
Research article

Evaluation of biogas production from water buffalo (*Bubalus bubalis*) manure under tropical climate conditions

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Abstract: In this study, we evaluated the feasibility of water buffalo (*Bubalus bubalis*) manure as a substrate for biogas production under tropical environmental conditions in southeastern Mexico. The manure was collected from a livestock unit in Chiapas and processed in a 64 m³ lagoon-type anaerobic digester. The substrate was characterized through proximate and elemental analyses, and its chemical formulas and theoretical biogas composition were estimated. Additionally, physicochemical parameters (pH, oxidation-reduction potential (ORP), electrical conductivity (EC), total dissolved solids (TDS), and chemical oxygen demand (COD)) and biogas composition (CH₄, CO₂, H₂S) were monitored throughout the study. Our results indicated that buffalo manure exhibits favorable properties for anaerobic digestion, with high moisture content (>80%), adequate volatile solids, and adequate carbon and nitrogen contents. Over 14 weeks of operation, the system maintained stable conditions of pH (7.7–8.0) and redox potential (–180 to –280 mV), reflecting an efficient transition toward the methanogenic phase. COD removal efficiency reached 86.6%, with a degradation constant of $k = 0.147 \text{ week}^{-1}$. The biogas produced showed a stable CH₄ content (58–60% v/v) and low H₂S concentrations (<22 ppm), which were eliminated after filtration, achieving calorific values up to 25.51 MJ/m³, highlighting its potential contribution to decentralized renewable energy systems in tropical rural regions.

Keywords: anaerobic digestion; calorific value; circular economy; chemical oxygen demand; methane

1. Introduction

Bioenergy accounts for about 7% of global primary energy, accounting for 60 exajoules (EJ) in 2023, of which 42 EJ corresponded to modern bioenergy. Under decarbonization scenarios, this demand could rise to nearly 100 EJ by 2050 [1]. Solid biomass continues to dominate the bioenergy share (~85%), whereas biogas and biomethane contribute only 2–3%. However, their consumption could double by 2035 (~90 billion of cubic meters equivalent), strengthening their role as renewable substitutes for natural gas and as sources of heat and electricity [2].

The rising demand for renewable energy, together with the urgent need to reduce the environmental impact of fossil fuels, has stimulated the development of sustainable technologies, including anaerobic digestion (AD). Bioenergy systems based on biomass utilization are increasingly recognized as key contributors to sustainable energy transitions, circular-economy frameworks, and climate-change mitigation, particularly when they rely on non-food feedstocks and locally available resources [3]. In this context, AD represents a mature biotechnological pathway capable of converting organic materials into high-value products, principally biogas and digestate, supporting decentralized and resource-efficient energy systems.

Biogas potential studies are essential to estimate bioenergy availability and assess multi-scale benefits. Technically, they help identify feasible technologies and optimal operating conditions. Economically, their implementation reduces dependence on conventional fossil fuels and generates added value through the use of digestate as fertilizer [4]. From an environmental standpoint, AD mitigates greenhouse gas emissions and fosters circular economy practices in waste management. Socially, it contributes to local energy security and supports the creation of green jobs, particularly in rural communities [5,6].

Lohani et al. [7] demonstrated that household biodigesters in Nepal reduced reliance on traditional biomass and improved living conditions in rural areas, confirming that animal manure can serve as a viable renewable resource. Similarly, Paolini et al. [8] reported that biogas from AD can be effectively upgraded by vacuum adsorption on zeolite, yielding biomethane with high methane purity and minimal impurities. These findings emphasize the need to address gas quality and purification at the design stage, particularly for substrates such as buffalo manure, where system stability is critical for energy recovery.

Anaerobic digestion is a flexible technology capable of converting a wide range of organic residues, including agricultural by-products, food waste, and sewage sludge, into biogas. Although lignocellulosic materials often require pretreatment to enhance degradability [9,10] animal manure is characterized by its high intrinsic biodegradability and essential nutrient content, which favor high energy yields [11,12]. Within this context, non-conventional substrates such as buffalo manure warrant greater scientific and technological attention, particularly in tropical regions. The manure of the water buffalo (*Bubalus bubalis*) represents a promising alternative feedstock due to its potential for efficient biogas generation in rural, agricultural, and agro-industrial contexts [5,13].

