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Anaerobic Co-digestion of Neem (*Azadirachta indica* A. Juss) Pulp with Cattle Manure: Effect of Mixing Ratio and Dry Matter Content on Biogas Production

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A B S T R A C T

Neem (*Azadirachta indica* A. Juss) pulp, a by-product of biopesticide production, constitutes a phytosanitary waste whose management poses environmental challenges. Its energy valorization through anaerobic digestion could transform this waste into a renewable resource. This study aimed to evaluate the effect of neem pulp/cattle manure co-digestion ratio and dry matter content on biogas production. Batch trials (200 L digesters) were conducted over 45 days under tropical ambient temperature (29-37°C). Four co-digestion ratios (P100: 100 % pulp; P75B25: 75 % pulp + 25 % manure; P50B50: 50 % pulp + 50 % manure; B100: 100 % manure) and two dry matter contents (TS10: 10 % dry matter; TS15: 15 % dry matter) were tested in triplicate. Results showed that pulp alone (P100) completely inhibited methanogenesis (12.2 L in 45 days, final pH 4.10-4.14) due to its bioactive compound content (azadirachtin, salanine, nimbin). Co-digestion with cattle manure progressively alleviated this inhibition through its buffering capacity and inhibitor dilution. The optimal ratio was P50B50 at TS15 (332.4 L in 45 days). A high significant interaction ($p < 0.001$) between co-digestion ratio and dry matter content was demonstrated. This study shows that controlled co-digestion enables energy valorization of neem based biopesticide waste under tropical climate, thus paving the way toward a circular economy in agricultural systems.

Introduction

Sub-Saharan Africa faces an unprecedented energy crisis, with approximately 60 % of its rural population lacking reliable access to electricity (Gbadeyan *et al.,* 2024). Concurently, population growth and rapid urbanization generate increasing volumes of organic waste, whose inadequate management poses major environmental and health challenge. Yet, these organic wastes represent considerable energy potential, estimated at 133 million GWh per year in sub-Saharan Africa (Rubagumya *et al.,* 2023) and could generate between 20 and 58 million MWh of electricity by 2060 (Longfor *et al.,* 2023). In this context, the transition toward renewable energy sources becomes imperative, with record investments of 15 billion dollards in 2023 in the African renewable

energy sector (BloombergNEF, 2024). Energy valorization of organic waste thus emerges as a promising solution, offering a dual benefit: clean energy production an sustainable waste management.

Anaerobic digestion (AD), constitutes a proven technology for organic waste valorization. This biological process occurs through four sequential stages (hydrolysis, acidogenesis, acetogenesis, and methanogenesis) during which complex microbial communities degrade organic matter into biogas, composed primarily of methane (CH_4) and carbon dioxide (CO_2) (Kumar *et al.,* 2024). Anaerobic digestion presents a dual advantage: it produce a renewable energy source while generating a nutrient-rich digestate usable as biofertilizer (Tolessa, 2024). In tropical context, elevated ambient temperatures (25-35°C) naturally favor methanogenic activity, thereby reducing energy requirement for digester heating. Studies have demonstrated that optimization of operational parameters, particularly through co-digestion of complementary substrates, can increase methane yield by 25 to 40 % (Longfor *et al.,* 2023). Despite this potential, adoption of anaerobic digestion in sub-Saharan Africa remains limited, primarily due to technical challenges related to the variable nature of available substrates.

However, anaerobic digestion of certain plant biomasses encounters major challenges related to the presence of inhibitory compounds. Methanogenic archaea, key microorganisms in methane production, are particularly sensitive to pH variations and the presence of bioactive compound (Cai *et al.,* 2024). Plant secondary metabolites, such as saponins, tannins, terpenoids, and alkaloids, can significantly inhibit methanogenic activity by disrupting microbial cell membranes or interfering with metabolic pathways (Patra & Saxena, 2010). This inhibition manifests through medium acidification, volatile fatty acid accumulation, and drastic decline in methane production (Ijoma *et al.,* 2024). Numerous agricultural biomasses rich in bioactive compounds, particularly those derived from medicinal or phytosanitary plants, thus present underexploited energy potential due to their intrinsic toxicity to methanogenic organisms. Anaerobic co-digestion, which involves simultaneously treating multiple complementary substrates, has emerged as an effective strategy to overcome mono-digestion limitations (Kadam *et al.,* 2024). This approach enables dilution of inhibitory compounds, balancing of the carbon/nitrogen (C/N) ratio, improvement of nutrient bioavailability, and reinforcement of reaction medium's buffering capacity. Animal manure, particularly cattle manure, constitutes a co-substrate of choice due to its high buffering capacity, nutrient richness, and natural microbial diversity derived from the rumen, which facilitates inoculation and process stabilization (Zhang *et al.,* 2017). Studies have demonstrated that co-digestion of organic waste with cattle manure can generate significant synergistic effects, with biogas production increases ranging from 24 to 47 % depending on mixing ratios (Argaw *et al.,* 2013; Baek *et al.,* 2020). This strategy has successfully enabled valorization of various biomasses initially considered difficult ti digest in mono-digestion.

