MICROBIOLOGY ARCHIVES
MICROBIOLOGY ARCHIVES
Original Research Article | ISSN: 3041-590X
Journal homepage: https://microjournal.researchfloor.org/

Influence of different plant powders on biogas yield and microbial communities during anaerobic digestion of cow dung

Oluwole O. Oladele*[1], John A. Victor Olumurewa[2], and Aina V. Jagun[3]

[1]*Department of Biology, School of Life Sciences, Federal University of Technology, P.M.B. 704, Akure, Nigeria*
[2]*Department of Food Science Technology, Federal University of Technology, P.M.B. 704, Akure, Nigeria*
[3]*School of General Studies, Biological Science Unit, Federal College of Agriculture, P.M.B. 724, Akure, Nigeria*

ARTICLE INFO

Citation: Oluwole O. Oladele, John A. Victor Olumurewa, and Aina V. Jagun (2026). Influence of different plant powders on biogas yield and microbial communities during anaerobic digestion of cow dung.
Microbiology Archives, an International Journal.
DOI: https://doi.org/10.51470/MA.2026.8.1.17

Received 13 October 2025
Revised 11 November 2025
Accepted 15 December 2025
Available Online January 17, 2026

Corresponding Author: **Oluwole O. Oladele**
E-Mail: **prophetoladele2014@gmail.com**

ABSTRACT

Anaerobic digestion of organic waste, such as cow dung, offers a sustainable solution for waste management and renewable energy production. In this study, four bioreactors were constructed using 25 L capacity gallons, with three containing cow dung mixed with each powder (50 g) of Azadirachta indica (neem), Curcuma longa (turmeric) and Carica papaya (papaya) and designated as CDNP (cow dung mixed with neem powders); CDTP (cow dung mixed with tumeric powders), and CDPP (cow dung mixed with papaya powders) while the fourth (CDTRL) served as control (cow dung without plant powders). Thereafter, the bioreactors were monitored over 30 days for biogas yield (kg) and microbial populations (cfu/ml). As digestion ended by day 30, CDNP had the highest biogas yield (0.63 ± 0.03), followed by CDPP (0.29 ± 0.09), then CDTP (0.25 ± 0.06) while CTRL had the least yield (0.17 ± 0.03) and the various yields obtained were significantly different (p<0.05) from one another. Similarly, CDNP had the highest bacteria counts (31.02 ± 0.03), followed by CDPP (26.01 ± 0.01), then CDTP (17.01 ± 0.01) and the least count was noticed in CTRL (13.01 ± 0.02). Notably, the various bacteria counts were significantly different (p< 0.05) from one another and the bacteria isolates identified included species of Bacillus, Pseudomonas, Methanobacterium, Lactobacillus and Escherichia coli.

Keywords: *bioreactor; neem; papaya; retention time; tumeric; waste.*

Introduction

Large volumes of total waste generation that are not properly collected and disposed pose a serious environmental and health risk. For instance, [1] reported that Ghana generates 810 tonnes of solid waste daily, but only 216 tonnes are collected, leaving 594 tonnes uncollected and posing serious hazards while Nigeria generates at least 32 million tonnes of solid waste annually. Unfortunately, only 30% of the waste is efficiently collected and disposed due to inadequate waste management services. As a matter of fact, waste generation in countries like Nigeria, Kenya, and Sub-Saharan Africa far exceeds collection and disposal [2]. Unfortunately, improper waste disposal leads to air, soil, and water pollution, exacerbating the greenhouse gas effect and negatively impacting human health, the environment, and the economy [3]. Besides, the growing volume of waste makes continuous landfill disposal unsustainable [4]. Even incineration, which reduces landfill wastes because it involves clearing and burning of materials at high temperatures, generates harmful and hazardous pollutants which are released into the air, and from there contaminate soil and groundwater, thus making landfills antithetical to circular economy. Hence, there is need for efficient solid waste management approach that will reduce contamination and significantly minimise environmental pollutions.

Consequently, anaerobic digestion is such a promising method. It is a biological process where microorganisms break down biodegradable organic matter without oxygen in a sealed system called an anaerobic bioreactor, producing biogas for energy and exemplifying the circular economy. The resulting biogas can be used for electricity, heating, or vehicle fuel. Meanwhile, cow dung is a popular substrate for biogas production due to its high organic matter content. Nonetheless, relying solely on cow dung for generating biogas may limit gas yield and process efficiency, necessitating the search for biocatalysts that can optimize digestion process because one of the major constraints in biogas production is low yields and slow digestion rates. Regrettably, the application of plant-based biocatalysts in the optimisation of biogas production has not been well documented. Remarkably, plant materials have been reported by many authors in several literatures to consist of bioactive compounds, eco-friendly, biodegradable, cost-effective and non-toxic, making them a good substitute to chemical catalysts for accelerating the digestion process and improving biogas yield. On this basis, the objective of this study is to investigate the influence of powders of *Azadirachta indica* (neem), *Curcuma longa* (turmeric) and *Carica papaya* (papaya) on biogas yield and microbial communities during anaerobic digestion of cow dung.

Materials and Method

Preparation of plant powders

Leaves of *Carica papaya, Azadirachta indica,* and the rhizomes of *Curcuma longa* were plucked from the parent plants, air-dried, and milled into powders.

