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Research article

Optimization of substrate mixing ratios and conditions for enhanced Biochemical Methane Potential (BMP) in small-scale biogas digesters

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Abstract: Biogas production through anaerobic digestion (AD) provides a dual solution to addressing energy scarcity and managing organic waste. However, performance strongly depends on substrate type, composition, and mixing ratios. This study evaluated the biochemical methane potential (BMP) and cumulative biogas yield (CBY) of five substrates—cow dung, swine manure, poultry droppings, food waste, and crop residues—both individually and in mixed ratios. Substrates were characterized for total solids (TS), volatile solids (VS), and pH, and performance was assessed over 15 days under mesophilic conditions. Results showed that individual substrates varied widely: food waste exhibited the highest BMP per g VS (350 ± 20 mL CH_4/g VS) but low CBY due to acidic inhibition, while crop residues, despite high TS (22.12%), produced limited methane from recalcitrant lignocellulose. In contrast, co-digestion of cow dung, swine manure, and poultry droppings in a 1:1:1 ratio achieved the most stable pH (6.9–7.3), moderate TS (11.56%), VS (63.72%), and the highest CBY (2.92 ± 0.03 m^3) with BMP of 380 ± 14 mL CH_4/g VS. These findings confirm that balanced co-digestion optimizes nutrient profiles, enhances stability, and maximizes methane yield, offering practical guidelines for efficient small-scale biogas systems.

Keywords: biogas production; organic waste; substrate; renewable energy; efficiency

1. Introduction

Biogas production has emerged as a pivotal component in the renewable energy landscape, offering a sustainable alternative to conventional fossil fuels and contributing to effective waste management. The process, which involves the anaerobic digestion of organic materials by microorganisms, generates biogas, a mixture of methane (CH_4), carbon dioxide (CO_2), and trace gases [1–3]. This renewable energy source not only reduces reliance on non-renewable energy but also mitigates greenhouse gas emissions and promotes environmental sustainability [4,5].

Despite its potential, the efficiency and effectiveness of biogas production can be significantly influenced by various factors, including substrate composition, process conditions, and system design [6,7]. Optimal biogas yield and quality depend on the careful management of these factors, making it essential to understand the interplay between different variables in the digestion process.

The primary goal of this study was to investigate the impact of different substrate types and compositions on biogas production. By examining these variables, the study aims to identify optimal conditions for maximizing biogas production and improving overall system performance.

Existing research has established foundational knowledge in the field of biogas production, but there remains a need for more nuanced insights into how specific substrate combinations influence the anaerobic digestion process. This study seeks to address this gap by providing a detailed analysis of substrate effects and offering practical recommendations for enhancing biogas production efficiency.

Furthermore, the research will contribute to a broader understanding of biogas system optimization, providing valuable insights for both small-scale and large-scale applications. By exploring the relationships between substrate composition, process parameters, and biogas yield, this study advances the field and supports the development of more effective and sustainable biogas production systems.

2. Literature review

Biogas production through anaerobic digestion (AD) has gained significant global interest because it combines renewable energy generation with on-site organic waste management and greenhouse gas reduction [8,9]. The performance of AD, measured by cumulative biogas yield (CBY), biochemical methane potential (BMP), and gas quality (CH_4/CO_2), is influenced by both substrate properties and operational conditions. Therefore, substrate selection and mixture design are crucial for maximizing energy recovery and maintaining process stability.

A wide variety of organic wastes have been examined as AD feedstocks. Manures (from cows, pigs, and poultry), food wastes, and crop residues are the most common in the literature due to their availability and organic content. Cow dung is frequently used because of its relative stability, availability, and generally favorable carbon-to-nitrogen ratio (C:N), which promotes balanced microbial growth in many small-scale digesters [10,11]. Swine manure offers significant biogas potential but often contains high nitrogen levels that can produce free ammonia under certain conditions, inhibiting methanogenesis if not properly managed [12–14]. Poultry droppings are nutrient-rich and can speed up digestion, but their high nitrogen and phosphorus levels require balancing to prevent inhibitor formation or nutrient imbalances in the digester [14,15]. Food waste generally contains highly biodegradable organics and therefore exhibits high BMP per unit of volatile solids, but its variable composition and high acidogenesis potential can lead to temporary process instability and inconsistent CBY across trials [16–18]. Crop residues are often rich in total solids and volatile solids but are mainly composed of lignocellulosic, resistant material, resulting in low BMP unless they undergo pretreatment [16–18].

A substantial body of research shows that co-digestion (mixing substrates) enhances AD performance by balancing nutrients, diluting inhibitors, and providing complementary hydrolysis kinetics [19–21]. Meta-analyses and systematic reviews also indicate co-digestion as a reliable way to boost methane yields and process stability across various feedstock mixes [22–24]. Empirical evidence supports that specific pairings (e.g., food waste + cow dung) reduce digestion time and increase yield [25], and that mixing lipid-rich or nitrogen-rich substrates with carbon-rich material can lower ammonia inhibition while boosting methane production [26]. Ratio optimization studies reveal that ideal proportions are context-dependent: the optimal ratio depends on feedstock chemistry, inoculum, retention time, and operating temperature [27]. Recent targeted studies have investigated statistical optimization of pretreatment and mixture ratios for cattle manure co-digested with food waste and pig manure, demonstrating that optimized pretreatment and mixture design can significantly increase yields [28].

Beyond feedstock composition, various operational factors significantly influence digestion results: temperature range (mesophilic 35–40°C versus thermophilic 50–60°C), hydraulic/solids retention time (HRT/SRT), pH, substrate loading rate, and reactor design [29–31]. The origin of microbial inoculum and the substrate-to-inoculum ratio also substantially impact lag phase, stability, and conversion efficiency. Studies show that inocula from stable dairy or manure digesters can speed up startup and enhance the breakdown of otherwise resistant feeds [32,33]. Pretreatment methods (mechanical, thermal, chemical, biological) for lignocellulosic or fat-rich wastes can notably increase BMP by boosting hydrolysis rates; recent reviews compile these advances and their implications for co-digestion [34,35]. Kinetic modeling and optimization studies further reveal how feedstock ratios and retention times interact to influence volumetric productivity and methane recovery [36].

Inhibitory compounds (free ammonia, sulfides, antibiotics, heavy metals, salts) can suppress methanogens; co-digestion dilutes inhibitors and can reduce their inhibitory effects if balanced with buffering substrates [12–14]. Conversely, trace elements (Fe, Ni, Co, Mo, Se) are essential for methanogenic enzyme systems; their deficiency can limit conversion even when bulk C and N are sufficient. Recent strategies, such as adding biochar, have been proposed to both adsorb inhibitors and create microhabitats for microbes; early results suggest biochar-enhanced AD can increase biomethane yields and offer agronomic co-benefits [37].

Energy value is determined not only by CBY but also by methane concentration. Gas quality depends on the balance between acidogenesis/acetogenesis and methanogenesis, hydrogen partial pressure, and CO_2 production during hydrolysis and fermentation. Stable, well-balanced digestion that encourages rapid conversion of Volatile Fatty Acids (VFA) usually results in higher and more consistent CH_4 levels. Research on sulfadiazine and other environmental antibiotics shows that pollutants can impact microbial activity and, consequently, gas composition, highlighting the importance of monitoring both the quantity and quality of biogas in co-digestion experiments [38].

Although several single-feed and co-digestion studies exist, important gaps remain. Many studies report BMP or CBY alone; fewer incorporate pH dynamics, TS/VS characterization, BMP per gram of VS, and daily yield kinetics across multiple substrate ratios within a single experimental framework. This study addresses these gaps by simultaneously reporting dynamic pH, TS, VS, CBY, and BMP for individual feeds and multiple co-digestion ratios (including 1:1:1, 1:2:1, 1:1:2, 2:1:1). It also uses repeated-measures ANOVA and Tukey HSD on daily yield data to rigorously compare temporal performance. While many optimization studies focus on either large-scale systems or specific pretreatments, fewer provide systematic ratio testing tailored to small-scale digesters with practical retention times (15 days in this study). The comparison of common rural substrates (cow dung, swine manure, poultry droppings, food waste, crop residues) under conditions relevant to household or community digesters is therefore novel and highly applicable to practitioners.