The potential of this feedstock is particularly relevant in Mexico, where water buffalo production has been rapidly expanding, with a national herd estimated at approximately 150,000 heads [14]. This emerging industry, primarily oriented toward high-value markets, generates around 8,200 tons of premium meat and dairy products annually [15]. The state of Tabasco alone accounts for nearly 20,000

buffalo, establishing itself as a strategic livestock region due to the species' remarkable adaptability to the country's tropical climate condition [16]. Beyond its agricultural significance, buffalo manure also represents a valuable bioenergy resource capable of supporting regional energy transition efforts, reducing dependence on fossil fuels, and strengthening decentralized renewable energy initiatives across southeastern Mexico.

From a biotechnological perspective, AD comprises four sequential stages: Hydrolysis, acidogenesis, acetogenesis, and methanogenesis, through which complex organic matter is degraded and transformed into biogas and digestate. Buffalo manure is considered a suitable feedstock owing to its favorable physicochemical characteristics, including high moisture (>80%), a substantial proportion of volatile solids (67–72%), carbon content (31–36%), nitrogen content (1.6–1.9%), and a variable carbon-to-nitrogen (C/N) ratio spanning 9.7 to 50 [17]. These properties support stable anaerobic digestion performance throughout the digestion process. Compared with cattle manure, buffalo manure contains a higher proportion of lignocellulosic fiber, which may increase hydraulic retention time (HRT) without compromising process stability. Unlike swine manure, which is readily degradable but often inhibited by high ammoniacal nitrogen [18,19], buffalo manure exhibits notable buffering capacity and intrinsic pH stability reported in the literature, achieving system stabilization without external additives even when initial pH values are as low as approximately 5.0 [17].

Nahar et al. [20] reported the anaerobic digestion of *Pistia stratiotes* inoculated with buffalo manure, showing high methanogenic potential. A lag phase of approximately 30 days was observed, attributed to initially low acetoclastic methanogen populations. Once the methanogenic community was established, biogas containing roughly 70% CH₄ was produced, achieving specific yields of 101 L m⁻³ d⁻¹ under continuous operation and 153 L m⁻³ d⁻¹ under semi-batch conditions. These performance levels corresponded to an estimated greenhouse gas mitigation potential of approximately 25 kg CO₂ per ton of processed biomass.

Based on this context, we aimed to characterize and evaluate buffalo manure as a feedstock for biogas production under tropical climate conditions in southeastern Mexico, using a lagoon-type anaerobic reactor. In addition, the relationships between key physicochemical parameters and process efficiency were analyzed to assess the feasibility of buffalo manure as a viable bioenergy alternative for livestock production systems in the region. To our knowledge, few researchers have investigated the anaerobic digestion of buffalo manure under real tropical field conditions, which underscores the novelty and practical relevance of this research. For example, Nahar et al. [20] reported methane concentrations ranging from approximately 55–60% during the digestion of buffalo manure under semi-batch operation, while other studies conducted in tropical or subtropical climates have highlighted the influence of temperature variability and substrate characteristics on process stability and biogas yield.

2. Materials and methods

Manure from water buffalo (*Bubalus bubalis*), Mediterranean-type (Murrah/Jafarabadi), was collected from the “La Carolina” ranch in Reforma, Chiapas, Mexico. The farm specializes in live animal management and milk production. Manure samples were collected from pens housing animals at different developmental stages (adults, weaned calves, and growing buffalo). Composite samples were subsequently prepared exclusively from adult and growing buffalo manure, following the quartering method described in the Mexican Standard NMX-AA-015-1985 [21].

2.1. Collection and transportation

Manure was manually collected using shovels and 200 L high-density polyethylene (HDPE) containers and immediately transported to the Center for Applied Technologies in Renewable Energy (CATRE) at the División Académica de Ciencias Biológicas (DACBio), Universidad Juárez Autónoma de Tabasco (UJAT). Transportation was completed on the same day to minimize pre-digestion, microbial fermentation, and compositional changes prior to incorporation into the anaerobic digester.

