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Co-Digestion of Neem (*Azadirachta indica*) Shoot Biomass with Poultry Droppings and Cow Rumen: Effect of Pretreatment Methods on Biogas Yield

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ABSTRACT

Inadequate energy supply, environmental pollution, and declining soil fertility are major challenges in developing nations like Nigeria. Despite the abundance of biomass, much of it ends up as unmanaged solid waste. This study evaluated the effects of pretreatment on biogas yield from the co-digestion of Neem (Azadirachta indica) shoot biomass with poultry waste and cow rumen (inoculum). Materials were sourced from LAUTECH and prepared by washing, blending (mechanical pretreatment). The blended neem shoot was pretreated in a water bath to about 60 degrees Celsius for 1hr 20 minutes (thermal pretreatment). Chemical pretreatment was adopted to aid in the degradation of the lignin content. 4g of NaOH was dissolved in distilled water and then added to the thermally blended biomass. Two batches were prepared from the chemically treated Neem shoots, with poultry waste (batch A) and cow rumen (batch B), and put into airtight biodigesters. Physicochemical parameters (pH, TN, TP, TC, BOD, COD, MC, TS, C/N, FS) of both slurry and digestate were analyzed using standard methods. Biogas production, pH, and temperature were monitored over 30 days, and gas composition was determined via Gas Chromatography-Mass Spectrometry. Batch A showed biogas yields of 0.1016–0.0326 L/day, pH 8.39–8.41, and 35.7–35.8°C; Batch B yielded 0.1628–0.0488 L/day, pH 8.36–8.39, and 35.7–35.8°C. Methane content was 61.29% in Batch A and 63.29% in Batch B. ANOVA indicated significant differences in yields (p = 0.0256 for A, p = 0.0200 for B). Results showed that co-digestion, particularly with cow rumen, improved methane output. The produced methane is suitable for use in cooking, heating, and electricity generation, offering a sustainable solution for waste-to-energy conversion in Nigeria.

INTRODUCTION

Environmental pollution by solid wastes and lack of access to adequate energy resources are some of the major challenges facing the human populace in some parts of Africa, like Nigeria. Weed can be found in many parts of the world. Plant weed, however, constitutes a conflict of interest to man, in which it may be beneficial or unfavourable to his health. Plant weeds act as threats to the growing process and survival of other plants, competing for nutrients and minerals. This research studies a particular species of plant weed, Neem (*Azadirachta indica*) Shoot Biomass, which dominates West Africa, most especially, Nigeria (Ngulde and Abubakar, 2012).

One of the major factors for national and international development is energy. Developing countries like Nigeria depend heavily on fuels from fossil origins. Research has discovered that there is a huge presence of enormous energy resources (crude oil, tar sands, natural gas and coal) in Nigeria. There are also a huge amount of renewable/sustainable energy resources, which include hydro, solar, wind, biomass etc. The global quest for environmentally friendly, ecologically balanced and sustainable energy has been on the increase over the last few decades, and this has forced the world to search for other alternate sources of energy (Okonkwo *et al.,* 2021).

Anaerobic digestion is a proven technological method of converting organic matter, thereby producing biogas and nutrient-rich digestate (Leite et al., 2016). It has been globally applied in the treatment of diverse wastes, agricultural residues, and energy crops and is a veritable means of abating environmental pollution. Animal wastes like piggery waste, cow dung, poultry droppings and so on are scattered all around and left to decompose, polluting the environment. If these wastes are put to effective use, they will reduce the pollution rate and improve the conservation system. One of the ways to control the activities of plant weeds is through their use in the biogas production process, in addition to animal waste. Biogas is the gas obtained from the decomposition of organic matter through anaerobic digestion of organic matter like plant and animal remains (Ugwoke *et al.,* 2020).