Although past meta-analyses and kinetic studies have shown ratio effects [28,6], our study explicitly connects TS and VS fractions, pH trajectories, and BMP per g VS to realized CBY over time. This provides mechanistic insight into why the 1:1:1 mix produced better outcomes, including nutrient balancing, buffering, dilution of inhibitors, complementary hydrolysis kinetics, and microbial diversity. This mechanistic integration, supported by statistical testing of daily yields, clarifies the empirical co-digestion literature. Building on findings that inoculum source and pretreatment influence outcomes [34,39,40], the study places results in the practical context of typical small-scale inocula and minimal pre-treatment, making conclusions immediately relevant to low-input settings and highlighting pretreatment and inoculum selection as opportunities for enhancing performance.

Considering the points above and the mixed evidence on optimal ratios, a controlled experimental comparison of commonly available manures and organic wastes, evaluating TS, VS, pH dynamics, BMP per g VS, and CBY across various ratios and days, offers significant practical value. The study design aligns with recent recommendations to combine laboratory BMP assays, short-term digester trials, and statistical optimization to identify robust, field-applicable co-digestion strategies [35–37]. Furthermore, by not relying solely on pretreatment or exotic additives, the study tests combinations that are feasible for low-resource, small-scale settings.

Overall, this work advances the AD literature by offering an integrated, statistically rigorous, and mechanism-focused comparison of small-scale co-digestion ratios using locally relevant substrates. It connects gaps between BMP assays, substrate characterization (TS/VS), and temporal production dynamics, and directly guides operators on practical co-digestion strategies that optimize yield, stability, and feasibility.

3. Materials and methods

3.1. Study area

The study was carried out in Nakisunga Parish, Nakasunga Sub-County, Mukono District, a neighbourhood of Kampala, the capital of Uganda. The district is located at coordinates 0.2835° N, 32.7633° E, with a temperature range varying from 17°C to 28°C. This district has roughly 140 people per square kilometre, and the neighbourhood is highly populated [41].

3.2. Determination of Total Solid (TS) and Volatile Solid (VS)

Clinical Five feedstock samples were collected, including swine manure, kitchen scraps, cow dung, chicken droppings, and crop residues. Each sample was placed in a polythene bag labeled clearly and sealed properly to prevent contamination. After four days of sun drying, the samples were crushed into powder using a crusher and pestle, then sieved and dried again for one day. The total solids (TS) were determined following ASTM D5907-18, “Standard Test Methods for Filterable Matter (Total Dissolved Solids) and Nonfilterable Matter (Total Suspended Solids) in Water,” and the APHA Standard Methods for the Examination of Water and Wastewater (2540 B, Total Solids Dried at 103–105 °C) [42,43]. The materials used included a crucible, laboratory oven, desiccator, electronic precision balance, dish tongs, wash bottles, and muffle furnace.

Procedure:

i. A clean crucible was dried in an oven at 105 °C for 1 hour, cooled in a desiccator, and weighed.
ii. 15 grams of homogenized substrate sample (consistent with ASTM and APHA protocols) were placed in the crucible.
iii. The sample was dried at 105 °C in the oven until a constant weight was reached (minimum of 1 hour, usually 2–4 hours depending on sample type).
iv. The crucible and residue were cooled in a desiccator to room temperature and weighed.

The percentage total solids (TS) were calculated [44–46]:

$$\%TS = \frac{m_{dry}}{m_{wet}} \times 100 \quad (1)$$

Where: m_{dry} is dry mass after drying at 105 °C (g) and m_{wet} is initial wet mass before drying (g)

$$TS(g) = m_{wet} \times \frac{\%TS}{100} \quad (2)$$

The volatile solids (VS) were determined following ASTM D2974-20, "Standard Test Methods for Moisture, Ash, and Organic Matter of Peat and Other Organic Soils" and APHA 2540 E (Fixed and Volatile Solids Ignited at 550 °C) [47,48].

Procedure:

i. The dried residue from TS determination was placed in a muffle furnace at 550 ± 25 °C.
ii. The crucible was held at this temperature for 30–60 min.
iii. The crucible and remaining ash were cooled partially in air, then transferred to a desiccator for final cooling and weighed.

The percentage volatile solids (VS) were calculated [44,49]:

$$\%VS = \frac{m_{dry} - m_{ash}}{m_{dry}} \times 100 \quad (3)$$

Where: m_{dry} is dry mass after drying at 105 °C (g) and m_{ash} is mass remaining after ignition at 550 °C.

$$VS(g) = TS \times \frac{\%VS}{100} \quad (4)$$

3.3. Biogas Yield and pH Monitoring

The experimental setup for biogas yield and pH monitoring consisted of:
Digester chamber: 20-L plastic bottle, airtight sealed.
Gas storage: Motorbike inner-tube serving as gas holder.
Inlet pipe: For slurry feeding.
Outlet pipe: For slurry removal and gas flow.

On/off valve, pipe plugs, adhesives: To prevent leakage and control gas release.
Funnel: For charging feedstock.
Temperature bath: Thermostatically controlled water bath (36 ± 1 °C).
Gas meter: For daily measurement of biogas yield, calibrated by water displacement at start and end.
Organic substrates: Cow dung, swine manure, poultry droppings, food wastes, crop residues, and mixtures.
Water: For slurry preparation at 1:1 (w/v) substrate-to-water ratio.
The experimental setup is shown in Figure 1.

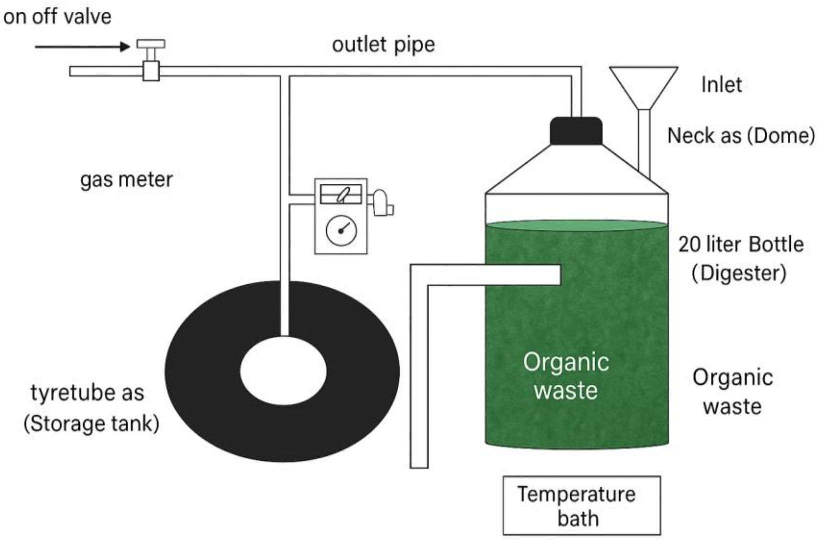

Figure 1. Experimental setup.

Each substrate (2 kg, crushed and homogenized) was mixed with distilled water in a 1:1 ratio to form slurry. The slurry was introduced into the 20-L digester through the inlet pipe with a funnel. The digester was then sealed airtight to maintain anaerobic conditions. The digester was immersed in a thermostatically regulated water bath maintained at 36 °C (mesophilic range). An immersion heater automatically switched on when the temperature dropped below 36 °C and off when the set temperature was restored, ensuring a narrow fluctuation range (±1 °C). Each substrate was digested separately for 15 days.