Neem (*Azadirachta indica* A. Juss), a tree native to South Asia and widely distributed in West Africa, is exploited on large scale for natural biopesticide production. Global production of azadirachtin, the main active compound of neem, reaches approximately 4.800 metric tons per year, with the Asia-Pacific region representing 62 % of production (Report Market, 2025). Neem seeds contain more than 186 bioactive compounds, including azadirachtin, salanine, and nimbin, which confer to neem its insecticidal, antifungal, and repellent properties (Chaudhary *et al.,* 2017; Dhakad *et al.,* 2025). Industrial extraction of these compounds generates substantial residual waste volumes including pulps, rich in fibers and bioactive residues. These wastes pose environmental management challenges due to their content of secondary metabolites potentially phytotoxic and hazardous to soil fauna. Paradoxically, although energy valorization of agricultural waste through anaerobic digestion is widely documented, no study has, to our knowledge evaluated the feasibility of neem pulp anaerobic digestion, leaving a major scientific and technological gap. This gap is all the more critical as neem is present in regions facing significant energy and environmental challenge.

In this context, the present study aims to evaluate, for the first time, the feasibility of energy valorization of neem pulp through anaerobic digestion in co-digestion with cattle manure. The specific objectives are: (i) to evaluate the effect of different ratios on the stability of digestion medium pH; (ii) to evaluate the effect of different neem pulp/cattle manure co-digestion ratios on biogas production over 45 days; (iii) to determine the influence of dry matter content on anaerobic digestion process performance. This research contributes to filling the gap identified in the literature and opens a pathway toward sustainable management of biopesticide waste in tropical contexts.

Materials and Methods

Study Site

The present study was conducted at the Lomé Agronomic Experimentation Station (SEAL) of the Higher School of Agronomy (ESA) at the University of Lomé (6°10'N,1°10'E, 19-60 m altitude), in southern Togo, in a humid tropical climate zone. A neem fruit processing unit for biopesticide production is installed at this location. This unit generates waste including pulps used in this experiment. Fresh cattle manure from the station's cattle farming operation was also used as co-substrate for digestion.

Substrates and Inoculum

For anaerobic digestion, two substrates were used. These consisted of pulps from neem (Azadirachta indica A. Juss) fruit depulping in the biopesticide production process and cattle manure from cattle farming within the same station. The pulp was used within 24 hours following depulping and the cattle manure within 24 hours following collection. The inoculum used was an anaerobic digestate from previous anaerobic digestion of cattle manure. Prior use, the inoculum was subjected to 15-day degassing in a sealed container without feeding, thus ensuring that measured biogas volumes were exclusively attributable to the experimental substrates. Both substrates as well as the inoculum were characterized before experiment initiation.

These analyses were performed in triplicate and covered dry matter (DM, %), pH, total organic carbon (TOC), total nitrogen (N), C/N ratio, and total sugar (on pulp only). Dry matter content was determined after placing a 5 g sample in an oven at 105°C until constant mass was obtained (AOAC 930.15). pH was measured using an electrode pH meter (VIVOSUN), calibrated with buffer solutions at pH 4.00 and 7.00. Nitrogen content was determined by the Kjeldahl method AOAC 955.04. Total organic carbon (TOC) content was determined according to ISO 10694 standard by high-temperature dry combustion with detection of produced CO_2. Results are expressed on a dry matter basis. Total sugar content was quantified by the classical phenol-sulfuric acid colorimetric method described by (DuBois *et al.,* 1956). This measurements enabled determination of the carbon/nitrogen (C/N) ratio of each substrate from TOC and N contents.

Experimental Design

The experimental design was based on two factors: neem pulp/cattle manure co-digestion ratio on a dry matter basis with 4 levels (100:0; 75:25; 50:50; 0:100) and mixture dry matter content with two levels (10 % and 15 %). Three replicates were performed per combination, totaling 24 digesters (4×2×3). Treatment codes were: P100 (pulp alone, negative control), P75B25 (75 % pulp and 25 % manure), P50B50 (50 % pulp and 50 % manure), B100 (manure only, positive control).

Each digester had a nominal volume of 250 liters including 200 liters of working volume (80 %), with the remaining 50 liters constituting the headspace for biogas collection. The total mass of the mixture introduced into each digester was 166.7 kg including 16.7 kg of fresh inoculum matter corresponding to 10 % of the total dry matter od the mixture. Borehole water was added as a supplement to reach the target dry matter content. Co-digestion ratios, expressed on a dry matter basis, were converted to fresh masses for experimental weighing. The detailed mass composition of each digester is presented in Table 1. Prior to digester loading, substrates and water were mixed in a tank for 5 minutes. The mixture was then transferred into the digester, which was hermetically sealed. The sealing date was recorded as day 0 (D0) of digestion.