Neem (*Azadirachta indica*) shoot biomass is abundantly available across Nigeria's six geopolitical zones. Despite its potential as a biofuel feedstock, there is limited documentation on its utilization for this purpose. A study has demonstrated the feasibility of producing bioethanol from neem leaves using Zymomonas mobilis as a fermenting organism, indicating its promise as a renewable energy source (Grace *et al.,* 2022). In Southwestern Nigeria, both neem shoot biomass and poultry waste are prevalent. However, these resources are often underutilized and end up as solid waste, contributing to environmental degradation. Research highlights that Nigeria's significant biomass resources, including agricultural residues and animal wastes, have not been effectively harnessed due to a lack of coherent policies and technologies (Nwankwo *et al.*, 2024).

MATERIALS AND METHODS

Sample collection

The Neem shoots were obtained from Ladoke Akintola University of Technology (LAUTECH), Ogbomoso environment, while the poultry waste and cow rumen, which were used as inoculum, were obtained in bulk from the Teaching/Research Farms, LAUTECH, Ogbomoso and slaughter slab from the abattoir in Ogbomoso, respectively, in a clean airtight plastic container and kept to preserve the microbes before laboratory analyses.

Procedure for pre-treatment of sample and loading

Due to the resistant lignocellulosic structure of biomass, pretreatment is essential to enhance biogas yield during anaerobic digestion. In this study, pretreatment methods used included mechanical, thermal, and chemical techniques. Neem shoots were prepared for pretreatments by washing and blending (mechanical pretreatment). The blended neem shoot was pretreated in a water bath to about 60 degrees Celsius for 1hr 20 minutes (thermal pretreatment). Chemical pretreatment was adopted to aid in the degradation of the lignin content. 4g of NaOH was dissolved in distilled water and then added to the thermally blended biomass. Two batches were prepared from the chemically treated Neem shoots, with poultry waste (batch A) and cow rumen (batch B) and put into airtight biodigesters.

For Samples A and B, neem shoots and poultry waste, and cow rumen, respectively, were each blended with distilled water in a 1:1 ratio to form slurries, mixed, and evaluated for pH and physicochemical properties before being charged into a digester. Digestion was conducted over 30 days with daily monitoring of gas production, pH, and temperature. At the end of the 30-day digestion period, both gas and digestate samples from each setup were analyzed for composition and fertiliser potential.

Determination of physiochemical parameters

Before and after the anaerobic digestion process, physicochemical analyses were carried out to quantify the elements /nutrients and other factors, on the fermenting substrates: neem biomass, inoculum and the digestates, and were carried out at the Environmental Engineering Laboratory of Landmark University, Omu Aran, Kwara State, while analyses on the biogas components were evaluated at Afe Babalola University, Ekiti State.

RESULTS AND DISCUSSION

Physiochemical analysis of neem shoot substrates

The analysis of neem shoots biomass and its co-digestion with animal wastes (cow rumen and poultry waste), as shown in Table 1, revealed that blending these substrates significantly improved their suitability for anaerobic digestion. The pH of neem shoots alone was 7.63, increasing to 8.49 with cow rumen and 8.46 with poultry waste more favourable for methanogenic bacteria. Total alkalinity also rose from 330 mg/L (neem alone) to 370 mg/L (cow rumen mix) and 365 mg/L (poultry mix), indicating better buffering capacity.

Total nitrogen content decreased slightly from 28.5 mg/kg in neem alone to 27.0 mg/kg and 24.5 mg/kg when mixed with cow rumen and poultry waste, respectively, while phosphorus increased from 3.52 mg/kg to 3.73 mg/kg (cow rumen) and 4.35 mg/kg (poultry waste), enhancing nutrient availability. Total carbon increased from 168.4 mg/kg in neem to 331.1 mg/kg and 320.7 mg/kg with cow dung and poultry waste, respectively, improving microbial energy sources. Biochemical Oxygen Demand (BOD) increased from 112 mg/L in neem shoots to 154 mg/L (cow dung) and 162 mg/L (poultry waste), while Chemical Oxygen Demand (COD) rose from 868 mg/L to 1340 mg/L and 1365 mg/L, respectively, both indicators of higher organic content available for digestion. The C/N ratio improved from an initial 6:1 in neem shoots to 12:1 and 13:1 with cow dung and poultry waste, respectively, suggesting a better nutrient balance.