Biogas generated was conveyed via the outlet pipe into a motorbike inner-tube gas holder, which inflated as gas accumulated. The daily gas yield was measured with a gas meter, and the cumulative biogas yield (CBY) was calculated using Equation (5):

$$CBY = \sum_{k=1}^{n} d \tag{5}$$

Where: *d* is the daily biogas yield (m^3) and *n* is the number of days in the retention period.

Digested slurry was expelled through the outlet port and collected as biofertilizer.

Alongside gas measurement, the digestate pH was dynamically monitored using the electrometric method (APHA 4500-H^+ B, APHA 2017) [50,51]: A calibrated glass electrode pH meter was used, standardized daily with pH 4.00, 7.00, and 10.00 buffers at the operating temperature (35 °C). pH was measured directly in the slurry at, day 0 (initial inoculation), daily during the first 5 days (acidogenesis phase), and every 48 h thereafter until day 15. For each measurement, approximately 10 mL of slurry was withdrawn from the outlet port, measured immediately at 36 °C, and logged with reactor ID.

In addition to single substrates, co-digestion was carried out with cow dung, swine manure, and poultry droppings at mixing ratios of 1:1:1, 1:2:1, 1:1:2, and 2:1:1 (maintaining total feedstock of 2 kg). Gas yield was measured daily and CBY tabulated. The three substrates cow dung, swine manure, and poultry droppings, were selected for co-digestion because they are among the most abundant livestock wastes available locally and based on their biochemical characteristics and preliminary screening results.

Replication and Controls

Single substrates: n = 3 replicates per substrate (18 total).
Co-digestion ratios: n = 3 replicates per ratio.
Blank controls: Water-only reactors (n = 3) to confirm no background gas and rule out leaks.
Positive control: Cellulose (n = 3) to confirm inoculum activity (for BMP runs).
Quality Control and Data Treatment
Gas meters were calibrated with water displacement at start and end.
pH meters were calibrated daily with standard buffers.
Data are reported as mean ± SD across replicates.
Statistical differences between groups (e.g., CBY of different substrates/ratios) were evaluated using one-way ANOVA followed by Tukey's HSD test (α = 0.05), via SPSS 30.

3.4. Biochemical Methane Potential (BMP)

The biochemical methane potential (BMP) test was performed to determine the ultimate methane yield of the selected substrates under anaerobic digestion. The BMP assay was carried out according to established protocols (VDI 4630; ISO BMP methods) [52,53], with modifications suited to the laboratory conditions. Each BMP test included the following reactors:

Inoculum blanks (n = 3): Reactors containing inoculum only, with no substrate, to quantify background gas production from the inoculum.

Positive control (n = 3): Reactors containing cellulose as a standard substrate, to verify inoculum activity and ensure the validity of the BMP test.

Substrate reactors (n = 3 per substrate): Reactors containing inoculum plus each test substrate at a constant substrate-to-inoculum (S/I) ratio.

This replication ensures statistical reliability of the results, while the inclusion of inoculum blanks and cellulose controls allows correction for background methane and verification that the inoculum is active.

All BMP reactors were incubated at mesophilic temperature (36 ± 1 °C) in a temperature-controlled water bath. Reactors were sealed airtight to maintain anaerobic conditions. Gas production was measured daily, and pH was monitored dynamically at different time intervals following the APHA 4500-H^+ B protocol (APHA, 2017). Daily methane volumes from the inoculum blanks were subtracted from those of the substrate reactors to obtain the net methane yield of each substrate. Methane production from the positive control (cellulose) confirmed inoculum activity and validated the BMP assay. Cumulative methane yields are reported as mean ± standard deviation (SD) across the triplicate reactors.

The theoretical methane potential was calculated using the Buswell equation, which relates methane yield to the volatile solids (VS) content of the substrate [54]:

$$BMP = 0.35 \times Vs \times (1 - e^{0.034 \times VS}) \quad (6)$$

Where: BMP is the biochemical methane potential (expressed in mL CH_4/g VS), V_S is the volatile solids content of the substrate (expressed in g/L), e is the base of the natural logarithm (approximately 2.71828).

4. Results

4.1. pH of sample substrates

Table 1 presents the pH of biodegradable organic wastes. Swine manure had an initial pH of 6.74 ± 0.05, which dropped slightly to a minimum of 6.40 ± 0.03 during days 2–3 before stabilizing and rising to 7.10 ± 0.04 by day 15. This near-neutral final pH was accompanied by a relatively high cumulative biogas yield (CBY) of 2.65 ± 0.02 m^3. Cow dung showed a slightly lower initial pH of 6.45 ± 0.04 and a minimum pH of 6.20 ± 0.02, reaching a final pH of 6.95 ± 0.03 at the end of digestion, with a CBY of 1.53 ± 0.02 m^3. Poultry droppings started with the highest initial pH (8.22 ± 0.06), decreased to 7.40 ± 0.05, and ended at 7.60 ± 0.04, producing 2.32 ± 0.03 m^3 of biogas. Food wastes had an initial pH of 6.40 ± 0.05, dropped to the lowest recorded pH (5.95 ± 0.04), and recovered to 6.85 ± 0.03 by day 15, but yielded the lowest CBY (1.22 ± 0.03 m^3). Crop residues had the most acidic initial pH (5.79 ± 0.03), with a minimum of 5.60 ± 0.02, but reached 6.70 ± 0.04 by day 15, producing 2.47 ± 0.02 m^3 of biogas. The mixed substrates (1:1:1 cow dung, swine manure, and poultry droppings) started near-neutral (7.20 ± 0.05), had a minimum pH of 6.80 ± 0.04, and ended at 7.30 ± 0.03, resulting in the highest CBY (2.92 ± 0.03 m^3) among all samples.

Table 1 highlights that pH dynamics are critical for successful anaerobic digestion. All substrates experienced an initial drop in pH within the first few days due to acidogenesis (organic acid accumulation), followed by a gradual rise as methanogens consumed acids and stabilized the system. Substrates that maintained pH close to neutral (6.8–7.3) by day 15, such as swine manure and mixed substrates, exhibited higher methane yields. Food waste and crop residues, which had more acidic starting conditions, produced lower CBY, likely due to partial inhibition of methanogens at lower pH. The co-digested mixture's near-neutral pH profile throughout digestion indicates improved buffering capacity and a more balanced microbial environment, which explains why it produced the highest biogas yield.

Table 1. Dynamic pH of biodegradable organic wastes during 15-day anaerobic digestion.

Material	Initial pH (Day 0)	Min pH (Day 2–3)	Final pH (Day 15)	CBY (m^3, 15 days)
Swine manure	6.74 ± 0.05	6.40 ± 0.03	7.10 ± 0.04	2.65± 0.02
Cow dung	6.45 ± 0.04	6.20 ± 0.02	6.95 ± 0.03	1.53± 0.02
Poultry droppings	8.22 ± 0.06	7.40 ± 0.05	7.60 ± 0.04	2.32± 0.03
Food wastes	6.40 ± 0.05	5.95 ± 0.04	6.85 ± 0.03	1.22± 0.03
Crop residues	5.79 ± 0.03	5.60 ± 0.02	6.70 ± 0.04	2.47± 0.02
Mixed	7.20 ± 0.05	6.80 ± 0.04	7.30 ± 0.03	2.92± 0.03

substrates*

Note: Mixed substrates are Cow dung, swine manure and poultry droppings in the ratios of 1:1:1.

4.2. ANOVA and Tukey HSD post-hoc test on the CBY values

Table 2 is a one-way ANOVA on the CBY values. It shows a very high F-value and a highly significant p-value ($p < 0.001$), indicating that there are statistically significant differences in methane yield among the different substrate types.

Table 2. One-way ANOVA on the CBY values.

Source of Variation	DF	F-Value	P-Value
Between Groups	5	2706.87	7.08×10^{-18}
Within Groups	12	–	–
Total	17	–	–

Table 3 is the Tukey HSD post-hoc results comparing all substrate pairs. Pairwise differences are statistically significant across all comparisons ($p < 0.001$).