Biogas Collection and Measurement System

During process, biogas produced in the digester passed successively through a hydrogen sulfide (H_2S) trap, then through a water vapor trap before being stored in a floating bell gasometer. Each digester was equipped with its own measurement system and the biogas thus collected corresponded to desulfurized crude biogas.

The H_2S trap consisted of a PVC tube filled with steel wool. This device selectively removes H_2S without affecting the methane and carbon dioxide contents of the biogas. The vapor trap was a condensation tube allowing collection of water vapor by gravity before entry into the gasometer.

The gasometer consisted of a polyethylene drum (100 liters) with an inverted floating bell in ordinary water. Volume measurement was performed by reading the level difference (Δh in cm) on a U-tube water manometer. Volume was calculated using the following

formula: V = Δh×S/1000, where S = internal cross-sectional area of gasometer. After each reading (every 3 days), the accumulated gas was burned on a burner connected to the gasometer to qualitatively confirm the presence of methane (blue/orange flame) and the system was reset to zero. This flammability test was performed at each reading. Biogas volumes were measured under ambient conditions and were not corrected to standard temperature and pressure conditions.

Monitoring Parameters Data Collection

Biogas volume was measured every three days over a total duration of 45 days of anaerobic digestion. pH was measured on the same day as biogas. A 50 mL sample was collected via the bottom valve of the digester, coarsely filtered through a sieve (mesh size > 1 mm), then pH was immediately measured to limit modifications related to aeration. Sampling through the bottom valve was preferred to minimize aeration of the reaction medium.

Internal temperature was measured using a submersible probe in the digester and ambient temperature using a sheltered thermometer. Temperatures were measured twice daily (6:00 A and 2:00 PM). No temperature or pH adjustment was performed throughout the process.

Cumulative volume was calculated over 45 days and productivity per 3-day period was determined. Specific yield was calculated by dividing cumulative biogas volume by the dry matter mass of substrates introduced (excluding inoculum), and expressed as L/kg DM. Organic matter was estimated using the formula OM = TOC×1.724 (Van Bemmelen factor) and specific yield was also calculated on an organic basis ($V_{cumulative}/OM_{substrates}$) and expressed as L/kg OM.

Statistical Analysis

Statistical analysis was performed using R software (Version 4.5.1). A two-way analysis of variance (ANOVA) at a significance level of 5 % was conducted with ratio and DM content as factors, and the following response variables: cumulative biogas volume (L), specific yield on a dry matter basis (L/kg DM), specific yield on an organic matter basis (L/kg OM), and final pH (D45). In case of significance, Tukey's post-hoc test was performed for multiple comparisons. ANOVA was preceded by verification of residuals normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test). For pH, descriptive analysis was performed with mean ± standard deviation followed by comparison of final values (D45) by ANOVA.

Results and Discussion

Physicochemical Characteristics of Substrates

Results from laboratory analysis of anaerobic digestion substrates are presented in Table 2.

These results shows that neem pulp is acidic (pH = 5.80) with TOC content (41.98 % DM) and total sugars (69.77 % DM). The dry matter content of this substrate is 18.20 % and the C/N ratio is 21.31.

Cattle manure has an alkaline pH (8.25) with higher dry matter content (27.23 %), total organic carbon (TOC) content of 36.62 %, and a C/N ratio of 21.94, similar to that of pulp. The C/N ratios of both substrates are close and fall within the range generally observed between substrates (5.80 versus 8.25).

Regarding the inoculum, it has a neutral pH (7.23), dry matter content of 15.13 %, and a C/N ratio of 17.70, lower than those of both substrates.

Biogas Production and Specific Yield

Results concerning cumulative biogas production are presented in Figure 1 and Table 3. Cumulative biogas production after 45 days of anaerobic digestion ranged from 12.2 L for neem fruit pulp (P100) to 527.4 L for cattle manure at 15 % dry matter content (B100-TS15). An increasing gradient of cumulative biogas production was observed following the order P100 < P75B25 < P50B50 < B100 for both DM contents (Figure 1). ANOVA revealed a highly significant effect of the substrate mixing ratio ($p < 0.001$), dry matter content ($p < 0.001$), and their interaction ($p < 0.001$) on cumulative biogas production. With neem fruit pulp alone, cumulative production remained low (12.2 L) and no significant difference was observed between the two DM contents according to Tukey's test.

Results from biogas production kinetics monitoring are presented in Figure 2. For B100, the production peak was reached at D15 for both DM contents, with a higher value for TS15 (112.4 L/3d) than for TS10 (94.2 L/3d). Production was concentrated between D3 and D30. For P50B50, the peak was reached at D15 for TS10 (39.7

L/3d) and at D27 for TS15 (49.4 L/3d). Production with TS15 started later (D9 versus D3) but extended longer, without complete stabilization at D45 (6.3 L/3d).