In terms of physical properties, biomass weight nearly doubled from 2422.5 g (neem alone) to 4865.4 g (cow rumen mix) and 4869.7 g (poultry mix), and volume increased from 2325 mL to 4650 mL in both co-digested samples. Moisture content decreased slightly from 68.4% to 65.7% and 64.8%, remaining within optimal ranges. Total solids content increased from 11.7% in neem alone to 14.1% (cow rumen) and 14.4% (poultry), and volatile solids rose from 79.6% to 81.3% and 81.7%, respectively, indicating more digestible organic matter.

Physiochemical analysis of neem shoot digestate

The physiochemical analysis of neem shoot digestate co-digested with cow dung and poultry waste revealed key differences in nutrient content and digestion suitability. Both digestates had slightly alkaline pH values 8.38 for the cow-based mixture and 8.35 for the poultry-based one, ideal for anaerobic microbial activity. Poultry and neem shoot digestate showed higher total alkalinity (348 mg/L vs. 326 mg/L), indicating stronger buffering capacity. Total nitrogen was slightly higher in the cow mixture (27.8 mg/L) than poultry (25.2 mg/L), while phosphorus was higher in the poultry mix (4.31 mg/L vs. 3.50 mg/L). Total carbon content was nearly identical (312.6 mg/L cow; 311.3 mg/L poultry), suggesting similar energy availability for microbes.

Potassium was higher in the cow digestate (5.8 mg/L), but phosphate was slightly greater in the poultry mix (3.18 mg/L vs. 3.10 mg/L). Sulfate levels were also slightly higher in the cow digestate (116 mg/L vs. 113 mg/L), and calcium was more abundant in the cow digestate (25 mg/L vs. 18 mg/L), promoting microbial stability. Conversely, magnesium was higher in the poultry digestate (86 mg/L vs. 79 mg/L), supporting enzyme activity. Micronutrients like manganese (0.022 mg/L vs. 0.017 mg/L), zinc (33.0 mg/L vs. 23.1 mg/L), aluminum (0.50 mg/L vs. 0.38 mg/L), and copper (4.58 mg/L vs. 2.64 mg/L) were all higher in the poultry digestate, enhancing its nutrient richness. However, iron was higher in the cow digestate (8.18 mg/L vs. 6.17 mg/L), important for methanogenic enzymes.

The biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were also higher in the poultry digestate (158 mg/L and 1364 mg/L) than in the cow digestate (150 mg/L and 1330 mg/L), indicating a greater load of biodegradable organic matter. C/N ratios were slightly better in the poultry mix (12:1) than in the cow mix (11:1), both within the optimal range for digestion. Moisture content was higher in the cow digestate (52.4%) than in poultry (50.8%), facilitating microbial activity. Total solids (T.S) were slightly higher in the poultry digestate (16.9% vs. 16.6%), as were fixed solids (19.6% vs. 19.2%). However, volatile solids (V.S), the key indicator of biodegradable content, were marginally higher in the cow digestate (77.3%) compared to the poultry mix (76.8%).