Table 3. Tukey HSD post-hoc results comparing all substrate pairs.

Group 1	Group 2	Mean Diff	95% CI (Lower–Upper)	p-adj	Significant?
Cow dung	Crop residues	0.9280	0.8674 – 0.9885	< 0.001	Yes
Cow dung	Food wastes	-0.3108	-0.3714 – -0.2502	< 0.001	Yes
Cow dung	Mixed substrates	1.3854	1.3248 – 1.4459	< 0.001	Yes
Cow dung	Poultry droppings	0.7761	0.7155 – 0.8367	< 0.001	Yes
Cow dung	Swine manure	1.1201	1.0595 – 1.1806	< 0.001	Yes
Crop residues	Food wastes	-1.2388	-1.2993 – -1.1782	< 0.001	Yes
Crop residues	Mixed substrates	0.4574	0.3968 – 0.5180	< 0.001	Yes
Crop residues	Poultry droppings	-0.1519	-0.2125 – -0.0913	< 0.001	Yes
Crop residues	Swine manure	0.1921	0.1315 – 0.2527	< 0.001	Yes
Food wastes	Mixed substrates	1.6961	1.6356 – 1.7567	< 0.001	Yes
Food wastes	Poultry droppings	1.0869	1.0263 – 1.1474	< 0.001	Yes
Food wastes	Swine manure	1.4309	1.3703 – 1.4914	< 0.001	Yes
Mixed substrates	Poultry droppings	-0.6093	-0.6698 – -0.5487	< 0.001	Yes
Mixed substrates	Swine manure	-0.2653	-0.3258 – -0.2047	< 0.001	Yes
Poultry droppings	Swine manure	0.3440	0.2834 – 0.4046	< 0.001	Yes

4.3. Total solids

Table 4 presents the total solids of biodegradable organic wastes. Swine manure (A) had a total solids (TS) content of 8.10 ± 0.24%, corresponding to 1.215 ± 0.036 g TS from the 15 g sample, and produced a cumulative biogas yield (CBY) of 2.65 ± 0.02 m^3 over 15 days. Poultry droppings (B) exhibited a higher TS content of 11.82 ± 0.29% (1.773 ± 0.044 g TS), with a CBY of 1.53 ± 0.02 m^3, which was comparatively lower than swine manure. Cow dung (C) had the highest TS among the animal manures at 14.77 ± 0.52% (2.216 ± 0.078 g TS) and produced 2.32 ± 0.03 m^3 of biogas. Food wastes (D) contained the highest TS overall, 24.62 ± 0.85% (3.693 ± 0.128 g TS), yet showed the lowest biogas yield at 1.22 ± 0.03 m^3, indicating poor bioconversion efficiency under the given conditions. Crop residues (E) had a TS of 22.12 ± 0.67% (3.318 ± 0.101 g TS) with a CBY of 2.47 ± 0.02 m^3, showing a better yield than food wastes despite having slightly lower TS content. The co-digested mixture had a TS of 11.56 ± 0.21% (1.734 ± 0.032 g TS) and achieved the highest biogas yield (2.92 ± 0.03 m^3) among all treatments.

The results suggest that total solids content alone does not determine biogas yield. While food waste and crop residues had the highest TS content, they did not produce the highest CBY. In contrast, co-digestion yielded the highest methane potential, indicating that mixing substrates can improve nutrient balance, enhance microbial activity, and optimize biogas production. This finding supports the strategy of substrate co-digestion to achieve higher methane yields in small-scale biogas digesters compared to single substrates.

Table 4. Total solids solid of biodegradable organic wastes.

Sample (code)	Mass used (g)	%TS (mean ± SD)	TS (g) mean ± SD	CBY (m^3, 15 days)
Swine manure (A)	15.000	8.10 ± 0.24 %	1.215 ± 0.036 g	2.65± 0.02
Poultry droppings (B)	15.000	11.82 ± 0.29 %	1.773 ± 0.044 g	1.53± 0.02
Cow dung (C)	15.000	14.77 ± 0.52 %	2.216 ± 0.078 g	2.32± 0.03
Food wastes (D)	15.000	24.62 ± 0.85 %	3.693 ± 0.128 g	1.22± 0.03
Crop residues (E)	15.000	22.12 ± 0.67 %	3.318 ± 0.101 g	2.47± 0.02
Mixed (F)	15.000	11.56 ± 0.21 %	1.734 ± 0.032 g	2.92± 0.03

4.4. Volatile solids

Table 5 presents the volatile solids of biodegradable organic wastes. Swine manure (A) had a volatile solids (VS) content of 85.38 ± 1.50%, corresponding to 1.037 ± 0.036 g VS from the measured total solids, and produced a cumulative biogas yield (CBY) of 2.65 ± 0.02 m^3 over 15 days. Poultry droppings (B) exhibited a much lower VS fraction of 41.03 ± 2.00% (0.727 ± 0.040 g VS), resulting in a comparatively low CBY of 1.53 ± 0.02 m^3. Cow dung (C) had a VS content of 64.76 ± 1.80%, corresponding to 1.435 ± 0.064 g VS, and yielded 2.32 ± 0.03 m^3 of biogas, reflecting moderate biodegradability. Food wastes (D) showed the lowest VS percentage (23.69 ± 1.20%) with 0.875 ± 0.054 g VS, and also the lowest CBY (1.22 ± 0.03 m^3), indicating a lower proportion of biodegradable organic matter. Crop residues (E) had a relatively high VS fraction (65.92 ± 1.70%) with 2.187 ± 0.087 g VS, leading to a CBY of 2.47 ± 0.02 m^3, which was higher than most individual substrates except for the mixture. The co-digested mixture (F) had a VS content of 63.72 ± 1.03% (1.105 ± 0.027 g VS) and achieved the highest CBY (2.92 ± 0.03 m^3) among all treatments.

Table 5 shows that volatile solids (VS) content is a key determinant of biogas production, since VS represent the organic fraction that microbes can degrade anaerobically. Swine manure, despite having lower TS than crop residues, had the highest VS percentage, which explains its relatively high CBY. Conversely, food wastes had the lowest VS percentage, aligning with its poor biogas performance. Most importantly, the co-digestion mixture (F) delivered the highest CBY despite having a VS content comparable to cow dung and crop residues, suggesting that the synergistic effect of co-digestion enhances biodegradability and methane potential beyond what individual substrates achieve. This reinforces the conclusion that substrate mixing improves process stability and overall methane yield in small-scale biogas digesters.

Table 5. Volatile solids of biodegradable organic wastes.

Sample (code)	TS (g) (from Table 1)	%VS (mean ± SD)	VS (g) mean ± SD	CBY (m^3, 15 days)
Swine manure (A)	1.215 ± 0.036 g	85.38 ± 1.50 %	1.037 ± 0.036 g	2.65± 0.02
Poultry droppings (B)	1.773 ± 0.044 g	41.03 ± 2.00 %	0.727 ± 0.040 g	1.53± 0.02
Cow dung (C)	2.216 ± 0.078 g	64.76 ± 1.80 %	1.435 ± 0.064 g	2.32± 0.03
Food wastes (D)	3.693 ± 0.128 g	23.69 ± 1.20 %	0.875 ± 0.054 g	1.22± 0.03
Crop residues (E)	3.318 ± 0.101 g	65.92 ± 1.70 %	2.187 ± 0.087 g	2.47± 0.02
Mixed (F)	1.734 ± 0.032 g	63.72 ± 1.03 %	1.105 ± 0.027 g	2.92± 0.03

4.5. Biochemical methane potential

Table 6 presents the sample substrates with their BMP. Swine manure (A) exhibited a biochemical methane potential (BMP) of 230 ± 12 mL CH_4/g VS, indicating moderate methane production from its volatile solids. Poultry droppings (B) had a higher BMP of 320 ± 15 mL CH_4/g VS, suggesting better methane-generating potential compared to swine manure. Cow dung (C) showed a BMP of 290 ± 10 mL CH_4/g VS, which is slightly lower than poultry droppings but higher than swine manure, reflecting its moderate biodegradability. Food wastes (D) displayed a high BMP of 350 ± 20 mL CH_4/g VS, highlighting that despite lower volatile solids content (from Table 3), it has a strong potential for methane production per gram of VS. Crop residues (E) had the lowest BMP at 180 ± 9 mL CH_4/g VS, indicating that their organic matter is less readily convertible to methane. The co-digested mixture (F) achieved the highest BMP of 380 ± 14 mL CH_4/g VS, demonstrating a clear synergistic effect of combining substrates, which enhances microbial activity and methane production efficiency beyond individual substrates.