P75B25 exhibited a longer lag phase, with peaks reached at D27 for TS10 (22.2 L/3d) and at D24 for TS15 (16.4 L/3d). For P100, production was very low, with a brief peak at D9 (4.4 L/3d) for TS10 and at D12 (4.3 L/3d) for TS15, followed by an almost complete cessation in the mixture, the peak became lower and the active production phase shorter.

Results regarding specific yields are presented in Table 3. Specific yields ranged between 30.05 $L.kg^{-1}$ DM for B100-TS10 and 0.54 $L.kg^{-1}$ DM for P100-TS15. The gradient of specific yields followed the same order as that of cumulative volumes (P100 < P75B25 < P50B50 < B100). However, unlike absolute volumes, specific yields were consistently higher with TS10 than with TS15, except for P100. ANOVA revealed highly significant effects ($p < 0.001$) of ratio, dry matter content, and their interaction on specific yields. Tukey's test revealed no significant difference between P100-TS10 and P100-TS15. Yields on an organic matter basis followed the same ranking from 0.74 $L.kg^{-1}$ OM (P100-TS15) to 48.24 $L.kg^{-1}$ OM (B100-TS10) (Table 3).

pH Evolution

pH evolution for different mixtures during the anaerobic digestion process is presented in Figure 3. Results show that all treatments exhibited a pH decrease during the digestion process, but the magnitude and trajectory varied markedly according to the ratio. For P100, rapid and irreversible pH acidification was observed: pH dropped from 6.63 (TS10) and 5.92 (TS15) to approximately 4.1 where it stabilized. B100 exhibited a slow and progressive pH decline from 8.17 to 7.25 (TS10) and from 8.26 to 7.35 (TS15), with pH remaining above 7.0 throughout the experiment. P50B50 exhibited a moderate decline (minimum of 6.18 at D18 for TS10 and 6.57 at D18 for TS15) followed by partial recovery toward 6.77 and 6.82, respectively. For P75B25, pH evolution was contrasted between TS10, which showed a marked pH recovery to 6.53 after declining to 5.44, and TS15, which exhibited low pH stabilization at 5.54. ANOVA revealed highly significant differences ($p < 0.001$) in ratio, dry matter content, and their interaction on final pH values.