2.2. Initial substrate proximal characterization

The collected samples were analyzed through proximate and elemental analyses to determine their initial composition. The proximate analysis included the quantification of moisture content, total solids (TS), volatile solids (VS), and ash content, following the ASTM D2974 [22] standard.

Moisture and total solids were determined by oven-drying the samples at 105 °C for 24 h, while volatile solids were quantified by calcination at 550 °C for 2 h. Ash content was subsequently determined by calcination at 800 °C for 1 h, as a complementary step to ensure complete oxidation of residual organic matter, following the general framework of ASTM D2974. Based on these results, total organic carbon (TOC) and organic matter (OM) were calculated using the Van Bemmelen factor (1.724), according to the following relationships:

$$TOC(\%) = 100 - Ash(\%) \quad (1)$$

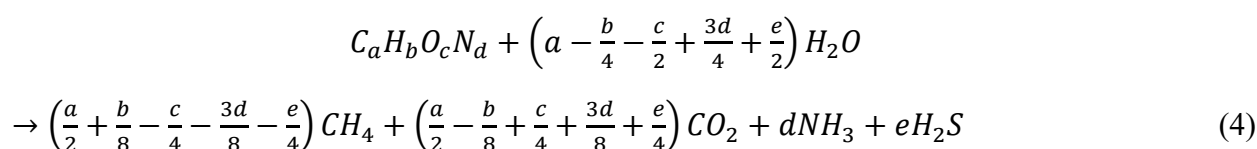
$$OM(\%) = \frac{TOC(\%)}{1.724} \quad (2)$$

Elemental analysis (C, H, N, S) was performed using a Perkin Elmer PE2400 CHNS/O analyzer (PerkinElmer, Waltham, MA, USA). Oxygen (O) content was calculated by difference, subtracting the sum of the measured elements and ash from the total (100%), according to the following equation:

$$O(\%) = 100 - \sum(CHNS) + Ash \quad (3)$$

2.3. Chemical formulas and stoichiometric calculations of biogas production

Based on elemental composition, the algebraic method was applied to derive general chemical formulas of organic matter ($C_aH_bO_cN_dS_e$). Molar fractions were calculated on an ash-free basis, and approximate molecular expressions were formulated with and without sulfur, considering one mole of nitrogen and sulfur as the reference basis. Using these formulas, the theoretical gas volumes generated during anaerobic degradation were estimated stoichiometrically. The equations proposed by Tchobanoglous [23] was applied to determine the principal gaseous products; methane (CH_4), carbon dioxide (CO_2), ammonia (NH_3), and hydrogen sulfide (H_2S), from which the theoretical biogas composition was obtained (Eq (4)):



2.4. Anaerobic reactor and feeding

The experimental setup consisted of a lagoon-type anaerobic digester with a total working volume of 64 m³ and a flexible biogas storage dome of approximately 40 m³, constructed from 1.5 mm-thick polyvinyl chloride (PVC) geomembrane, as reported by Laines and Sosa [24]. The reactor was equipped with a biogas conduction system of 2" diameter PVC Schedule 40, and two 4" diameter PVC Schedule 40 modules for hydrogen sulfide (H₂S) removal.

The initial feeding stage involved loading 0.5 t of buffalo manure homogenized with digestate directly inside the reactor. Homogenization was performed using a 9 HP, 4" ø centrifugal pump, with suction connected to a recirculation pipe and discharge directed to feed inlet. Two additional feedings of 0.4 t and 0.6 t, respectively, were carried out at approximately one-month intervals, resulting in a total loading of 1.5 t of buffalo manure.

2.5. Agitation operation

One week after the initial loading, internal recirculation was initiated twice weekly (on Mondays and Fridays) to prevent stratification, homogenize the feedstock, and enhance microbial activity, in accordance with lagoon-type digester operating criteria. This operation was performed with the 9 HP centrifugal pump, which was sequentially connected to each of the four recirculation lines, discharging into the adjacent line. Each pair of lines was recirculated for 15 minutes, resulting in a total mixing time of approximately 45 minutes per session.