Table 6. Substrates BMP.

Sample (code)	BMP (mL CH_4 / g VS) ± SD
Swine manure (A)	230 ± 12
Poultry droppings (B)	320 ± 15
Cow dung (C)	290 ± 10
Food wastes (D)	350 ± 20
Crop residues (E)	180 ± 9
Mixed (F)	380 ± 14

Table 6 shows that BMP per gram of volatile solids varies considerably among substrates, reflecting differences in biodegradability and nutrient composition. Food wastes, despite having lower VS fraction, show high BMP, likely due to a higher content of easily degradable carbohydrates and fats. Crop residues have the lowest BMP, suggesting high lignocellulosic content that resists anaerobic degradation. Co-digestion optimizes substrate balance, providing both nutrients and buffer capacity, resulting in the highest methane potential per unit VS. The higher BMP obtained from the 1:1:1 co-digestion of cow dung, swine manure, and poultry droppings is attributable to synergistic effects rather than the absolute TS or VS content. Mixing these substrates improves the overall C/N ratio, ensuring balanced nutrient availability for anaerobic microorganisms and reducing the risk of ammonia inhibition that may arise from poultry droppings alone. Co-digestion also dilutes inhibitory compounds and provides complementary organic fractions: cow dung contributes buffering capacity, swine manure supplies easily degradable organic matter, and poultry droppings add nitrogen-rich content that stimulates microbial growth. This balanced environment enhances microbial diversity and activity across hydrolytic, fermentative, and methanogenic pathways, leading to more efficient degradation of volatile solids and higher methane yields than those from individual substrates.

4.6. Optimal mixing ratios for co-digestion

Table 7 shows the daily and cumulative biogas yields from different co-digestion ratios of cow dung (C), poultry droppings (PD), and swine manure (SM) over a 15-day retention period. The ratios tested were 1:1:1, 1:2:1, 1:1:2, and 2:1:1. For the 1:1:1 ratio, daily biogas production started slowly (0.00–0.40 m^3/day in the first three days), peaked around day 6–7 (3.30 and 3.22 m^3/day), and gradually declined, resulting in a cumulative biogas yield (CBY) of 35.89 m^3 by day 15. The 1:2:1 ratio showed faster initial production (0.50–1.00 m^3/day) but had highly variable spikes (e.g., day 5, 144 m^3/day) and lower overall CBY (25.28 m^3) compared to the 1:1:1 ratio, suggesting instability in methane production. The 1:1:2 ratio had moderate daily yields with peaks around day 6–7 (220–300 m^3/day), giving a cumulative yield of 23.53 m^3. The 2:1:1 ratio displayed daily production that was relatively consistent but slightly lower than the 1:1:1 ratio, with a CBY of 22.25 m^3. Overall, the 1:1:1 co-digestion ratio consistently achieved the highest cumulative biogas yield over the 15-day period, indicating it is the most effective substrate balance among those tested. The other ratios produced lower CBY, likely due to either nutrient imbalance or inhibitory effects from an excess of a single substrate.

Table 7. Mixing ratios and their biogas yields.

Retention time (days)	Daily biogas for each ratio CW:PD:SM (m^3)				Cumulative biogas yield for each ratio (m^3)			
	1:1:1	1:2:1	1:1:2	2:1:1	1:1:1	1:2:1	1:1:2	2:1:1
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.50	0.30	0.40	0.00	0.50	0.30	0.40
3	0.40	1.00	0.98	0.97	0.40	1.50	1.28	1.37
4	1.54	1.40	1.00	1.28	1.94	2.90	2.28	2.65
5	2.98	144	158	170	4.92	4.34	3.86	4.35
6	3.30	200	220	200	8.22	6.34	6.06	6.35
7	3.22	298	300	294	11.44	9.32	9.06	9.29
8	3.19	3.00	3.18	2.72	14.63	12.32	12.24	12.01
9	3.15	2.50	2.71	1.58	17.78	14.82	14.95	13.59
10	3.11	2.40	2.45	1.74	20.89	17.22	17.40	15.33
11	3.08	2.44	1.97	1.60	23.97	19.66	19.37	16.93
12	3.05	1.58	1.00	1.67	27.02	21.24	10.37	18.60
13	3.00	1.44	0.97	1.23	30.02	22.68	21.34	19.83
14	2.95	1.40	1.05	1.42	32.97	24.08	22.39	21.25

15	2.92	1.20	1.14	1.00	35.89	25.28	23.53	22.25

The results highlight that balanced substrate mixing (1:1:1) optimizes microbial activity and ensures a stable anaerobic environment, leading to maximum biogas production. Ratios with higher proportions of a single substrate (e.g., 1:2:1 or 2:1:1) were less efficient, likely due to imbalanced carbon-to-nitrogen ratios, accumulation of inhibitory compounds, or uneven degradation rates. The daily production profiles indicate that peak biogas production occurred around days 6–7, after which yields gradually declined, reflecting the typical kinetics of small-scale anaerobic digestion.

4.7. Repeated Measures ANOVA and Tukey's HSD post-hoc test comparing daily biogas yields across substrate mixing ratios

Table 8 is the Repeated Measures ANOVA result for daily biogas yield across the different substrate mixing ratios. There is a highly significant effect ($p < 0.001$) of substrate mixing ratio on daily biogas yield. This means the choice of mixing ratio significantly influences methane production over the retention period.

Table 8. Repeated Measures ANOVA on Daily Biogas Yield.

Source	Num DF	Den DF	F Value	p-value
Ratio	3	42	12.70	0.000005

Table 9 is the Tukey's HSD post-hoc test comparing daily biogas yields across substrate mixing ratios. Pairwise differences are statistically significant across all comparisons ($p < 0.001$).

Table 9. Tukey's HSD Pairwise Comparisons daily biogas yields.

Comparison	Mean Diff	p-adj	95% CI Lower	95% CI Upper	Significant?
1:1:1 vs 1:1:2	-0.8240	< 0.001	-1.7673	0.1193	Yes
1:1:1 vs 1:2:1	-0.7073	< 0.001	-1.6507	0.2360	Yes
1:1:1 vs 2:1:1	-0.9093	< 0.001	-1.8527	0.0340	Yes
1:1:2 vs 1:2:1	0.1167	< 0.001	-0.8267	1.0600	Yes
1:1:2 vs 2:1:1	-0.0853	< 0.001	-1.0287	0.8580	Yes
1:2:1 vs 2:1:1	-0.2020	< 0.001	-1.1453	0.7413	Yes

5. Discussion

Our results indicate that co-digesting cow dung (C), swine manure (SM), and poultry droppings (PD) in a 1:1:1 ratio yielded the best overall performance across several metrics: the highest cumulative biogas yield (CBY: 2.92 ± 0.03 m^3 over 15 days for the mixed substrate), the highest biochemical methane potential (BMP = 380 ± 14 mL CH_4/g VS), and a nearly neutral, well-buffered pH trend (start ≈7.20, minimum≈6.80, end≈7.30). Individual substrates presented tradeoffs: food waste had a high BMP per g VS but a low VS fraction and low CBY; crop residues had high TS and VS but the lowest BMP per g VS (180 ± 9 mL CH_4/g VS). These findings demonstrate that substrate composition and mixing, rather than TS alone, determine overall digester performance.