Collection of substrate

Cow dung, used as substrate was collected freshly from the Livestock Section of the Teaching and Research Farm of the Federal University of Technology, Akure.

Construction of bioreactors

Four bioreactors were constructed using yellow gallons of 25 litre capacity according to the methods of [5]. Three of the gallons contained cow dung mixed with 50 g of each of the plant powders and designated as CDNP (cow dung mixed with neem powders); CDTP (cow dung mixed with tumeric powders), and CDPP (cow dung mixed with papaya powders) while the fourth gallon (CDTRL) served as control (cow dung without plant powders). The original opening of the gallons was used as the slurry inlet and an outlet (gas outlet) was fabricated on each of gallon's lid using a half inch pipe connected to 6.36mm diameter gas transparent hose using the method of [6] and then connected to a tyre tube of 0.8kg as the gas collector [7]. Each of the connections was made airtight using hose clamps.

Charging of bioreactors

The mixtures were charged into the 25 litre prototype batch bioreactor where the waste mixture was loaded once and sealed for a retention period of 30 days, allowing for anaerobic digestion to occur without further input or output. The waste was charged up to three-quarters of the bioreactor volume, leaving headspace for gas collection. The bioreactor contents were stirred adequately daily to ensure substrate homogeneity.

Determination of Quantity of Biogas Produced

The quantity of biogas produced (kg) was obtained by taking daily measurements of the tubes (gas collector) using a digital measuring scale (CAMMRY Model).

Determination of microbial counts and isolation

One (1) ml of 10^{-4} dilutions of the homogenates prepared from the various bioreactors at days 0, 15 and 30 were pipetted aseptically and dispensed into sterilized Petri dishes. This was followed by pouring the already prepared nutrient agar medium into the Petri dishes. The Petri dishes were swirled gently for homogeneity of the contents, then allowed to solidify and later incubated upside down at 37°C for 24 hours. After 24 hours, observed colonies were counted with a colony counter device (Gallenkamp Model, UK) and each count expressed as colony-forming units per millilitre (cfu/ml).

Identification and characterization of isolates

The observed colony of each bacterial isolate was sub cultured severally until a pure culture was obtained using the method of [8]. The pure colonies were later examined using their cultural and morphological attributes [9]. Additionally, biochemical tests which included Gram staining, catalase, coagulase, sugar fermentation, starch hydrolysis and spore staining assay was carried out to assist in the characterisation and identification of the isolated bacteria.

pH determination

The pH was determined by inserting the electrode of a digital pH-meter (Jenway 550 model) directly into each digestate / bioreactor sample at days 0, 15, 30 and readings taken thereafter.

Statistical analysis

All the data on biogas yield, microbial populations and pH of digesting materials obtained from this study were subjected to Analysis of Variance (ANOVA) and where significant, the means were compared at 5% level of probability using Duncan's Multiple Range Test (SPSS Version 20). The results were expressed as mean ± standard deviation.

Results

Total biogas yield from the different bioreactors

The total biogas yield (kg) from the 4 bioreactors throughout the 30-day retention time is shown in Table 1. At day 0, there was no biogas yield (0.00 ± 0.00) across the cow dung treated with all the different plant powders and cow dung without treatment. However, there was significant increase in the biogas yield in all the bioreactors as observed by day 15 with CDNP having the highest biogas yield (0.38 ± 0.03), followed by CDTP (0.16 ± 0.06), then CDPP (0.09 ± 0.08), while CTRL had the least (0.07 ± 0.03) and the various biogas yields in each bioreactor were significantly different ($p<0.05$) from one another (Table 1). Finally, on day 30, CDNP still maintained the highest yield (0.63 ± 0.03), next was CDPP (0.29 ± 0.09), then CDTP (0.25 ± 0.06kg) while CTRL had the least yield (0.17 ± 0.02). Again, the various biogas yield in each bioreactor were significantly different ($p<0.05$) from one another (Table 1).

Microbial population of digesting materials

The bacteria count ($\times 10^4$ cfu/ml) of each digestate in the different bioreactors is shown in Table 2. At day 0, cow dung without plant powders (CTRL) had the highest bacteria counts (13.02 ± 0.02), followed by cow dung with papaya powders (CDPP) having 12.01 ± 0.23 as their bacteria counts, then cow dung with neem powders (CDNP) having bacteria counts of 11.02 ± 0.03 while the least bacteria counts (10.01 ± 0.05) was observed in cow dung with tumeric powders (CDTP). Notably, the various bacteria counts were significantly different ($p<0.05$) from one another.

However, as digestion continued till day 15, the reverse was the case. For instance, CDTP had the highest bacteria counts (14.02 ± 0.02), followed by CDNP (13.00 ± 0.01), CDPP (11.01 ± 0.02) while CTRL had the least bacteria counts (9.01 ± 0.01). Specifically, the various bacteria counts were significantly different ($p<0.05$) from one another (Table 2). Finally, as digestion ended by day 30, CDNP now had the highest bacteria counts (31.02 ± 0.03), followed by CDPP (26.01 ± 0.01), then CDTP (17.01 ± 0.01) and the least count was still noticed in CTRL (13.01 ± 0.02). Notably, the various bacteria counts were significantly different ($p<0.05$) from one another (Table 2).