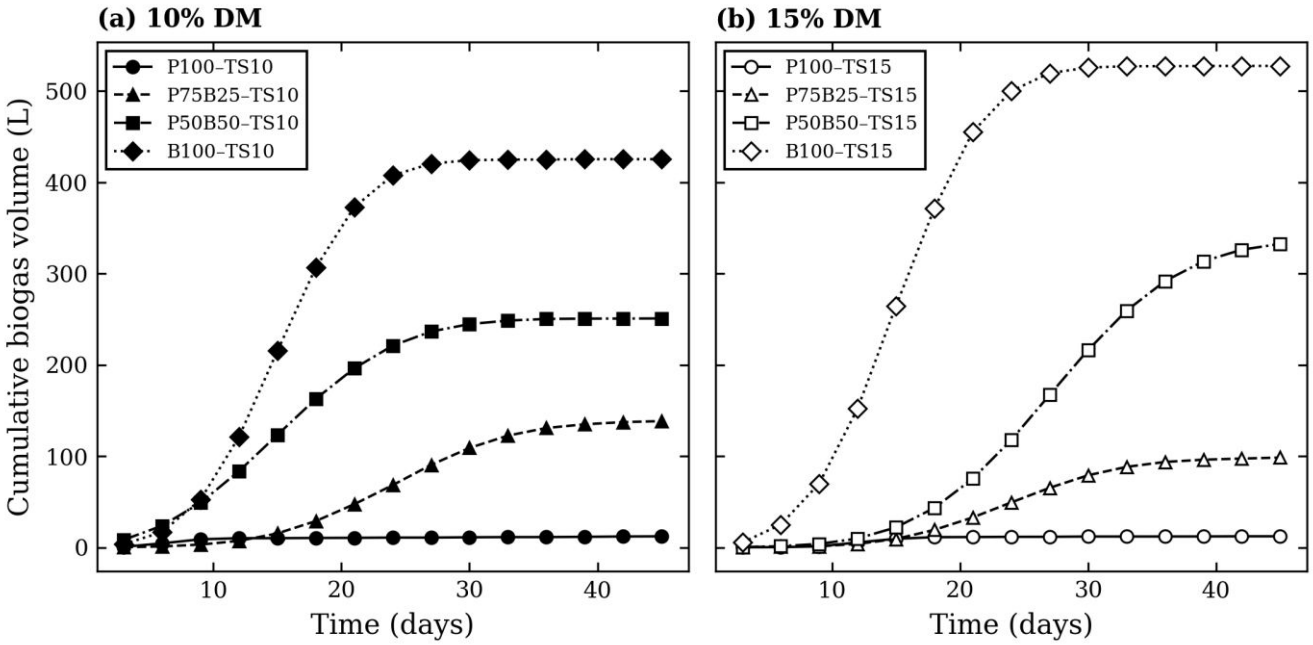
Discussion

Monitoring biogas production with pulp alone (P100-TS10 and P100-TS15) yielded near-zero volumes with cumulative production of 12.2 L over 45 days and yields ranging between 0.54 and 0.86 $L.kg^{-1}$ DM. Irreversible acidification was observed with pH dropping from 5.92-6.63 at the beginning of the experiment (D0) to a final pH of 4.10-4.14 at D45. This rapid decline suggests active substrate hydrolysis but with complete methanogenesis blockage. Biogas production ceased quickly with very low peaks (4.3-4.4 L/3days between D9 and D12). These results are explained by the concentrated nature of bioactive compounds in neem pulp, a residue from neem fruit depulping for biopesticide production. Neem residues contain limonoids such as azadirachtin, salanine, and nimbin, known for their broad-spectrum antimicrobial properties. These bioactive compounds likely directly inhibited methanogenic archaea (Ijoma *et al.,* 2024; Patra & Saxena, 2010), microorganisms responsible for methane production. Studies on plant bioactive compounds have demonstrated that these compounds can inhibit methanogenesis by suppressing methanogen activity and abundance (Patra & Saxena, 2010). Work on anaerobic digestion has confirmed that methanogenesis is inhibited by bioactive compounds at concentration above 0.3 $g.L^{-1}$ (Ngoma *et al.,* 2015). Medium acidification (pH < 6.5) likely exacerbated this inhibition, as methanogens are particularly sensitive to pH variations with optimal activity between 6.5 and 7.5 (Cai *et al.,* 2024). Final pH values (4.10-4.14) indicate stabilization under persistent acidic conditions without any recovery, unlike treatments P75B25-TS10 and P50B50-TS10 where partial pH recovery was observed.

In the absence of bioactive compound quantification, their inhibitory role remains inferred from process behavior rather than directly demonstrated. Probable accumulation of unmeasured volatile fatty acids (VFA) would have created a negative feedback loop, worsening acidification and permanently blocking methanogenesis. Co-digestion with cattle manure enabled partial alleviation of this inhibition.

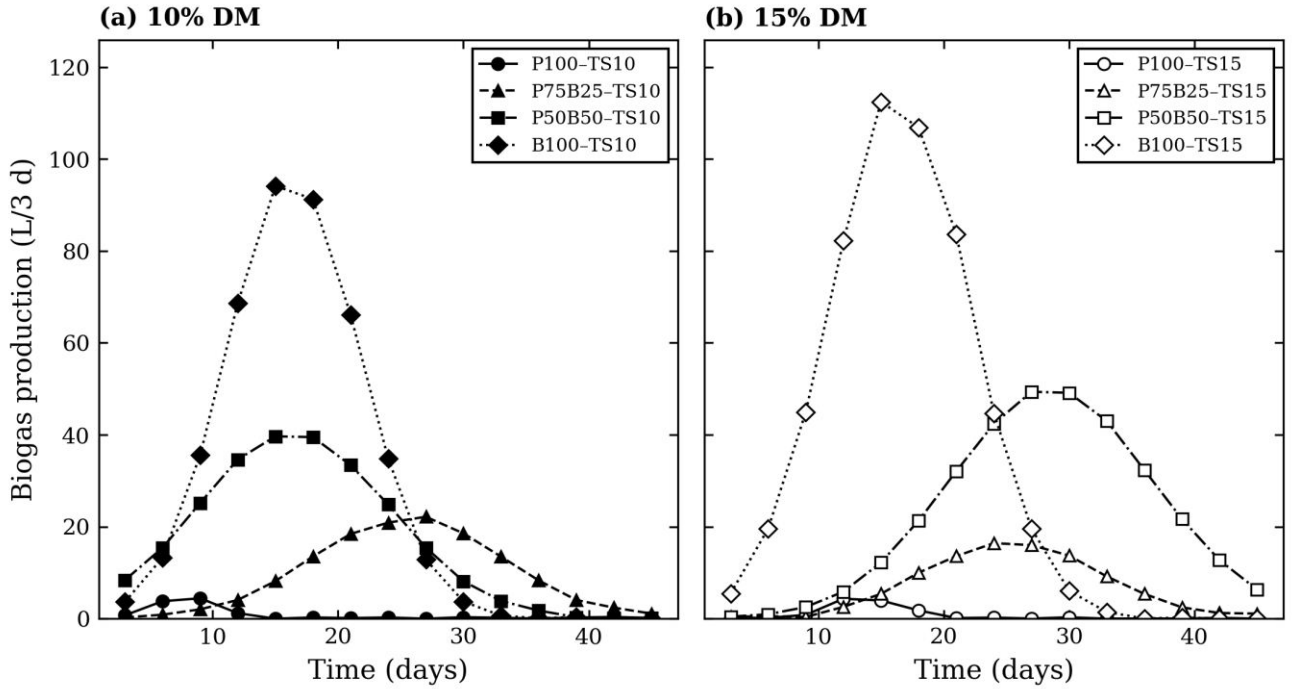
Indeed, cattle manure would have diluted medium acidity not only through its high pH (pH = 8.25) but also through strong buffering capacity. Studies have shown that anaerobic digestion system stability is improved during co-digestion with bovine manure due its high buffering capacity (J. Zhang *et al.,* 2017).