The pH time-series for each substrate shows the typical acidogenesis to methanogenesis transition (initial drop during acid formation, followed by recovery as methanogens consume VFAs). Substrates that remained near-neutral pH by day 15 (swine manure final pH≈7.10; mixed substrate final pH≈ 7.30) produced the highest CBY and BMP. This supports the long-standing conclusion that neutral to slightly alkaline pH encourages methanogenic activity and higher methane yields. Extremes—such as very alkaline poultry start pH 8.22 or more acidic crop residues and food waste—were linked to reduced or unstable production, which aligns with previous reports that both low and high pH limit methanogen metabolism and community stability [29–31]. Maintaining buffer capacity (through co-substrates with higher alkalinity or incremental additions) helps prevent extended low-pH periods that can inhibit methanogenesis.

Our TS data show that the highest TS substrates (food wastes 24.62%, crop residues 22.12%) did not produce the highest CBY. Conversely, the mixed substrate with TS ≈ 11.56% produced the highest CBY. This supports the idea that moderate TS (≈10–15%) creates a favorable physical environment (sufficient free water for microbial mobility and mass transfer) while keeping organic content accessible, which aligns with the literature stating that higher TS can hinder microbial contact and mass transfer, reducing effective digestion [8,9]. Therefore, TS should be optimized rather than maximized: too high TS → limited microbial access/diffusion; too low TS may dilute substrates and decrease volumetric productivity.

VS represents the biodegradable organic fraction; our data show a positive relationship between VS content and CBY at both the substrate and mixture levels. Swine manure and crop residues had relatively high VS fractions, which corresponded with good CBY; the co-digested mixture combined a competitive VS fraction (≈63.72%) with other favorable properties such as buffering and balanced nutrients, resulting in the highest CBY and BMP. This aligns with previous findings that VS is a major predictor of methane potential when bioavailability and degradability are sufficient [3,7]. However, the percentage of VS alone does not tell the whole story: food wastes had a lower VS fraction in the measured TS but a high BMP (350 ± 20 mL CH_4/g VS), indicating that the quality of VS—such as ease of biodegradation and chemical composition—is just as important as its quantity.

BMP per g VS measures the innate convertibility of volatile organic matter to methane. Food waste had a high BMP per g VS (350 ± 20 mL CH_4/g VS), likely because it contains readily degradable carbohydrates, sugars, and lipids. However, it showed low CBY in the 15-day assays because its low VS fraction and/or process inhibition (transient acidity) limited total conversion within that retention time. Crop residues (low BMP) reflect the presence of recalcitrant lignocellulosic fractions. The co-digested mix combined fairly high VS with a substrate matrix that supported conversion through nutrient balance and buffering, delivering both high BMP and high cumulative yields. This confirms that co-digestion can enhance both the methane potential per VS and the overall cumulative yield by improving degradability and process stability [19,20].

The 1:1:1 mixture consistently performed the best. Mechanistic reasons supported by the current dataset include:

Nutrient balancing (C:N ratio and macro/micro nutrients): Different substrates vary in their C:N ratios, such as poultry droppings being high in nitrogen and crop residues high in carbon. A 1:1:1 mix helps bring the overall C:N ratio closer to the optimal range for methanogens (around 20–30:1), reducing nitrogen or carbon limitations and preventing ammonia inhibition caused by overly N-rich feeds. Balanced nutrients enhance microbial growth and enzyme production, increasing conversion rates [21].

Buffering and pH stabilization: Swine manure and cow dung typically offer alkalinity and minerals that buffer VFAs. The mixed substrate's pH trend (minimum ≈ 6.80, end ≈ 7.30) demonstrates stronger buffering than many single feeds, preventing prolonged acidification that can halt methanogenesis. Buffering reduces the amplitude and duration of harmful low pH events, allowing methanogens to recover more quickly [29–31].

Dilution of inhibitors and toxic compounds: Some substrates may contain inhibitory levels of ammonia, salts, antibiotics, or other antimicrobials. Mixing dilutes these inhibitors below toxic levels, reducing the inhibition of sensitive methanogenic populations. This aligns with the observed increased stability (more consistent daily production curve) for 1:1:1 ratios compared to those dominated by a single substrate.

Complementary substrate biodegradability and sequential feeding: Fast-degrading soluble organics (e.g., food waste in PD or SM) supply readily available substrates for hydrolytic and acidogenic bacteria, while slower-releasing organics (such as cow dung particulate matter) sustain methanogens over longer periods, smoothing production peaks and supporting higher overall yields.

Enhanced microbial diversity and syntrophic interactions: Each substrate carries unique microbial inocula and different fermentable compounds. Combining them expands ecological niches, fostering a richer community of hydrolyzers, syntrophs, and methanogens (both acetoclastic and hydrogenotrophic). Syntrophy—where fermenters and H_2-consuming methanogens cooperate—is more effective when substrates supply both electrons and electron sinks in balanced amounts. Increased microbial diversity boosts resilience to disturbances and can improve overall conversion efficiency [19,20].

Optimal solids and mass transfer conditions: The 1:1:1 mix resulted in TS ≈ 11.56%, within the 10–15% range specified in your pattern. This TS level enhances liquid-phase diffusion and microbe-substrate contact, improving hydrolysis and subsequent processes [8,9].

Overall, these mechanisms explain why the balanced mixture produced the highest BMP per g VS and the highest cumulative yields during the 15-day testing period.

Some single substrates display conflicting indicators (e.g., high BMP but low CBY). Food waste exemplifies this: it had high BMP per g VS but low CBY under the 15-day retention and experimental conditions. Reasons may include low VS content in the sample, such as fewer available grams in the reactor volume, transient inhibition (VFAs buildup or temporary low pH) limiting VS conversion within the retention time, or particle size and pretreatment needs. Some food fractions may require longer hydrolysis or mechanical/thermal pretreatment for full conversion. This underscores the difference between intrinsic potential (BMP/g VS) and actual performance (CBY over fixed retention time and load).

6. Operational recommendations

Adopt balanced co-digestion (1:1:1 C:SM: PD) where feasible. It yields the highest CBY and BMP while maintaining a stable pH [17,18,21]. Maintain total solids (TS) in the range of approximately 10–15% to ensure proper mass transfer and microbial contact (the mixed substrate TS is approximately 11.56%, which supports this) [8,9]. Monitor and regulate pH and buffering capacity. Keep the system near neutral to prevent methanogen inhibition, aiming for a final pH of about 6.8–7.3, as observed in the mixed substrate [25–27]. Measure gas composition (CH_4/CO_2) during scale-up—

total volume alone isn't enough to evaluate energy value and safety; periodically sample headspace gas using GC or portable sensors. Avoid dominance of a single substrate unless its chemistry is well understood and pretreated. Excessive amounts of one feed can lead to nutrient imbalance or inhibitor buildup; our tests with 1:2:1 and 2:1:1 ratios showed instability and lower CBY.

7. Limitations

Experiment Scale: Small-scale laboratory digesters were used in this study, which might not accurately reflect the conditions and complexities of large-scale biogas systems. As a result, the findings may have limited applicability to larger, industrial-scale processes, since factors like heat transfer, mixing efficiency, and substrate handling can differ significantly.

Specific Substance Types: The study focused on a particular group of substrates, such as cow dung, swine manure, chicken droppings, and food wastes. While these substrates are commonly used, the results may not apply to other types of organic waste or unusual substrates. Additionally, the effects of other factors, such as the addition of additives or different kinds of waste, were not examined.

Statistical Limitations: Although the study included comprehensive statistical analysis, the results are based on data from a limited number of experiments. Increasing the sample size and repeating the tests under different conditions could enhance the statistical power and reliability of the findings.