2.6. Monitoring and evaluation of physicochemical parameters

The anaerobic digestion process was operated and monitored over a 14-week period (approximately 3.5 months). Physicochemical parameters and biogas composition were measured to evaluate system performance with the selected feedstock. Monitored parameters included pH, oxidation-reduction potential (ORP), electrical conductivity (EC), total dissolved solids (TDS), temperature (°C), chemical oxygen demand (COD), biogas composition (CH₄, CO₂, H₂S), and calorific value. Volatile fatty acids and microbial community structure were not assessed, as we focused on field-scale engineering performance.

Physicochemical analyses were performed by sampling approximately 48 h after each recirculation event. One-liter samples were collected from each recirculation pipe using a filling-trap tube with a 1 L capacity. Measurements were performed with handheld multiparametric instruments. COD was analyzed weekly using 3 mL aliquots with the small-scale sealed-tube method and quantified by UV-Vis spectrophotometer at 550 nm, following the Mexican Standard NMX-AA-03/2-SCFI-2011 [25] based on ISO 15705:2002 [26].

COD removal efficiency, average degradation rate, and first-order degradation kinetics were calculated using the following expressions:

$$Remotion(\%) = \frac{COD_{initial} - COD_{final}}{COD_{initial}} * 100 \quad (5)$$

where COD_{initial} = initial COD concentration (g/L); COD_{final} = final COD concentration (g/L).

$$\tau = \frac{COD_{initial} - COD_{final}}{time_{initial} - time_{final}} \quad (6)$$

where τ = average degradation rate; $time_{initial} - time_{final}$ = time interval (weeks).

$$\frac{d(COD)}{dt} = -k * COD \quad (7)$$

$$COD_t = COD_0 * e^{-kt} \quad (8)$$

where k = degradation constant (1/week); COD_0 = COD initial; COD_t = COD at time.

$$k = -\frac{\ln\left(\frac{COD_t}{COD_0}\right)}{t} \quad (9)$$

2.7. Biogas characterization

Biogas composition and calorific value were determined weekly using an Optima® Biogas 7000 analyzer. The reactor was equipped with two monitoring points directly connected to the gas dome, and two additional points along the conduction pipeline downstream of the H₂S removal modules. All points had a diameter of ½". The H₂S removal stage employed a filtering material patented at the Mexican Institute of Industrial Property (IMPI) under file MX/a/2019/008094 [27].

The produced biogas was stored in the reactor's dome and subsequently transferred through the conduction pipeline, passing through the H₂S removal modules. Finally, the biogas was delivered to a domestic stove with two burners, where combustion performance was demonstrated through controlled cooking tests. The stove, 15 m from the digester, consisted of two 12" star-type burners with a ¾" supply connection. A thermographic camera was employed during the combustion tests to record the surface temperatures reached by the burners and to evaluate the thermal distribution during operation.

Data analysis was based on descriptive statistics. Experimental results are reported as mean values with corresponding standard deviations, calculated from repeated measurements collected during the operational period.

3. Results and discussion

The proximate and elemental characteristics of buffalo manure are summarized in Table 1. These parameters provide the baseline physicochemical composition used to evaluate its suitability as a feedstock for anaerobic digestion under tropical conditions.

The percentages of volatile solids, carbon, and nitrogen for both buffalo sizes were consistent with those reported by Carotenuto [17], confirming the reliability of the proximate and elemental analyses. In contrast, the moisture observed in this study was notably lower, which may be attributed to the tropical climatic conditions and partial dehydration during manure handling prior to reactor loading.

The pH evolution (Figure 1) exhibited a typical pattern characteristic of stable anaerobic digestion. The process began with slightly alkaline values (pH < 8.0), reaching a maximum of 8.42 during the second week, likely due to the release of ammoniacal compounds during the early stages of organic matter degradation. Subsequently, the pH decreased to approximately 7.60 in week 6, typically associated with acidogenic activity. However, because volatile fatty acids (VFA) were not quantified, the driver of this fluctuation (e.g., transient accumulation of individual VFAs) cannot be confirmed

within the scope of this field-scale study.

Table 1. Proximate and elemental characteristics buffalo manure by animal category.