Table 1: Physiochemical analysis of neem shoots substrates and digestates

		Neem shoot substrate			**Neem shoot digestate**	
S/N	**Parameters**	**Neem shoot**	**Cow dung + Neem shoot**	**Poultry waste + Neem shoot**	**Cow dung + Neem shoot**	**Poultry waste + Neem shoot**
1	pH	7.63	8.49	8.46	8.38	8.35
2	Total alkalinity (mg/L)	330	370	365	326	348
3	Total Nitrogen (mg/L)	28.5	27.0	24.5	27.8	25.2
4	Total Phosphorous (mg/L)	3.52	3.73	4.35	3.50	4.31
5	Total Carbon (mg/L)	168.4	331.1	320.7	312.6	311.3
6	Potassium (mg/L)	5.9	8.4	6.9	5.8	4.7
7	Phosphate (mg/L)	3.0	4.65	4.52	3.10	3.18
8	Sulphate (mg/L)	114	145	120	116	113
9	Calcium (mg/L)	22	40	36	25	18
10	Magnesium (mg/L)	80	96	108	79	86
11	Manganese (mg/L)	0.017	0.022	0.023	0.017	0.022
12	Iron (mg/L)	8.20	9.60	9.24	8.18	6.17
13	Zinc (mg/L)	23.0	27.0	33.0	23.1	33.0
14	Aluminum (mg/L)	0.39	0.52	0.51	0.38	0.50
15	Copper (mg/L)	2.65	4.40	4.60	2.64	4.58
16	BOD (mg/L)	112	154	162	150	158
17	COD (mg/L)	868	1340	1365	1330	1364
18	Carbon to Nitrogen Ratio (C/N)	6:1	12:1	13:1	11:1	12:1
20	Volume of Sample (m^3)	68.4	65.7	64.8	4638	4631
21	Moisture Content (%)	11.7	14.1	14.4	52.4	50.8
22	Total Solids (%)	19.6	17.9	17.6	16.6	16.9
23	Fixed Solids (%)	79.6	81.3	81.7	19.2	19.6
24	Volatile Solids (%)	-	-	-	77.3	76.8

Gas Chromatography-Mass Spectrometry (G C-MS) Analysis

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of neem shoots co-digested with poultry wastes and cow rumen, displayed in Tables 2 and 3, reveals the presence of various volatile compounds and organic acids, highlighting their potential contribution to enhanced biogas production efficiency through the breakdown of complex organic materials.

Neem shoots with poultry wastes

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of neem shoots co-digested with poultry waste (Table 2) reveals a significant proportion of methane (CH4), which dominates the gas composition at 61.29%. This high methane content underscores the biogas potential of the co-digestion process, as methane is the primary energy carrier in biogas. The substantial methane yield reflects an efficient anaerobic digestion process, which is essential for optimizing biogas production. Following methane, carbon dioxide (CO2) makes up 33.54% of the gas composition, which is typical in biogas production as CO2 is a natural by-product of microbial digestion. The balance between methane and carbon dioxide content in the gas mixture aligns with previous studies, such as that of Bonilla *et al.* (2020), which also reported methane as the predominant gas in the co-digestion of biomass and organic wastes. These findings indicate that neem shoots combined with poultry waste create favourable conditions for microbial activity, resulting in substantial methane production.

In contrast, gases like nitrogen (N2) and hydrogen (H2) are present in smaller proportions, 0.72% and 0.04%, respectively. These trace gases, while not significant contributors to the biogas's energy content, play supporting roles in maintaining microbial health and balance during digestion. Similarly, carbon monoxide (CO) and oxygen appear in minimal amounts, 0.02% and 0.05%, suggesting the presence of minor impurities, which are often found in biogas mixtures. The low retention times for hydrogen and nitrogen in the GC-MS analysis reflect their minimal contribution to the overall gas composition as presented in Table 2. These results echo findings from recent research, such as that by Aworanti *et al.* (2023), which highlighted the importance of optimising methane production through the careful management of digestion conditions to reduce impurities and enhance the quality of the biogas. The presence of these trace gases indicates the need for further refinement in the digestion process to minimize non-energy-producing gases and maximize the efficiency of biogas as a renewable energy source.

Neem Shoots with Cow Rumen

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of neem shoots co-digested with cow rumen (Table 3) shows a dominant methane (CH4) concentration of 63.29%, which emphasizes the co-digestion's effectiveness in biogas production. Methane is critical for biogas energy potential, and this high percentage highlights the efficiency of the neem shoot and cow dung mixture in producing substantial methane. Carbon dioxide (CO2), the second most prevalent gas at 32.18%, supports the microbial processes driving anaerobic digestion, consistent with prior studies like that of Bonilla et al. (2020), which documented methane dominance in biogas systems with organic co-digestion. These proportions between methane and carbon dioxide in the neem and cow dung mix suggest that the substrate offers optimal conditions for microbial activity, fostering enhanced biogas yield.