Isolation of microorganisms

A total of five (5) bacteria were isolated over 30 days retention time of biogas generation, based on morphological and biochemical characteristics (Table 3). The various bacteria isolates included *Bacillus* species, *Pseudomonas* species, *Escherichia coli*, *Lactobacillus* species and *Methanobacterium* species.

Bacteria counts of different isolates from the various bioreactors

The bacteria counts (×10^4cfu/ml) of different isolates from the bioreactor of Cow Dung with Neem Powders (CDNP) are shown in Table 4. At day 0, It was observed that, *Bacillus* sp had the highest count (4.02 ± 0.01), followed by *Escherichia coli (*3.07 ± 0.00), then *Pseudomonas* sp (2.08 ± 0.00) and *Methanobacterium* sp (2.04 ± 0.01) while *Lactobacillus* sp had the lowest counts (1.02 ± 0.0010). Notably, the various bacteria counts were significantly different (p<0.05) from one another, aside *Pseudomonas* sp and *Methanobacterium* sp that were significantly not different (p>0.05). However, as digestion continued till day 15, *Pseudomonas* sp had the highest count (4.00 ± 0.01), then *Lactobacillus* sp (3.00 ± 0.00), *Methanobacterium* sp (3.00 ± 0.00) and *Bacillus* sp (2.00 ± 0.00) while the least counts were observed in *Escherichia coli* (1.00 ± 0.00).

The various bacteria counts were significantly different (p<0.05) from one another, except *Lactobacillus* sp and *Methanobacterium* sp that were significantly not different (p>0.05) (Table 4). Finally, after digestion by day 30, *Methanobacterium* sp was observed having the highest bacteria counts (15.05 ± 0.01) followed by *Bacillus* sp (7.05 ± 0.01), then *Escherichia coli* (6.06 ± 0.01), *Pseudomonas* sp (2.03 ± 0.00) then *Lactobacillus* sp had the least bacteria counts (1.04 ± 0.00) and the various bacteria counts were significantly different (p<0.05) from one another (Table 4).

Table 1: Weight (kg) of biogas produced in each bioreactor over 30 days retention time

Retention time (days)	CDTP	CDNP	CDPP	CTRL
0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
15	0.16 ± 0.06^{c}	0.38 ± 0.03^{d}	0.09 ± 0.08^{b}	0.07 ± 0.02^{a}
30	0.25 ± 0.05^{b}	0.63 ± 0.03^{d}	0.29 ± 0.09^{c}	0.17 ± 0.00^{a}

Note: *Each value represents a mean of 5 replicates and where significant, the means were separated using Duncan multiple range test (DMRT) at p≤ 0.05. Values with the same alphabet across the rows are not significantly different (p≤ 0.05).* ***CDNP*** *- cow dung mixed with neem powders;* ***CDTP*** *- cow dung mixed with tumeric powders;* ***CDPP*** *- cow dung mixed with papaya powders;* ***CTRL*** *- control (cow dung without any powder)*

Table 2. Bacterial loads of each digestate

	Bacteria Counts (×10^4 cfu/ml)		
Digestate / days	0	15	30
CDNP	11.02 ± 0.03^{b}	13.00 ± 0.01^{c}	31.02 ± 0.03^{d}
CDTP	10.01 ± 0.02^{a}	14.02 ± 0.02^{d}	17.01 ± 0.01^{b}
CDPP	12.01 ± 0.23^{c}	11.01 ± 0.02^{b}	26.01 ± 0.01^{c}
CTRL	13.02 ± 0.02^{d}	9.01 ± 0.01^{a}	13.01 ± 0.02^{a}

Note: *Each value represents a mean of 5 replicates and where significant, the means were separated using Duncan multiple range test (DMRT) at p≤ 0.05. Values with the same alphabet in the same column are not significantly different (p≤ 0.05).* ***CDNP*** *- cow dung mixed with neem powders;* ***CDTP*** *- cow dung mixed with tumeric powders;* ***CDPP*** *- cow dung mixed with papaya powders;* ***CTRL*** *- control (cow dung without any powder)*

Table 3. Morphological and biochemical characteristics of the bacteria isolates during the anaerobic digestion of cow dung

Test	Morphological and biochemical characteristics of the isolates				
Colour	whitish	Creamy	whitish	whitish	Whitish
Elevation	Flat	raised	flat	Flat	Flat
Edge	smooth	Smooth	crenated	crenated	Crenated
Texture	Hard	Hard	soft	Hard	Slimy
Gram stain	+	+	+	+	-
Catalase	+	+	+	+	+
Coagulase	-	-	-	-	-
Spore	+	+	+	-	-
Starch hydrolysis	+	+	-	-	-
Glucose	OF	F	F	OF	NF
Fructose	OF	OF	NF	NF	NF
Sucrose	OF	OF	OF	F	NF
Lactose	F	OF	OF	F	NF
Galactose	F	OF	F	F	NF
Likely isolates	*Bacillus* sp	*Lactobacillus* sp	*Pseudomonas* sp	*Esherichia coli*	*Methanobacterium* sp