Characteristic		Size	
		Adult	Growth
Proximal analysis	Total solids (%)	8.92 ± 1.06	9.36 ± 0.29
	Volatil solids (%)	67.04 ± 4.83	71.79 ± 1.15
	Fixed solids (%)	8.60 ± 0.05	0.51 ± 0.09
	Ash (%)	20.88 ± 15.19	27.69 ± 1.21
	TOC (%)	79.12 ± 15.19	72.31 ± 16.36
	OM (%)	45.89 ± 8.81	41.94 ± 9.49
Elemental analysis	C (%)	35.81 ± 0.16	36.55 ± 0.49
	H (%)	4.83 ± 0.04	5.02 ± 0.11
	O (%)	34.24 ± 10.11	30.37 ± 1.64
	N (%)	1.93 ± 0.01	1.60 ± 0.05
	S (%)	0.56 ± 0.04	0.46 ± 0.09

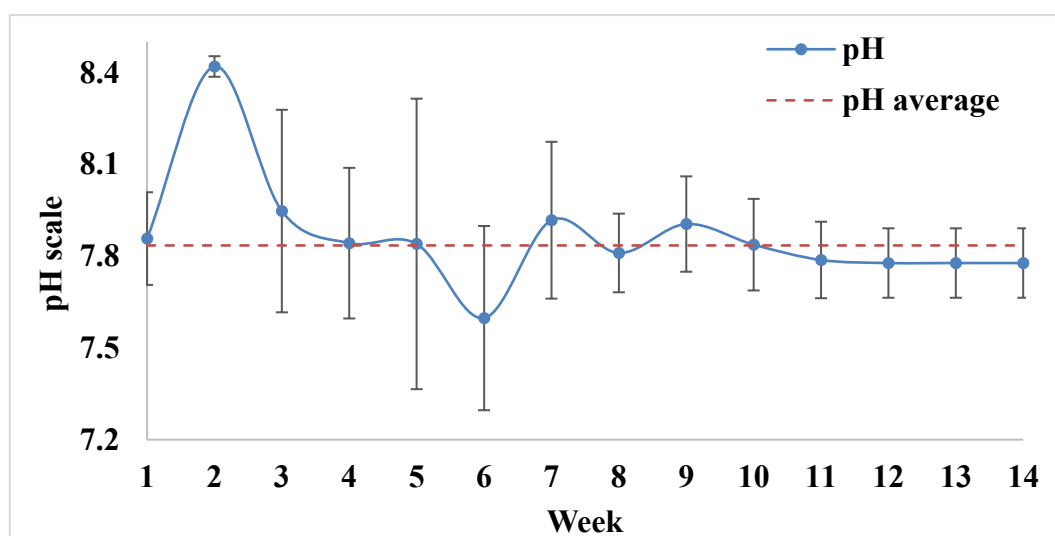


Figure 1. Variation of pH during the anaerobic digestion process of buffalo manure.

Electrical conductivity (EC) is presented in Figure 2. EC initial values declined from 6.98 dS/m in the first week to 6.46 dS/m in week 5, reflecting a temporary reduction of ionic salts in solutions. Subsequently, fluctuations around the overall average of 6.64 dS/m indicated ionic equilibrium conditions typical of the methanogenic stage.

Total dissolved solids (TDS) started at 3,443 ppm and decreased to 3,208 ppm by week 6, followed by a temporary increase between weeks 10 and 12 (3,488 ppm) and final stabilization at 3,252 ppm, close to the overall average of 3,331 ppm. These variations reflected the initial solubilization of organic compounds and salts, followed by their transformation and consumption during digestion, before equilibrium was reached in the methanogenic stage (Figure 3).