Figure.1 Cumulative biogas production curves



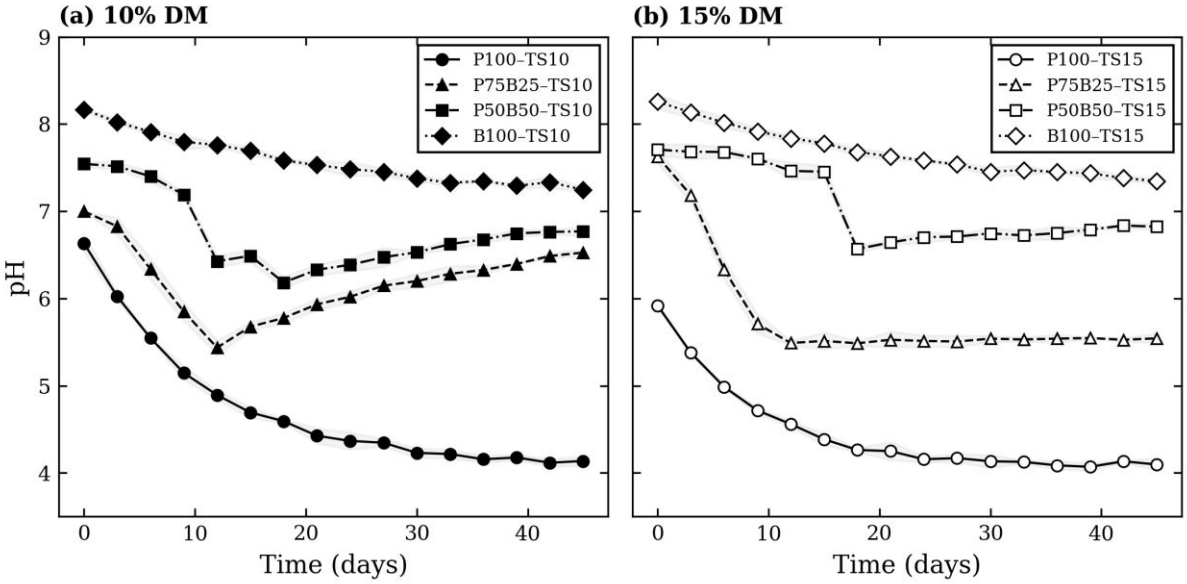
P100 = 100 % pulp; P75B25 = 75 % pulp + 25 % manure; P50B50 = 50 % pulp + 50 % manure; B100 = 100 % manure; TS10 = 10 % dry matter; TS15 = 15 % dry matter.

Figure.2 Daily bigas productivity



P100 = 100 % pulp; P75B25 = 75 % pulp + 25 % manure; P50B50 = 50 % pulp + 50 % manure; B100 = 100 % manure; TS10 = 10 % dry matter; TS15 = 15 % dry matter.

Figure.3 pH evolution during anaerobic digestion



P100 = 100 % pulp; P75B25 = 75 % pulp + 25 % manure; P50B50 = 50 % pulp + 50 % manure; B100 = 100 % manure; TS10 = 10 % dry matter; TS15 = 15 % dry matter.

Table.1 Mass composition of each digester

Treatment	Neem pulp (kg DM)	Cattle manure (kg DM)	Pulp (kg FM)	Fumier (kg FM)	Inoc. (kg FM)	Water (L)	Total (kg)	Target DM (%)
P100-TS10	14.15	0.00	75.67	0.00	16.7	74.33	166.7	10
P100-TS15	22.49	0.00	120.27	0.00	16.7	29.73	166.7	15
P75B25-TS10	10.61	3.54	56.74	13.01	16.7	80.25	166.7	10
P75B25-TS15	16.87	5.62	90.21	20.66	16.7	39.13	166.7	15
P50B50-TS10	7.08	7.08	37.86	26.03	16.7	86.11	166.7	10
P50B50-TS15	11.25	11.25	60.16	41.35	16.7	48.49	166.7	15
B100-TS10	0.00	14.15	0.00	52.02	16.7	97.98	166.7	10
B100-TS15	0.00	22.49	0.00	82.68	16.7	67.32	166.7	15

DM: calculated dry matter; FM: weighed fresh masse; Inoc.: inoculum. Water value include water provided by fresh substrates. Value are rounded to two decimals places. P100 = 100 % pulp; P75B25 = 75 % pulp + 25 % manure; P50B50 = 50 % pulp + 50 % manure; B100 = 100 % manure; TS10 = 10 % dry matter; TS15 = 15 % dry matter.

