop to 6.35 aligns with Li *et al.* (2023), who observed fluctuations due to microbial activity shifts, and Maurus *et al.* (2021), who reported temporary pH decreases caused by transient VFA accumulation.

The subsequent pH increase to 6.55 in week five corresponds with Shu *et al.* (2022), who observed pH ranges of 6.5–6.7 in poultry manure digesters at this stage. Similar findings were reported by Massé *et al.* (2014), indicating pH recovery due to continued methanogenesis.

The final pH observed at week seven aligns with Ma *et al.* (2015), who reported that poultry manure digesters stabilize within a pH range of 6.5–6.8 during the final digestion phase.

In this study, *Staphylococcus aureus* showed a 21.42% occurrence rate, which is higher than the 13.0% reported by Nwachukwu *et al.* (2024) in poultry farms in Umuahia, Nigeria. Conversely, Islam *et al.* (2019) detected *Staphylococcus* spp. in manure and bio-slurry from biogas plants in Bangladesh, with bacterial loads ranging from 3.14 to 7.68 log CFU/g, a trend not consistent with this study's findings.

On the other hand, Ezeagu *et al.* (2023) reported a much higher prevalence of 47% in poultry feeds, suggesting possible contamination originating from feed that persists through digestion. The 21.42% prevalence of *S. aureus* in this study is slightly higher than the 15.3% reported by Goualié et al., but comparable to the 22.8% found by Ona et al. (2019) in poultry-cow dung co-digestion studies.

The presence of *Pseudomonas aeruginosa* in this study, with a prevalence of 14.28%, is slightly lower than the 18.6% reported by Hammad *et al.* (2018), but higher than the 11.2% found by Nwachukwu *et al.* (2024) in poultry-cow manure co-digestion. Odoi *et al.* (2020) reported a relatively low prevalence of *P. aeruginosa* in poultry litter, with only 1.8% testing positive in the Ashanti Region of Ghana. In contrast, Ndubuisi-Nnaji *et al.* (2023) detected *P. aeruginosa* in 6.5% of diseased and dead chicken litter, 7.3% of dead chicken litter, and 4% of droppings from one-day-old chicks, suggesting a moderate presence in poultry populations.

The detection of *Shigella* spp. at a frequency of 7.14% in this study is not in line with the findings of Ugwu and Nwankwo (2022) in Ishiagu, Ebonyi State, Nigeria, who reported a significantly higher prevalence of 40.03% in poultry feeds, droppings, and drinking water. Similarly, Obi and Ike (2017) recorded a 3.3% occurrence of *Shigella* spp. in cloacal swabs from chickens in Nsukka, Enugu State, with a higher rate in free-range chickens (6.7%) compared to intensively reared ones (0%). The 7.14% prevalence found in this study aligns with Dike *et al.* (2020), who recorded a 6.9% occurrence in poultry digestate, though it is slightly lower than the 10.5% reported by Watanabe *et al.* (2015).

Bacillus subtilis was the most prevalent bacterium in this study, with a frequency of 35.71%. This finding is supported by Ugwu *et al.* (2022), who investigated anaerobic digestion of poultry and goat manure to estimate biochemical methane potential and digestate biosafety. Their study found that, apart from methanogens, *Bacillus* species were the most predominant bacterial genera, with a prevalence of 40.91% in the digestate.

The presence of *Escherichia coli* at 21.42% in this study is lower than the 35.00% reported by Ezeagu *et al.* (2023) in poultry feed samples, and significantly lower than the 62.18% found by Nwachukwu *et al.* (2024) in poultry farm environments. However, Islam *et al.* (2019) detected *E. coli* in both manure and bio-slurry samples, with bacterial loads ranging from 3.82 to 6.96 log CFU/g, indicating that *E. coli* can persist through anaerobic digestion, albeit at reduced levels. Variations in *E. coli* prevalence across studies may reflect differences in poultry management practices, environmental conditions, and digestion efficiency.

CONCLUSION

The results of this study indicate that anaerobic digestion of poultry droppings can yield significant amounts of biogas, particularly during the second week of retention. However, ammonia inhibition and microbial imbalances contribute to temporary cessation in gas production. Temperature and pH fluctuations align with microbial activity phases, influencing digestion efficiency. The presence of pathogenic bacteria in the digestate highlights the need for proper post-treatment to ensure biosafety. Overall, poultry droppings have the potential to serve as a viable feedstock for biogas production, provided process parameters are well managed.

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