Note: *+ = positive reaction; - = negative reaction; OF = fermentation with oxygen formation; F = fermentation without oxygen formation; NF = no fermatation*

Table 4. Microbial counts of different bacteria isolates from the bioreactor of cow dung with neem powders (CDNP)

	Bacteria Counts (×10^4 cfu/ml)		
Isolates / days	0	15	30
Bacillus sp	4.02 ± 0.01^{d}	2.00 ± 0.00^{b}	7.05 ± 0.01^{d}
Pseudomonas sp	2.08 ± 0.01^{b}	4.00 ± 0.01^{d}	2.03 ± 0.00^{b}
Escherichia coli	3.07 ± 0.00^{c}	1.00 ± 0.00^{a}	6.06 ± 0.01^{c}
Lactobacillus sp	1.02 ± 0.00^{a}	3.00 ± 0.00^{c}	1.04 ± 0.00^{a}
Methanobacterium sp	2.04 ± 0.01^{b}	3.00 ± 0.00^{c}	15.05 ± 0.01^{e}

Note: *Each value represents a mean of 5 replicates and where significant, the means were separated using Duncan multiple range test (DMRT) at p≤ 0.05. Values with the same alphabet in the same column are not significantly different (p≤ 0.05).*

The bacterial counts (×10^4cfu/ml) of different isolates from the bioreactor of cow dung with tumeric powders (CDTP) were reported in Table 5. At day 0, *Escherichia coli* had the highest counts (4.03 ± 0.01), followed by *Bacillus* sp (3.03 ± 0.00), then *Pseudomonas* sp (2.05 ± 0.00), *Methanobacterium* sp (1.02 ± 0.00) while *Lactobacillus sp* had the lowest counts (0.00 ± 0.00). Interestingly, the various bacteria counts were significantly different (p<0.05) from one another (Table 5).

However, by day 15, *Lactobacillus* sp had the highest counts (5.04 ± 0.01), followed by *Pseudomonas* sp (4.04 ± 0.00) and *Methanobacterium* sp (4.09 ± 0.01), then *Escherichia coli* (1.00 ± 0.00), while *Bacillus* sp had the least count (0.00 ± 0.00). The various bacteria counts were significantly different (p<0.05) from one another, except *Pseudomonas* sp and *Methanobacterium* sp that are significantly not different (p>0.05) (Table 5). At day 30, when the digestion ended, it was noted that *Methanobacterium* sp had the highest bacteria counts (12.00±0.01), followed by *Bacillus* sp (3.01±0.00), then *Escherichia coli* (2.00 ± 0.00) while *Pseudomonas* sp and *Lactobacillus* sp both had least counts (0.00 ± 0.00). Notably, the various bacteria counts were significantly different (p<0.05) from one another, except *Pseudomonas* sp and *Lactobacillus* sp that were significantly not different (p>0.05) (Table 5).

Furthermore, the bacterial counts (×10^4cfu/ml) of different isolates from the bioreactor of cow dung with papaya powders (CDPP) was presented in Table 6.

It was observed at day 0 that *Bacillus* sp had highest counts (5.00 ± 0.06), then *Escherichia coli* (3.00 ± 0.04), followed by *Pseudomonas* sp (2.00 ± 0.07) and the various counts were significantly different (p<0.05) from one another. Meanwhile, *Lactobacillus* sp and *Methanobacterium* sp had the least bacteria counts (1.00 ± 0.00) that were not significantly different (p>0.05). As the digestion process advanced to day 15, the highest counts were observed in *Lactobacillus* sp (4.00 ± 0.01), then *Pseudomonas* sp (3.00 ± 0.00), *Methanobacterium* sp (3.00 ± 0.01), and *Bacillus* sp (1.00 ± 0.00) while *Escherichia coli* had the least counts (16.00 ± 0.00). Notably, the various bacteria counts were significantly different (p<0.05) from one another, except *Pseudomonas* sp and *Methanobacterium* sp that were significantly not different (p>0.05) (Table 6).

After the digestion by day 30, it was noted that *Methanobacterium* sp had the highest counts (16.00 ± 0.00), next was *Bacillus* sp (7.00 ± 0.00), then *Escherichia coli* (3.00±0.00), while *Pseudomonas* sp (0.00 ± 0.00) and *Lactobacillus* sp (0.00 ± 0.00) had the least count. The various bacteria counts were significantly different (p<0.05) from one another, aside *Pseudomonas* sp and *Lactobacillus* sp which were significantly not different (p>0.05) (Table 6).

The bacterial counts (×10^{4}cfu/ml) of different isolates from the bioreactor of cow dung without plant powders (CTRL) are shown in Table 7. At day 0, *Bacillus* sp had the highest counts (6.00 ± 0.00), followed by *Escherichia coli* (4.00 ± 0.00) then *Pseudomonas* sp (2.00 ± 0.00), followed by *Methanobacterium* sp while *Lactobacillus* sp had the least counts (0.00 ± 0.00) and the various bacteria counts were significantly different (p<0.05) from one another.