Moreover, other gases present in minimal amounts, such as nitrogen (N2) at 0.70% and hydrogen (H2) at 0.01%, indicate typical trace gas occurrences in biogas production as depicted in Table 3. Their minor presence reflects the overall purity of the gas mixture, further supported by the low concentrations of carbon monoxide (CO) and oxygen, measured at 0.01% and 0.03%, respectively. The small presence of these impurities aligns with the findings of Svane and Karring, (2019), who identified similar trace elements in cow dung digestion processes. The retention times for these trace gases, like nitrogen and hydrogen, suggest their limited role in the biogas' energy profile. The neem shoot and cow dung co-digestion proves to be a potent substrate combination for producing high methane concentrations, positioning it as a promising feedstock for renewable energy production.

Biogas Production Analysis

The analysis of biogas production focuses on the performance of neem biomass and cow rumen (Table 4), as well as the combination of neem biomass with poultry wastes (Table 5), evaluating their efficiency in enhancing biogas yields.

Neem Shoots and Poultry Waste

The biogas production from co-digesting neem shoot biomass and cow rumen was tracked over 32 days, showing typical anaerobic digestion dynamics. Initially, gas production was negligible, with microbial adaptation evident in the slow increase during the first week (lag phase), reaching 0.1628 liters by day 7. This gradual onset of biogas production is a characteristic observation in the anaerobic digestion of biomass, as similarly reported by Schnürer and Jarvis (2018), who highlighted that initial gas yields tend to be low as microorganisms begin hydrolyzing complex organic matter.

A steady rise in production occurred between days 8 and 19, peaking at a cumulative 0.63492 liters, indicating optimal microbial activity and substrate breakdown. During this active phase, stable pH (8.39–8.42) and temperature (35.7°C) supported efficient methane generation. Studies such as that of Thakur et al. (2022) corroborate this pattern, where the mid-phase of anaerobic digestion typically exhibits the highest gas yields due to the complete breakdown of volatile solids into simpler compounds like methane (CH4) and carbon dioxide (CO2). From days 20 to 30, gas production stabilized, with daily outputs ranging from 0.03256 to 0.04884 liters, culminating in 1.26984 liters by day 30. This observation is consistent with the findings of Choudhary et al. (2020), who reported that biogas production in mesophilic conditions remains steady as long as temperature and pH are maintained within optimal ranges.

The final days (31–32) showed a slight decline in gas output as most biodegradable material had been digested, leading to a total cumulative yield of 1.3024 liters. These trends reflect the typical stages of anaerobic digestion lag, exponential, and plateau and underscore the importance of maintaining consistent pH and temperature for sustained biogas output. This plateau phase is characteristic of anaerobic digestion processes and aligns with the results from similar studies that explored co-digestion of biomass and animal waste, such as the research conducted by Maurus *et al.* (2021), which found that biogas production tapers off as the availability of organic matter decreases.

Neem Shoots and Cow Rumen

The co-digestion of neem shoots and poultry waste over 32 days followed a typical anaerobic digestion pattern, starting with a lag phase of no gas production on day one. By day two, a small amount of biogas (0.01628 liters) was recorded, with stable and optimal conditions for methanogens (pH 8.38, temperature 35.7°C). Gas production gradually increased, reaching 0.04884 liters by day eight and a cumulative yield of 0.21164 liters, indicating effective hydrolysis and acidogenesis. Similar patterns were reported by Khadka *et al.* (2022), who observed steady increases in biogas yield during the mid-phase of digestion in co-digestion systems involving plant-based biomass and animal waste due to the enhanced nutrient availability provided by the animal waste component. By day 18, production peaked, with cumulative yield at 0.60236 liters and steady daily output, supported by consistent pH (8.39) and temperature, signalling optimal methanogenic activity. Hoang *et al.* (2022) reported similar findings, where the steady pH levels and optimal temperature in a co-digestion setup fostered sustained methane generation, with poultry waste acting as an excellent buffer to maintain the pH within the ideal range for methanogenesis.

In the final days (28–32), gas production declined as substrates were depleted, with the cumulative yield reaching 1.0582 liters. The process was stable throughout, demonstrating that poultry waste effectively supports digestion by maintaining ideal conditions for microbial activity. These observations are consistent with findings by Rocamora *et al.* (2020), who also documented a gradual decline in biogas production during the final stages of anaerobic digestion due to the reduced availability of organic material for microbial conversion.