8. Conclusions

This study examined how to optimize substrate mixing ratios and conditions to boost biochemical methane potential (BMP) and cumulative biogas yield (CBY) in small- scale anaerobic digesters. Five biodegradable organic wastes were tested: swine manure, cow dung, poultry droppings, food waste, and crop residues, along with their co- digested mixtures. In all analyses- including pH dynamics, total solids, volatile solids, BMP, and daily yield patterns- the co- digestion of cow dung, swine manure, and poultry droppings in a 1: 1: 1 ratio consistently surpassed the performance of individual substrates.

Swine manure and cow dung showed good buffering capacity and moderate volatile solids levels, while poultry droppings offered higher nitrogen content and more readily degradable organic matter. Food waste, despite its high BMP per gram of volatile solids (350 ± 20 mL CH_4/g VS), produced the lowest CBY due to its low VS fraction and acidic nature that partly inhibited methanogens. Crop residues had high total solids (22.12%) and VS (65.92%) but low BMP (180 ± 9 mL CH_4/g VS), reflecting their resistant lignocellulosic makeup. In contrast, the 1: 1: 1 mixed substrate had moderate total solids at 11.56. 56%, a volatile solids fraction of 63. 72%, and a nearly neutral pH, leading to the highest CBY (2. 92 ± 0. 03 m^3) and BMP (380 ± 14 mL CH_4/g VS) among all treatments.

These findings show that total solids or volatile solids alone do not determine methane yield; instead, yield is maximized when substrates are mixed to balance nutrients, improve buffering, dilute inhibitors, and support diverse microbial communities. The excellent results of the 1: 1: 1 ratio demonstrate the synergistic effects of co-digestion, where combined microbial and chemical environments enable more complete and efficient substrate breakdown than single substrates.

The key message of this study is that balanced co- digestion (1: 1: 1 ratio of cow dung, swine manure, and poultry droppings) provides the best approach for increasing methane production in small-scale biogas systems. Operators should aim for a total solids content of about 10–15% and keep pH near neutral (6. 6.8–7.3. 3) to maintain stable methanogenesis conditions. These insights offer practical guidance for rural and small- scale biogas projects, showing that strategic substrate management can significantly boost energy extraction from organic waste while promoting sustainable waste handling and renewable energy efforts.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

Conflict of interest

The authors declare there is no conflict of interest.

References

1. David M, Uzorka A, Makeri YA (2022) Optimisation of a renewable energy system for rural electrification. *J Power Energy Eng* 10: 1–15. https://doi.org/10.4236/jpee.2022.1011001
2. Lee J, Hong J, Jeong S, et al. (2020) Interactions between substrate characteristics and microbial communities on biogas production yield and rate. *Bioresource Technol* 303: 122934. https://doi.org/10.1016/j.biortech.2020.122934
3. Vakalis S, Georgiou A, Moustakas K, et al. (2022) Assessing the effect of hydrothermal treatment on the volatile solids content and the biomethane potential of common reed (Phragmites australis). *Bioresour Technol Rep* 17: 100923. https://doi.org/10.1016/j.biteb.2021.100923
4. Djellouli N, Abdelli L, Elheddad M, et al. (2022) The effects of non-renewable energy, renewable energy, economic growth, and foreign direct investment on the sustainability of African countries. *Renewable Energy* 183: 676–686. https://doi.org/10.1016/j.renene.2021.10.066
5. Rather KN, Mahalik MK, Mallick H (2024) Do renewable energy sources perfectly displace non-renewable energy sources? Evidence from Asia–Pacific economies. *Environ Sci Pollut Res* 31: 25706–25720. https://doi.org/10.1007/s11356-024-32820-1
6. Dhull P, Lohchab RK, Kumar S, et al. (2024) Anaerobic digestion: Advance techniques for enhanced biomethane/biogas production as a source of renewable energy. *BioEnergy Res* 17: 1228–1249. https://doi.org/10.1007/s12155-023-10621-7
7. Ahmadi-Pirlou M, Mesri Gundoshmian T (2021) The effect of substrate ratio and total solids on biogas production from anaerobic co-digestion of municipal solid waste and sewage sludge. *J Mater Cycles Waste Manag* 23: 1938–1946. https://doi.org/10.1007/s10163-021-01264-x
8. Wang Z, He H, Yan J, et al. (2024). Influence of temperature fluctuations on anaerobic digestion: Optimum performance is achieved at 45°C. *Chem Eng J* 492: 152331. https://doi.org/10.1016/j.cej.2024.152331
9. Gao J, Li Z, Chen H (2023) Untangling the effect of solids content on thermal-alkali pre-treatment and anaerobic digestion of sludge. *Sci Total Environ* 855: 158720. https://doi.org/10.1016/j.scitotenv.2022.158720
10. Fetta MM, Ancha VR, Fantaye FK, et al. (2024) Experimental evaluation of biogas production from anaerobic co-digestion of cactus cladodes, cow dung, and goat manure. *Brazilian J Chem Eng*, 1–16. https://doi.org/10.1007/s43153-024-00437-z

11. Mazurkiewicz J (2022) Energy and economic balance between manure stored and used as a substrate for biogas production. *Energies* 15: 413. https://doi.org/10.3390/en15020413
12. Yellezuome D, Zhu X, Wang Z, et al. (2022) Mitigation of ammonia inhibition in anaerobic digestion of nitrogen-rich substrates for biogas production by ammonia stripping: A review. *Renewable Sustain Energy Rev* 157: 112043. https://doi.org/10.1016/j.rser.2021.112043
13. Xiao Y, Yang H, Zheng D, et al. (2022) Alleviation of ammonia inhibition in dry anaerobic digestion of swine manure. *Energy* 253: 124149. https://doi.org/10.1016/j.energy.2022.124149
14. Wang Y, Zhang Y, Li J, et al. (2021) Biogas energy generated from livestock manure in China: Current situation and future trends. *J Environ Manag* 297: 113324. https://doi.org/10.1016/j.jenvman.2021.113324
15. Rahman MA, Shahazi R, Nova SNB, et al. (2023) Biogas production from anaerobic co-digestion using kitchen waste and poultry manure as substrate—part 1: Substrate ratio and effect of temperature. *Biomass Conversion Biorefinery* 13: 6635–6645. https://doi.org/10.1007/s13399-021-01604-9
16. Chew KR, Leong HY, Khoo KS, et al. (2021) Effects of anaerobic digestion of food waste on biogas production and environmental impacts: A review. *Environ Chem Letters* 19: 2921–2939. https://doi.org/10.1007/s10311-021-01220-z
17. Rajput AA, Hassan M (2021) Enhancing biogas production through co-digestion and thermal pretreatment of wheat straw and sunflower meal. *Renewable Energy* 168: 1–10. https://doi.org/10.1016/j.renene.2020.11.149
18. Cheng H, Li Y, Hu Y, et al. (2021) Bioenergy recovery from methanogenic co-digestion of food waste and sewage sludge by a high-solid anaerobic membrane bioreactor (AnMBR): Mass balance and energy potential. *Bioresource Technol* 326: 124754. https://doi.org/10.1016/j.biortech.2021.124754
19. Elsayed M, Andres Y, Blel W (2023) Anaerobic co-digestion of linen, sugar beet pulp, and wheat straw with cow manure: Effects of mixing ratio and transient change of co-substrate. *Biomass Conversion Biorefinery* 13: 11831–11840. https://doi.org/10.1007/s13399-021-02229-8
20. Ibro MK, Ancha VR, Lemma DB (2022) Impacts of anaerobic co-digestion on different influencing parameters: A critical review. *Sustainability* 14: 9387. https://doi.org/10.3390/su14159387
21. Tumusiime E, Kirabira JB, Musinguzi WB (2022) Optimization of substrate mixing ratios for wet anaerobic digestion of selected organic waste streams for productive biogas systems. *Energy Rep* 8: 10409–10417.https://doi.org/10.1016/j.egyr.2022.08.189
22. Thirumalaivasan N, Gopi S, Karthik K, et al. (2024) Nano-PCM materials: Bridging the gap in energy storage under fluctuating environmental conditions. *Process Safety Environ Protect* 189: 1003–1021. https://doi.org/10.1016/j.psep.2024.06.079
23. Soman K, Kanagaraj K, Senthilkumar N, et al. (2025) Nitrogen-doped carbon dots from Brahmi (Bacopa monnieri): Metal-free probe for efficient detection of metal pollutants and methylene blue dye degradation. *Green Process Synth* 14: 20240182. https://doi.org/10.1515/gps-2024-0182
24. Thirumalaivasan N, Nangan S, Verma D, et al. (2025) Exploring the diverse nanomaterials employed in dental prosthesis and implant techniques: An overview. *Nanotechnol Rev* 14: 20250140. https://doi.org/10.1515/ntrev-2025-0140
25. Malik W, Mohan C, Annachhatre AP (2020) Community based biogas plant utilizing food waste and cow dung. *Mater Today Proceed* 28: 1910–1915.