At day 15, *Lactobacillus* sp was observed to have the highest counts (4.00 ± 0.00), followed by *Pseudomonas* sp (3.00 ± 0.0032), then *Methanobacterium* sp (2.00 ± 0.00), while *Bacillus* sp and *Escherichia coli* had the lowest counts (0.00 ± 0.00). The various bacteria counts were significantly different (p<0.05) from one another, other than *Bacillus* sp and *Escherichia coli* which are significantly not different (p>0.05) (Table 7).

However, at day 30, *Methanobacterium* sp had the highest counts (8.00 ± 0.01) then *Bacillus* sp (4.00 ± 0.01) and *Escherichia coli* (1.00 ± 0.00), while *Lactobacillus* sp and *Pseudomonas* sp were observed to have the least bacteria counts (0.00 ± 0.00) and the various bacteria counts were significantly different (p<0.05) from one another, aside *Lactobacillus* sp and *Pseudomonas* sp that are significantly not different (p>0.05) (Table 7).

Table 5: Microbial counts of different bacteria isolates from bioreactor of cow dung with tumeric powders (CDTP)

	Bacteria counts (×10^{4} cfu/ml)		
Isolates / days	0	15	30
Bacillus sp	3.03 ± 0.00^{d}	0.00 ± 0.00^{a}	2.00 ± 0.00^{c}
Pseudomonas sp	2.05 ± 0.00^{c}	4.04 ± 0.00^{c}	0.00 ± 0.00^{a}
Escherichia coli	4.03 ± 0.00^{e}	1.00 ± 0.00^{b}	3.01 ± 0.00^{b}
Lactobacillus sp	0.00 ± 0.00^{a}	5.04 ± 0.01^{d}	0.00 ± 0.00^{a}
Methanobacterium sp	1.02 ± 0.00^{b}	4.09 ± 0.01^{c}	12.00 ± 0.01^{d}

Note: *Each value represents a mean of 5 replicates and where significant, the means were separated using Duncan multiple range test (DMRT) at p≤ 0.05. Values with the same alphabet in the same column are not significantly different (p≤ 0.05).*

Table 6: Microbial counts of different bacteria isolates from the bioreactor of cow dung with papaya powders (CDPP)

	Bacteria counts (×10^{4} cfu/ml)		
Isolates / days	0	15	30
Bacillus sp	5.00 ± 0.07^{d}	1.00 ± 0.00^{b}	7.00 ± 0.01^{c}
Pseudomonas sp	2.00 ± 0.07^{b}	3.00 ± 0.00^{c}	0.00 ± 0.00^{a}
Escherichia coli	3.00 ± 0.04^{c}	0.00 ± 0.00^{a}	3.00 ± 0.00^{b}
Lactobacillus sp	1.00 ± 0.00^{a}	4.00 ± 0.01^{d}	0.00 ± 0.00^{a}
Methanobacterium sp	1.00 ± 0.05^{a}	3.00 ± 0.01^{c}	16.00 ± 0.00^{d}

Note: *Each value represents a mean of 5 replicates and where significant, the means were separated using Duncan multiple range test (DMRT) at p≤ 0.05. Values with the same alphabet in the same column are not significantly different (p≤ 0.05).*

Table 7: Microbial counts of different bacteria isolates from the bioreactor of cow dung without plant powders (CTRL)

	Bacteria counts (×10^{4} cfu/ml)		
Isolates / days	0	15	30
Bacillus sp	6.00 ± 0.00^{e}	0.00 ± 0.00^{a}	4.00 ± 0.01^{c}
Pseudomonas sp	2.00 ± 0.00^{c}	3.00 ± 0.00^{c}	0.00 ± 0.00^{a}
Escherichia coli	4.00 ± 0.00^{d}	0.00 ± 0.00^{a}	1.00 ± 0.00^{b}
Lactobacillus sp	0.00 ± 0.00^{a}	4.00 ± 0.00^{d}	0.00 ± 0.00^{a}
Methanobacterium sp	1.00 ± 0.01^{b}	2.00 ± 0.00^{b}	8.00 ± 0.01^{d}

Note: *Each value represents a mean of 5 replicates and where significant, the means were separated using Duncan multiple range test (DMRT) at p≤ 0.05. Values with the same alphabet in the same column are not significantly different (p≤ 0.05).*

Table 8: pH of each digestate

	Retention time (days)		
Digestate	0	15	30
CDNP	6.80 ± 0.06^{a}	5.21± 0.02^{a}	7.35 ± 0.01^{d}
CDTP	7.18 ± 0.02^{c}	6.00 ± 0.01^{d}	7.09 ± 0.06^{b}
CDPP	7.20 ± 0.01^{d}	5.89 ± 0.02^{c}	7.15 ± 0.01^{c}
CTRL	6.90 ± 0.02^{b}	5.63 ± 0.01^{b}	6.95 ± 0.01^{a}