Table 2: Gas Chromatography-Mass Spectrometry Analysis of Neem Shoots with Poultry Wastes

Peak	Retention time (days0	Name of Gas (Molecular Formula)	Molecular Mass	Peak Area (%)	% Composition
1	14.53	Hydrogen (H_2)	2	22.16	0.04
2	17.51	Nitrogen (N_2)	28	9.66	0.72
3	22.50	Methane (CH_4)	16	19.31	61.29
4	23.25	Carbon dioxide (CO_2)	44	17.05	33.54
5	24.80	Standard (STD)	STD	13.63	STD
6	28.33	Carbon monoxide (CO)	28	5.68	0.02
7	29.98	Oxygen	32	12.50	0.05

Table 3: Gas Chromatography-Mass Spectrometry Analysis of Neem Shoots with Cow Rumen

Peak	Retention time	Name of Gas (Molecular Formula)	Molecular Mass	Peak Area (%)	% Composition
1	13.25	Hydrogen (H_2)	2	5.52	0.01
2	16.50	Nitrogen (N_2)	28	13.50	0.70
3	20.41	Methane (CH_4)	16	17.79	63.29
4	22.20	Carbon dioxide (CO_2)	44	20.24	32.18
5	25.62	Standard (STD)	STD	16.56	STD
6	27.41	Carbon monoxide (CO)	28	10.42	0.01
7	31.54	Oxygen	32	15.95	0.03

Table 4: Biogas Production from Neem Shoot with Cow Rumen

Days	Production per day (mm)	Quantity of Gas per Day (mm)	Production Per Day (litre)	Communitive of gas Produced	pH	Temp (° C)
1	280mm	0mm	0.00000	0.00000	8.42	35.8
2	279mm	1mm	0.01628	0.01628	8.41	35.8
3	278mm	1mm	0.01628	0.03256	8.40	35.7
4	276mm	2mm	0.03256	0.06512	8.41	35.7
5	274mm	2mm	0.03256	0.09768	8.41	35.7
6	272mm	2mm	0.03256	0.13024	8.41	35.7
7	270mm	2mm	0.03256	0.16280	8.41	35.7
8	267mm	3mm	0.04884	0.21164	8.40	35.7
9	264mm	3mm	0.04884	0.26048	8.41	35.7
10	262mm	2mm	0.03256	0.29304	8.40	35.7
11	260mm	2mm	0.03256	0.32560	8.40	35.7
12	257mm	3mm	0.04884	0.37444	8.40	35.7
13	255mm	2mm	0.03256	0.40700	8.41	35.7
14	253mm	2mm	0.03256	0.43956	8.41	35.7
15	251mm	2mm	0.03256	0.47212	8.41	35.7
16	249mm	2mm	0.03256	0.50468	8.41	35.7
17	247mm	2mm	0.03256	0.53724	8.40	35.7
18	243mm	3mm	0.04884	0.58608	8.41	35.7
19	240mm	3mm	0.04884	0.63492	8.41	35.7
20	238mm	2mm	0.03256	0.66748	8.40	35.7
21	236mm	2mm	0.03256	0.70004	8.40	35.7
22	234mm	2mm	0.03256	0.73260	8.39	35.7
23	232mm	2mm	0.03256	0.76516	8.40	35.7
24	230mm	2mm	0.03256	1.09076	8.39	35.7
25	228mm	2mm	0.03256	1.12332	8.39	35.7
26	226mm	2mm	0.03256	1.15588	8.39	35.7
27	224mm	2mm	0.03256	1.18844	8.40	35.7
28	222mm	2mm	0.03256	1.22100	8.39	35.7
29	220mm	2mm	0.03256	1.25356	8.39	35.7
30	219mm	1mm	0.01628	1.26984	8.39	35.7
31	218m	1mm	0.01628	1.28612	8.39	35.7
32	217mm	1mm	0.01628	1.30240	8.39	35.7

Statistical Analysis

The statistical analysis of the 2FI model for biogas yield from the co-digestion of neem shoot and cow rumen demonstrated that the model is statistically significant, with an F-value of 5.22 and a p-value of 0.0256 (Table 6).