https://doi.org/10.1016/j.matpr.2020.05.312

26. Buivydas E, Navickas K, Venslauskas K, et al. (2022) Biogas production enhancement through chicken manure co-digestion with pig fat. *Appl Sci* 12: 4652. https://doi.org/10.3390/app12094652
27. Pax ME, Muzenda E, Lekgoba T (2020) Effect of co-digestion of food waste and cow dung on biogas yield. In: *E3S Web of Conferences,* EDP Sciences, 181: 01005. https://doi.org/10.1051/e3sconf/202018101005
28. Uzorka A, Wonyanya M (2025) Design and performance evaluation of small-scale biogas digesters using locally available materials in rural Uganda. *Renewable Energy* 246: 122994. https://doi.org/10.1016/j.renene.2025.122994
29. Chen B, Azman S, Crauwels S, et al. (2024) Mild alkaline conditions affect digester performance and community dynamics during long-term exposure. *Bio Resource Technol,* 131009. https://doi.org/10.1016/j.biortech.2024.131009
30. Kou Q, Yuan Q, Chen S, et al. (2024) Alkaline pre-fermentation promotes anaerobic digestion of Enhanced Membrane Coagulation (EMC) sludge: Performance and microbial community response. *Water* 16: 2057. https://doi.org/10.3390/w16142057
31. Jiang W, Jiang Y, Tao J, et al. (2024) Enhancement of methane production from anaerobic co-digestion of food waste and dewatered sludge by thermal, ultrasonic and alkaline technologies integrated with protease pretreatment. *Bioresource Technol,* 131357. https://doi.org/10.1016/j.biortech.2024.131357
32. Wi J, Lee S, Ahn H (2023) Influence of dairy manure as inoculum source on anaerobic digestion of swine manure. *Bioengineering* 10: 432. https://doi.org/10.3390/bioengineering10040432
33. Demichelis F, Tommasi T, Deorsola FA, et al. (2022) Effect of inoculum origin and substrate-inoculum ratio to enhance the anaerobic digestion of organic fraction municipal solid waste (OFMSW). *J Cleaner Product* 351: 131539. https://doi.org/10.1016/j.jclepro.2022.131539
34. Sunar SL, Kumara MK, Oruganti RK, et al. (2025) Pretreatment and anaerobic co-digestion of lignocellulosic biomass: Recent developments. *Bioresource Technol Rep,* 102133. https://doi.org/10.1016/j.biteb.2025.102133
35. Jo S, Bae J, Kadam R, et al. (2024) Enhanced anaerobic co-digestion of cattle manure with food waste and pig manure: Statistical optimization of pretreatment condition and substrate mixture ratio. *Waste Manag* 183: 32–41. https://doi.org/10.1016/j.wasman.2024.04.043
36. Mohammadianroshanfekr M, Pazoki M, Pejman MB, et al. (2024) Kinetic modeling and optimization of biogas production from food waste and cow manure co-digestion. *Results Eng* 24: 103477. https://doi.org/10.1016/j.rineng.2024.103477
37. Fiore M, Demichelis F, Deorsola FA, et al. (2025) Optimizing biomethane production and plants growth with biochar-enhanced anaerobic digestion. *Results Eng* 26: 1–14. https://doi.org/10.1016/j.rineng.2025.104883
38. Liang J, Li C, Luo L, et al. (2025) Deciphering impacts of sulfadiazine on anaerobic co-digestion of pig manure and food waste for methane production. *Bioresource Technol* 432: 132671. https://doi.org/10.1016/j.biortech.2025.132671
39. Makumbi D, Uzorka A, Ukagwu JK (2025) Mathematical modeling of the biomass waste to energy conversion technology. *Int J Res Innov Appl Sci (IJRIAS)* 10: 1251–1263. https://doi.org/10.51584/IJRIAS.2025.10060095
40. Makumbi D, Uzorka A, Ajiji Makeri Y (2022) Number of cattle for commercialising electricity

from cattle waste to energy technology. *Int Res J Appl Sci Eng Technol* 8: 1–14. Available from: https://cirdjournals.com/index.php/irjaset/article/view/824

41. Ministry of Finance, Planning and Economic Development (MOFEPD) under population and Housing Census, 2022 Final Report.
42. ASTM D5907 (2018) Standard test methods for filterable matter (total dissolved solids) and nonfilterable matter (total suspended solids) in water.
43. Lipps WC, Braun-Howland EB, Baxter TE (2023) Standard methods for the examination of water and wastewater, 24th edition.
44. Sudiartha GAW, Imai T, Hung YT (2022) Effects of stepwise temperature shifts in anaerobic digestion for treating municipal wastewater sludge: A genomic study. *Int J Environ Res Public Health* 19: 5728. https://doi.org/10.3390/ijerph19095728
45. Zhao J, Hou T, Lei Z, et al. (2020) Effect of biogas recirculation strategy on biogas upgrading and process stability of anaerobic digestion of sewage sludge under slightly alkaline condition. *Bioresource Technol* 308: 123293. https://doi.org/10.1016/j.biortech.2020.123293
46. Nwokolo NL, Enebe MC (2024) An insight on the contributions of microbial communities and process parameters in enhancing biogas production. *Biomass Conversion Biorefinery* 14: 1549–1565. https://doi.org/10.1007/s13399-022-02580-4
47. ASTM D (2020) Standard test methods for determining the water (moisture) content, ash content, and organic material of peat and other organic soils. USA: ASTM West Conshohocken, PA.
48. American Public Health Association, American Water Works Association, & Water Environment Federation (2017) Standard Methods for the Examination of Water and Wastewater (23rd ed.). Part 2540E: Fixed and Volatile Solids Ignited at 550 °C. Washington, DC: APHA.
49. Ferdeș M, Zăbavă BȘ, Paraschiv G, et al. (2022) Food waste management for biogas production in the context of sustainable development. *Energies* 15: 6268. https://doi.org/10.3390/en15176268
50. Martínez-Orozco E, Nápoles-Armenta J, Gortáres-Moroyoqui P, et al. (2024) Treatment of tequila distillation volatile residues by electrochemical oxidation using titanium electrodes. *Environ Technol* 45: 3048–3061. https://doi.org/10.1080/09593330.2023.2206527
51. Adams VD (2017) Water and wastewater examination manual. Routledge. https://doi.org/10.1201/9780203734131
52. Ingenieure VD (2016) VDI 4630: Fermentation of Organic Materials: Characterization of the Substrate, Sampling, Collection of Material Data, Fermentation Tests. November. German.
53. Üveges Z, Damak M, Klátyik S, et al. (2023) Biomethane potential in anaerobic biodegradation of commercial bioplastic materials. *Fermentation* 9: 261. https://doi.org/10.3390/fermentation9030261
54. Gübitz GM, Gronauer A, Oechsner H (2010) Editorial: Biogas science—State of the art and future perspectives. *Eng Life Sci* 10: 491–492. https://doi.org/10.1002/elsc.201090027