Note: *Each value represents a mean of 5 replicates and where significant, the means were separated using Duncan multiple range test (DMRT) at p≤ 0.05. Values with the same alphabet in the same column are not significantly different (p≤ 0.05).* ***CDNP*** *- cow dung mixed with neem powders;* ***CDTP*** *- cow dung mixed with turmeric powders;* ***CDPP*** *- cow dung mixed with papaya powders;* ***CTRL*** *- control (cow dung without any powder)*

pH values of the different bioreactors

pH change of each digestate within 30 days retention time was shown in Table 8. At day 0, CDPP had highest pH value (7.20 ± 0.01), followed by CDTP (7.18 ± 0.02), then CTRL (6.90 ± 0.02) while CDNP was observed to have the least pH value (6.80 ± 0.06). Interestingly, various pH values were significantly different (p<0.05) from one another. However, there was a general decrease in the pH values in all the bioreactors at day 15, though CDTP now had the highest value (6.00 ± 0.00), followed by CDPP (5.89 ± 0.02), then CTRL (5.63 ± 0.01) while CDNP had the least value (5.21 ± 0.01) and the various pH values were significantly different (p<0.05) from one another. Finally at day 30, the pH values increased notably in all bioreactors again from CDNP (7.35 ± 0.01), then CDPP (7.15 ± 0.01) to CDTP (7.09 ± 0.01) while CTRL had the least pH value (6.95 ± 0.01) and various pH values were significantly different (p<0.05) from one another (Table 8).

Discussion

The study showed the influence of the various plant powders in enhancing biogas production because all the different bioreactors (CDNP, CDTP and CDPP) yielded more biogas when compared with the untreated cow dung (CTRL) over 30 days retention time. This is supported by the works [10] who reported that plant extracts are said to maintain favourable conditions for enhanced biogas production in anaerobic digestion by enhancing enzymes that extensively degrade lignin and break down cellulose and hemicellulose. Among all the different bioreactors, CDNP had the highest biogas weight. This may not be unconnected with the activity of azadirachtin, a bioactive compound present in neem, ultimately boosting biogas yield [11-12]. The swift hydrolysis and acidogenesis may be enhanced by the stronger antimicrobial effects of azadirachtin present in neem, which enriched the growth of methanogens.

Again, it has been reported in literatures [13-14] that anaerobic digestion relies on the combined efforts of hydrolytic, acidogenic, acetogenic, and methanogenic bacteria to break down organic matter.

Undoubtedly, findings from this study showed that CDNP, CDTP and CDPP digester systems had more bacterial loads when compared with CTRL. Remarkably, CDNP had the highest bacterial loads. The notable increase in microbial counts at day 30 of digestion suggests adaptation and proliferation of resilient microbes, contributing to enhanced biogas production especially in CDNP [15]. This finding is consistent with [16], who noted that anaerobic digestion involves a sequence of bacterial groups, including hydrolyzing, acidifying, acetogenic, and methanogenic bacteria.

A total of five bacterial species were isolated from the anaerobic digestion of the cow dung and the identified isolates included species of *Bacillus*, *Pseudomonas*, *Methanobacterium*, *Lactobacillus* and *Escherichia coli*. This was supported by the findings of [17] who confirmed the presence of hydrolytic bacteria such as *Bacillus* spp., *Pseudomonas* spp.; acidogenic bacteria such as *Escherichia coli, Lactobacillus* spp.; acetogenic bacteria such as *Lactobacillus* sp and methanogenic bacteria such as *Methanobacterium* sp from microbial community composition in staged anaerobic digesters fed with food waste. This is also in accordance with [18] who reported that bacteria are responsible for anaerobic digestion in the production process of biogas. In facts, research conducted by [19] on biogas production from co-digestion of cow dung and chicken droppings isolated *Bacillus*, *Pseudomonas* spp, *Serratia*, *Enterococcus*, and *Klebsiella* as cellulose and amylase producing bacteria while [20] equally identified *Bacillus* spp, *Pseudomonas* spp, *Micrococcus* spp, and *Proteus* spp in their own work. Meanwhile the presence and identification of *Methanobacterium* spp in this study is equally buttressed by the works of [21] who isolated and identified *Methanobacterium* spp from chicken droppings while [22] also isolated and identified *Methanobacterium* spp from human wastes.

Meanwhile, the presence and dominance of *Bacillus* spp in all the four bioreactors (CDNP, CDTP, CDPP, and CTRL) before digestion (day 0) might not be unconnected with their ability to develop resistant spores, enabling survival in oxygen-rich environments and tolerance to transitions between aerobic and anaerobic conditions [23]. They also produce extracellular hydrolytic enzymes that facilitate the breakdown of polymer compounds through hydrolysis [24]. Furthermore, *Lactobacillus* spp. dominated CDTP, CDPP, and CTRL digester systems by day 15. These bacteria excel at lactic acid fermentation, breaking down simple sugars mainly into lactic acid, and sometimes producing acetate, ethanol, and carbon dioxide. Their metabolic activity acidifies the environment, which is significant because methanogenic bacteria are sensitive to pH. This acidification by *Lactobacillus* spp. influences the emergence of methanogens [25].

In contrast, *Pseudomonas* spp only occurred in CDNP at day 15. *Pseudomonas* species are versatile, utilizing various carbon sources, degrading aromatic compounds, forming biofilms, and exhibiting anaerobic respiration using nitrate. Their dominance may be attributed to specific substrate or nutrient conditions, as well as tolerance to inhibitory compounds [26].