Table.2 Physicochemical properties of anaerobic digestion substrates

Substrate	DM (%)	pH	TOC(% DM)	N (% DM)	C/N	Total sugars (% DM)
Neem pulp	18.20 ± 0.36	5.80 ± 0.04	41.98 ± 0.44	1.99 ± 0.03	21.31 ± 0.18	69.77 ± 2.31
Cattle manure	27.23 ± 0.06	8.25 ± 0.11	36.62 ± 0.37	1.61 ± 0.06	21.94 ± 0.43	-
Inoculum	15.13 ± 0.32	7.23 ± 0.08	31.25 ± 0.47	1.79 ± 0.08	17.70 ± 0.84	-

DM: dry matter; TOC: total organic carbon; N: total nitrogen; C/N: carbon-to-nitrogen ratio. Values are expressed as mean ± standard deviation (n=3). (-) indicates not measured.

Table.3 Biogas production and specific yields

Treatment	$V_{cumulative}$ (L)	Yield MS (L.kg^{-1} DM)	Yield MO (L.kg^{-1} MO)
P100-TS10	12.2 ± 0.2^{g}	0.86 ± 0.01^{g}	1.18 ± 0.02^{g}
P100-TS15	12.2 ± 1.4^{g}	0.54 ± 0.06^{g}	0.74 ± 0.08^{g}
P75B25-TS10	138.4 ± 2.3^{e}	9.78 ± 0.16^{e}	13.86 ± 0.23^{e}
P75B25-TS15	98.5 ± 5.2^{f}	4.38 ± 0.23^{f}	6.21 ± 0.33^{f}
P50B50-TS10	250.8 ± 13.1^{d}	17.72 ± 0.92^{c}	26.14 ± 1.37^{c}
P50B50-TS15	332.4 ± 5.0^{c}	14.78 ± 0.22^{d}	21.80 ± 0.33^{d}
B100-TS10	425.2 ± 18.1^{b}	30.05 ± 1.28^{a}	48.24 ± 2.06^{a}
B100-TS15	527.4 ± 27.6^{a}	23.45 ± 1.23^{b}	37.64 ± 1.97^{b}

Values are expressed as mean ± standard deviation (n=3). Values followed by different letters are significantly different (Tukey HSD, α = 0.05). $V_{cumulative}$: cumulative biogas volume over 45 days; Yield DM: specific yield on dry matter basis; Yield OM: specific yield on organic matter basis; P100 = 100 % pulp; P75B25 = 75 % pulp + 25 % manure; P50B50 = 50 % pulp + 50 % manure; B100 = 100 % manure; TS10 = 10 % dry matter; TS15 = 15 % dry matter.

Thus, the biogas production gradient followed the order P100 < P75B25 < P50B50 < B100. Beyond stabilizing reaction medium pH, cattle manure would have diluted the antimicrobial and inhibitory effects of bioactive compounds. This synergy would result from a set of coupled effects including improved nutritional balance, toxic compound dilution, increased buffering capacity, and co-metabolism-based detoxification (Zhang *et al.,* 2021). Increased biogas production with increasing cattle manure incorporation rate would also be due to supplemental nutrient supply and especially microorganisms to the reaction medium. Cattle manure, dominated by phyla Firmicutes, Bacteroidetes, Lentisphaerae, Protobacteria, and Verrucomicrobia, as well as methanogenic archaea such as *Methanocorpusculum* (Kadam *et al.,* 2024), is recognized for its microbial diversity facilitating initiation and stability of anaerobic digestion processes (Kadam *et al.,* 2024). Anaerobic co-digestion thus generates synergistic interactions through nutrient balancing, inhibitory compound dilution, and increased microbial diversity (Kadam *et al.,* 2024).

Dry matter content (DM) constitutes a determining operational parameter in anaerobic digestion, influencing reaction medium properties and biogas production. In the present study, two dry matter contents (TS10 and TS15) were evaluated for each co-digestion ratio. With pulp alone, biogas production obtained for both contents was very low with cumulative volume of 12.2 L ±0.2 for TS10 and 12.2 L ±1.4 for TS15. With cattle manure alone, TS15 enabled higher biogas production (527.4 L ±27.6 versus 425 L ±18.1). For co-digestion, reduced biogas production was observed with TS15 when neem pulp incorporation rate was high, revealing an interaction between DM content and mixing ratio. Thus for P75B25, cumulative biogas volume was 1138.4 L ±2.3 for TS10 versus 98.5 L ±5.2 for TS15, whereas for P50B50, cumulative volume obtained was 250.8 L ±13.1 for TS10 versus 332.4 L ±5.0 for TS15. This shows that high dry matter content (TS15) is beneficial for cattle manure alone but problematic with pulp alone, and that at low dilution with P100 and P75B25, it can worsen inhibition unlike P50B50 and B10. These contrasting results according to ratio highlight a significant interaction between DM content and cattle manure proportion. This interaction is explained by several mechanisms identified in the literature. Indeed high dry matter content increases medium viscosity, limiting microorganism access to substrates. Moreover, bioactive compounds concentration in neem pulp increases proportionally to incorporation rate in the mixture, worsening methanogen inhibition. These results agree with those of Abbassi-Guendouz *et al.,* (2012) who report that increasing solids content increases viscosity, which makes mixing difficult and limits diffusion of soluble substrates and nutrients toward microorganisms. Furthermore, according to Gao *et al.,* (2023), high dry matter content reduces water mobility and promotes volatile fatty acid accumulation, leading to pH decline detrimental to methanogens. Although VFA were not measured in our study, acidification observed in P100 digesters (pH 4.10-4.14) corroborates this mechanism. Also, microbial community structure is likely modified in high dry matter media. In this regard, (Wang *et al.,* 2020) report that dry matter content modifies microbial community structure. Acetoclastic methanogens, predominant in liquid media, are progressively replaced