Table 5: Biogas Production from Neem Shoot with Poultry Wastes

Days	Production per day (mm)	Quantity of Gas per Day (mm)	Production Per Day (litre)	Communitive of gas Produced	pH	Temp (º C)
1	280	0	0.00000	0.00000	8.38	35.8
2	279	1	0.01628	0.01628	8.38	35.7
3	278	1	0.01628	0.03256	8.39	35.8
4	276	2	0.03256	0.06512	8.38	35.7
5	274	2	0.03256	0.09768	8.39	35.7
6	272	2	0.03256	0.13024	8.39	35.7
7	270	2	0.03256	0.1628	8.39	35.7
8	267	3	0.04884	0.21164	8.39	35.7
9	264	3	0.04884	0.26048	8.39	35.7
10	262	2	0.03256	0.29304	8.38	35.7
11	259	3	0.04884	0.34188	8.38	35.7
12	256	3	0.04884	039072	8.37	35.7
13	254	2	0.03256	0.42328	8.37	35.7
14	252	2	0.03256	0.45584	8.37	35.7
15	250	2	0.03256	0.48840	8.37	35.7
16	248	2	0.03256	0.52096	8.37	35.7
17	246	2	0.03256	0.55352	8.37	35.7
18	243	3	0.04884	0.60236	8.37	35.7
19	240	3	0.04884	0.6512	8.37	35.7
20	237	3	0.04884	0.70004	8.37	35.7
21	235	2	0.03256	0.7326	8.37	35.7
22	232	2	0.03256	0.76516	8.36	35.7
23	230	2	0.03256	0.79772	8.37	35.7
24	228	2	0.03256	0.83028	8.36	35.7
25	226	2	0.03256	0.86284	8.36	35.7
26	224	2	0.03256	0.8954	8.36	35.7
27	222	2	0.03256	0.92796	8.37	35.7
28	220	2	0.03256	0.96052	8.36	35.7
29	218	2	0.03256	0.99308	8.36	35.7
30	216	2	0.03256	1.02564	8.36	35.7
31	215	1	0.01628	1.04192	8.36	35.7
32	214	1	0.01628	1.0582	8.36	35.7

This finding aligns with the results obtained by Atelge *et al.* (2020), who found that similar factors such as pH and total solids played critical roles in biogas production from organic waste. Key factors such as pH, total solids (TS), and volatile solids (VS) were identified as significant contributors to biogas production, with pH showing particularly strong influence (F = 18.32, p = 0.0052).

This finding is supported by Siddique and Wahid (2018), who reported that maintaining an optimal pH is essential for methanogenic bacteria, especially in co-digestion systems, where variations in feedstock composition can lead to pH fluctuations. Furthermore, the model further explored the interaction between different factors. Significant interactions such as AE (p = 0.0372), BE (p =

0.0200), and CD (p = 0.0195) point to the combined effects of these variables on biogas yield. Specifically, the interaction between VS and TS (CD) suggests that variations in substrate composition and solid content can have a compounded effect on gas production. In contrast, other interactions like AB (p = 0.0774) and AC (p = 0.2101) were found to be insignificant, indicating that retention time and temperature, either alone or in combination with other factors, did not significantly impact the overall yield. These findings contrast with the work of Bumharter *et al.* (2023), where temperature was reported to have a pronounced effect on biogas production from agricultural waste, suggesting that the specific feedstock combination in this study may be less sensitive to thermal variations.