At day 30, *Methanobacterium* spp. had the highest loads in CDNP, CDTP, CDPP, and CTRL digester systems. This shows they were dominant in the methanogenesis phase of anaerobic digestion. [23] reported that *Methanobacterium* spp. can thrive in different environmental conditions, allowing them dominate in various digester setups. Their growth by day 30 marked the methanogenesis stage where these microbes use hydrogen and carbon dioxide to make methane, the key gas for biogas production.

Their dominance by day 30 cannot help but be connected with the fact that methanogens are extremely sensitive to conditions like pH, shaping digester microbiome dynamics.

For pH readings, the values varied throughout the retention period with the pH values of CDNP, CDTP and CDPP considerably higher when compared with the CTRL. This is in accordance with the work of [27] who reported that anaerobic conditions caused organic matter to ferment, producing resultant products with varying pH values in a biogas digester [28]. The initial acidic pH levels can be attributed to the activity of aerobes and facultative anaerobes producing acidic metabolites, a crucial step for methane production. No wonder, there was decreased pH on 15th day of the digestion process and perhaps the reason why acid-tolerant organisms like *Bacillus* and *Lactobacillus* predominated in the digestion process by day 15. The observed pH decline can be linked to metabolite accumulation, including acetate, hydrogen gas, carbon dioxide, and volatile fatty acids [29].

However, on the day 30 of the retention duration, the pH increased due to methanogenesis which is the fourth stage of biogas production and methanogenesis stage typically requires a neutral- alkaline pH environment. Hence, these pH fluctuations, according to [30] are normal in biogas systems and can serve as an indicator of biogas production status. In the same vein, the observed pH is consistent with earlier research by [31], who reported that methanogens thrive best within a pH range of 5 to 8 during biogas production and the same case was also noticed in this work during methanogenesis. Even an optimal pH range of 5 to 8 for biogas production has been reported [32]. Likewise, [33] and [34] also observed that methanogenesis thrives within a pH range of 5-7.8. Hence, effective pH management is essential for optimizing anaerobic digestion efficiency and advancing biogas production.

Conclusions

This study demonstrates the potential of cow dung catalyzed by plant powders as a valuable resource for biogas production over 30 days retention time. The selected plant powders positively influenced the production of biogas with neem powder being the most effective for optimising biogas yield than other powders, as the cow dung treated with neem plant powders had the highest biogas weight. Thus, neem powders being natural, affordable and eco-friendly could be explored and utilized by biogas operators in farm waste digestion.

Authors' Contributions

Conceptualisation: OO and JA; Investigation: OO, OJ and JA; Supervision: OO and OJ; Visualisation: OO, OJ and JA; Writing original draft: OO; Writing-review and editing: OO, OJ and JA. All authors read and approved the final manuscript.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