by hydrogenotrophic methanogens in high-solids media, the latter being more tolerant to high volatile fatty acid concentrations. Interactions observed between dry matter content and co-digestion ratio are consistent with observations of Wang *et al.,* (2020), who studied co-digestion of food waste with pig slurry.

These authors showed that a 1:1 co-digestion ratio combined with dry matter content between 5 % and 15 % enabled pH stabilization through the high buffering capacity of slurry, leading to better biogas production. Beyond dry matter content, tropical ambient temperature conditions (29-37°C) under which our experiment was conducted also played a determining role.

The experiment was conducted under tropical ambient temperature conditions ranging between 29 and 37°C, without thermal control system. This temperature range, although slightly below standard mesophilic temperatures (35-38°C), nevertheless falls within the extended mesophilic range (20-45°C). Under these conditions, biogas volumes obtained after 45 days of anaerobic digestion for P50B50-RS15 were 332.4 L and 527 L for B100-TS15. These results are consistent with those of Deago *et al.,* (2023), who demonstrated the feasibility of anaerobic digestion under tropical conditions in Panama (ambient temperatures 32.4-34.1°C), with acceptable methane production even at 25°C. Despite temperature fluctuations, biogas volumes produced demonstrate the feasibility of anaerobic digestion at ambient temperature in tropical environments. This approach presents the advantage of energy savings, requiring no heating. However, biogas production is subject to certain variability due to daily temperature fluctuations (Deago *et al.,* 2023). Ultimately, tropical ambient conditions offer a relevant compromise between technical simplicity and performance. Beyond the operational conditions discussed, several methodological limitations of our study deserve to be highlighted.

Study limitations

Despite the promising results obtained, several methodological limitations must be acknowledged. On the analytical level, several key parameters were not measured. First, the absence of chromatographic quantification of neem bioactive compound (azadirachtin, salanine, nimbin) limited our ability to precisely quantify inhibitory concentrations. Second, volatile fatty acids were not directly measured, although observed acidification (pH 4.10-4.14 for pulp alone) strongly suggest their accumulation. Third, the absence of methanogenic analyses did not allow confirmation of hypothesized microbial community changes.

In conclusion, this study aimed to evaluate energy valorization of neem pulp, a by-product of biopesticide production, through anaerobic digestion in co-digestion with cattle manure. The challenge was to transform a potentially environmentally problematic waste into a renewable energy resource. Results demonstrate that neem pulp alone completely inhibits methanogenesis due its high bioactive compound content (azadirachtin, salanine, nimbin). However, co-digestion with cattle manure enables progressive alleviation of this inhibition through the buffering capacity of cattle manure, toxic compound dilution, and contributed microbial diversity. The optimal ratio identified is P50B50 (50 % pulp, 50 % manure) at 15 % dry matter content, generating a biogas volume of 332.4 L over 45 days. Experimentation under tropical ambient temperature conditions (29-37°C) confirmed the technical feasibility of this approach without heating system. This study provides an original contribution to the literature by demonstrating for the first time the feasibility of neem pulp anaerobic digestion through controlled co-digestion. It identifies methanogenic inhibition mechanisms and strategies for alleviating this inhibition, while highlighting the importance of the interaction between co-digestion ratio and dry matter content. Moreover, its confirms the applicability of this approach under tropical conditions without thermal control, thereby reducing energy costs.

In perspective, notably, characterization of neem bioactive compounds by liquid chromatography coupled with mass spectrometry (HPLC-MS) would enable precise quantification of toxicity thresholds and optimization of dilution ratios. Furthermore, exploration of neem pulp detoxification pretreatments could improve their energy valorization. This work thus demonstrates the feasibility of sustainable energy valorization of biopesticide waste in tropical environments.

Authors' Contributions

MEMOKO Djalalou-Dine: study design, data collection, analysis, interpretation, writing, and revising the article. NENONENE Yawo Amen: study design, supervision, critically reviewing, final approval of the submitted version. AMENUTI Yao Félicité: study design, data analysis, and interpretation.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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