Analysis of Methane Gas Percentage

The difference between the methane gas percentage produced from the co-digestion of Neem shoots with poultry waste and cow rumen demonstrates the efficiency of different substrates in enhancing methane production, as illustrated in Figure 1. At a temperature of 35.7°C, the methane gas percentage for Neem shoots co-digested with poultry waste was 61.29%, while that of Neem shoots with cow dung reached a slightly higher value of 63.29%. Similarly, at 35.8°C, the methane yield from Neem shoots with poultry waste increased marginally to 62.00%, while co-digestion with cow dung led to a methane percentage of 64.10%. These results underscore the impact of substrate type on methane generation, indicating that cow dung might be a more efficient co-substrate for enhancing methane production than poultry waste under similar anaerobic digestion conditions. This finding aligns with the results reported by Awasthi *et al.* (2020) and Oladejo *et al.* (2020, 2024 and 2025), who observed that cow dung, due to its higher microbial diversity and nutrient content, can enhance the anaerobic digestion process and improve methane yield more effectively than poultry waste. The higher methane yield associated with the cow rumen may be attributed to the presence of more diverse anaerobic microorganisms, which are essential for the breakdown of complex organic matter and the subsequent production of biogas.

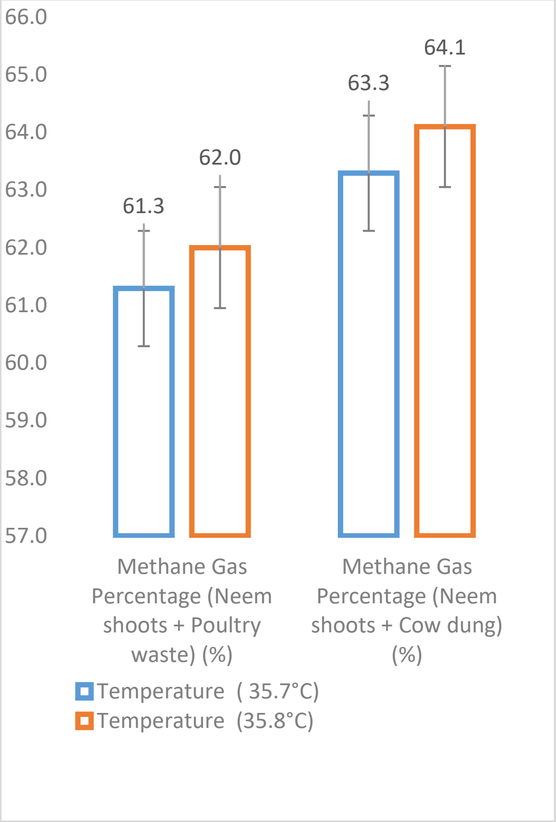

Figure 1: Percentage gas analysis of methane.

CONCLUSION AND RECOMMENDATION

This study concluded that co-digesting neem shoots with poultry waste and cow rumen supports favourable conditions for anaerobic digestion, producing high methane yields (61.29- 63.29%). The use of cow rumen slightly outperformed poultry. Furthermore, for systems aiming to maximize methane output, cow dung should be considered the preferred co-substrate over poultry waste, owing to its higher microbial diversity and stronger performance in methane generation under mesophilic conditions.

Table 6: ANOVA for 2FI model of Neem shoot and cow rumen

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	0.2203	15	0.0147	5.2200	0.0256 significant
A-retention	0.0002	1	0.0002	0.0586	0.8167
B-pH	0.0516	1	0.0516	18.320	0.0052
C-temp	0.0020	1	0.0020	0.7014	0.4344
D-TS	0.0888	1	0.0888	31.540	0.0014
E-VS	0.0191	1	0.0191	6.8000	0.0402
AB	0.0128	1	0.0128	4.5300	0.0774
AC	0.0055	1	0.0055	1.9700	0.2101
AD	0.0055	1	0.0055	1.9500	0.2116
AE	0.0200	1	0.0200	7.1100	0.0372
BC	0.0012	1	0.0012	0.4432	0.5303
BD	0.0062	1	0.0062	2.2000	0.1884
BE	0.0278	1	0.0278	9.8800	0.0200
CD	0.0281	1	0.0281	10.000	0.0195
CE	0.0086	1	0.0086	3.0600	0.1306
DE	0.0005	1	0.0005	0.1741	0.6910
Residual	0.0169	6	0.0028		
Lack of Fit	0.0164	5	0.0033	6.17	0.2961 not significant
Pure Error	0.0005	1	0.0005		
Cor Total	0.2371	21			

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