1. Amoah ST, Kosoe EA (2014). Solid waste management in urban areas of Ghana: issues and experiences from Wa. Journal of Environmental Pollution and Humam Health 2:110–117.
2. Kumar A, Agrawal A (2020). Recent trends in solid waste management status, challenges, and potential for the future Indian cities - A Review: Curriculum Research Environmental Sustainainable 2:100011. doi: 10.1016/j.crsust.2020.100011
3. Kwun Omang DI, Egbe John G, Alain Inah S, Owan Bisong J (2021). Public health implication of solid waste generated by households in Bekwarra local government area. Journal of African Health Science 21:1467–1473. doi: 10.4314/ahs.v21i3.58
4. Basu R (2009). Solid Waste Management-A Model Study. Sies Journal of Management 6:20-24.
5. Ofoefule AU, Uzodinma EO (2006). Optimization of the qualitative and quantitative biogas yield from poultry waste. Proceedings of the IX World Renewable Energy Congress. University of Florence Italy. pp 9–25.
6. Kalia AK, Singh SP (2004). A biogas plant is a suitable technology for developing countries. Journal of Applied Energy 77(1):33-43. https//doi.org/10.1016/S0306-2619(04)00023-2
7. Adeyanju AA (2012). Performance evaluation of a modified low-cost anaerobic digester with tyre tube gas storage. International Journal of Renewable Energy Research 2 (3): 476-481. https//doi.org/10.18818/ijerr.v2i3.35
8. Cheesebrough M (2005). District laboratory practices in tropical countries. Low price ed., Cambridge University Press, London. pp 64-69.
9. Holt JG, Krieg NR, Sneath PH, Staley JT, Williams ST (1994). Bergey's Manual of Determinative Bacteriology.
10. Sang-Ryong S, Mo-kwon L, Seongwon I, Dong-Hoon K (2019). Effect of seaweed addition on enhanced anaerobic digestion of food waste and sewage sludge. Journal of Environmental Engineering Research 24(3):449-455. http://dx.doi.org/10.4491/eer.2018.275
11. Fuentes G, Santamaría JM (2014). Papaya (*Carica papaya* L.): Origin, domestication, and production. In Genetics and Genomics of Papaya. Springer: Berlin/Heidelberg, Germany. pp 3-15. https://doi.org/10.1007/978-1-4614-8087-7_1
12. Soudamini NK, Kuttan R (1989). Inhibition of chemical carcinogenesis by curcumin. Journal of Ethnopharmacology 27:227-233. https://doi.org/10.1016/0378-8741(89)90094-9.
13. Angelidaki I, Ellegaard L, Ahring BKA (2004). Mathematical model for dynamic simulation of anaerobic digestion of complex substrates with a focus on ammonia inhibition. Journal of Biotechnology and Bioengineering 42:159–166. https://doi.org/10.1002/bit.260420203
14. Appels L, Baeyens J, Degrève J, Dewil R (2008). Principles and potential of the anaerobic digestion of waste-activated sludge. Progress in Energy and Combustion Science 34 (6):755-781. https//doi.org/10.1016/j.pecs.2008.06.002
15. Parawira W, Murto M, Zvauya R, Mattiasson B (2004). Anaerobic digestion of solid potato waste alone and in combination with sugar beet leaves. Journal of Renewable Energy 29:1811-1823. https://doi.org/10.1016/j.renene.2004.02.005
16. Demirel B, Scherer P (2008). Roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane. Review of Environmental Science and Technology 7:73-901. https://doi.org/10.1007/s11313-008-0088-5
17. Gaby J, Zamanzadeh M, Horn S (2017). The effect of temperature and retention time on methane production and microbial community composition in staged anaerobic digesters fed with food waste. Journal of Biotechnology and Biofuels 10:302. https://doi.org/10.1186/s13068-017-0989-4.
18. Agunwamba JC (2001). Waste engineering and management tool. Immaculate Publication Limited Nigeria. pp 56.
19. Mazzucotelli CA, Ponce AG, Kotlar CE (2013). Isolation and characterization of bacterial strains with a hydrolytic profile with potential use in bioconversion of agro industrial by-products and waste. Journal of Food Science and Technology 33(2):295-303. https://doi.org/10.1590/S0101-20612013005000038
20. Ramsay IR, Pullammanappallil PC (2001). Protein degradation during anaerobic waste water treatment: deviation of stoichiometry. Journal of Renewable Energy 12:247-257. https://doi.org/10.1023/A:1015417501822
21. Oyewole OA (2010). Biogas production from chicken droppings. Science World Journal 5(4): 1597-6343. https//doi.org/10.12691/ajer-11-2-1
22. Dahunsi SO, Oranusi US (2013). Co digestion of food waste and human excreta for biogas production. British Biotechnology Journal 3(4):485-499. https://doi.org/10.9734/BBJ/2013/4476
23. Jabbari S, Heap JT, King JR (2011). Mathematical modelling of the sporulation-initiation network in *Bacillus subtilis* revealing the dual role of the putative quorum-sensing signal molecule PhrA. Bulletin of Mathematical Biology 73(1): 181–211. https://doi.org/10.1007/s11538-010-9530-7
24. Errington J, Aart LT (2020). Microbe Profile: *Bacillus subtilis*: model organism for cellular development, and industrial workhorse. Microbiology 166(5):425–427. https://doi.org/10.1099/mic.0.000922
25. Sonomoto K, Yokota A (2012). Lactic Acid Bacteria and Bifidobacteria: Current Progress in Advanced Research. Caister Academic Press. ISBN 978-1-904455-82-0. https://doi.org/10.1111/j.1471-0307.2012.00825.x
26. Dolan SK, Kohlstedt M, Trigg S, Vallejo P, Kaminski CF (2020). Contextual flexibility in Pseudomonas aeruginosa central carbon metabolism during growth in single carbon sources. https://doi.org/10.1128/mBio.02684-19
27. Yuan H, Zhu N (2016). Progress in inhibition mechanisms and process control of intermediates and by-products in sewage sludge anaerobic digestion. Journal of Renewable and Sustainable Energy Review 58:429–438. https://doi.org/10.1016/j.rser.2015.12.038
28. Lindner J, Zielonka S, Oechsner H, Lemmer A (2015). Effect of different pH-values on process parameters in two-phase anaerobic digestion of high-solid substrates.
1. Journal of Environmental Technology 36(2):198-207. https://doi.org/10.1080/01436536.2014.979221
29. Tsunatu D, Yavini U, Usman H, Taura N, James M (2014). Comparative study of mesophilic biogas production potentials of selected agro-wastes. International Journal of Engineering and Science 3(2):1-6.
30. Refai S, Berger S, Wassmann K, Hecht M, Dickhaus T, Deppenmeier U (2017). Profiles as rapid test system for status analysis and early detection of process incidents in biogas plants. Journal of the Society for Industrial Microbiology and Biotechnology 44(3): 465-476. https://doi.org/10.1007/s10295-016-1894-8
31. Yerima MB, Rahman AT, Ekwenchi MM (2001). Effect of Buffering on Biogas Fermentation of Chicken Droppings. Journal of Applied Microbiology and Biotechnology 54:844-849
32. Garba B, Sambo AS (2002). Effect of some operating parameters on Biogas production rate. Nigeria Journal of Renewable Energy 6(3):343-344.
33. Karthikeyan KG, Gungor K (2005). Influence of anaerobic digestion on dairy manure phosphorus extractability. Applied Journal of Environmental Microbiology 48:1497-1507. https://doi.org/10.13031/2013.19182
34. Farrel AE, Plevin RJ, Turner BT, Jones AD, O'Hare M, Kammen DM (2006). Ethanol can contribute to energy and environmental goals. *Science direct journal*. 3(11):506-508. https://doi.org/10.1126/science.1121416