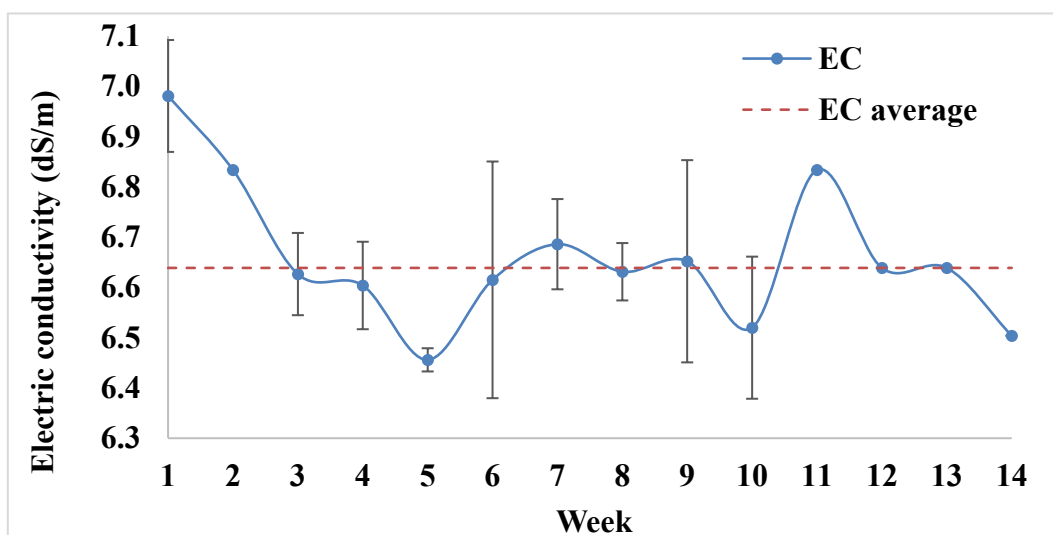


Figure 2. Variation of electrical conductivity (EC) values during anaerobic digestion of buffalo manure.

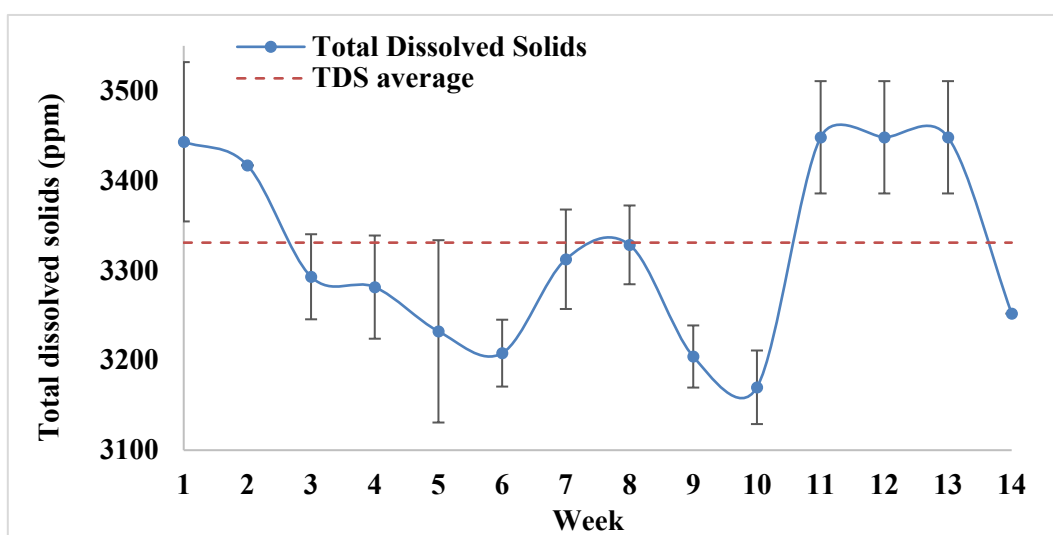


Figure 3. Variation of total dissolved solids (TDS) during anaerobic digestion of buffalo manure.

The oxidation-reduction potential (ORP) initially measured -360 mV prior to digester feeding, indicating strongly reducing conditions. ORP values progressively increased to -167 mV by week 10 and subsequently decreased, then stabilizing around -280 mV toward the end of the monitoring period (Figure 4). This dynamic pattern reflected the transition from the highly reducing conditions favorable for hydrolytic and acidogenic activity to a stable environment suitable for methanogenesis, while remaining within the reducing range typically associated with AD.

Chemical oxygen demand (COD) increased from 74.69 g/L to 166 g/L during the first week, coinciding with the initial reactor feeding, and reached a maximum value of 954 g/L in week 4 (Figure 5). This peak reflected the release of soluble organic compounds during the hydrolytic phase. Subsequently, COD concentrations decreased progressively, with slight fluctuations, and stabilized between 150 and 300 g/L during the final weeks of operation.

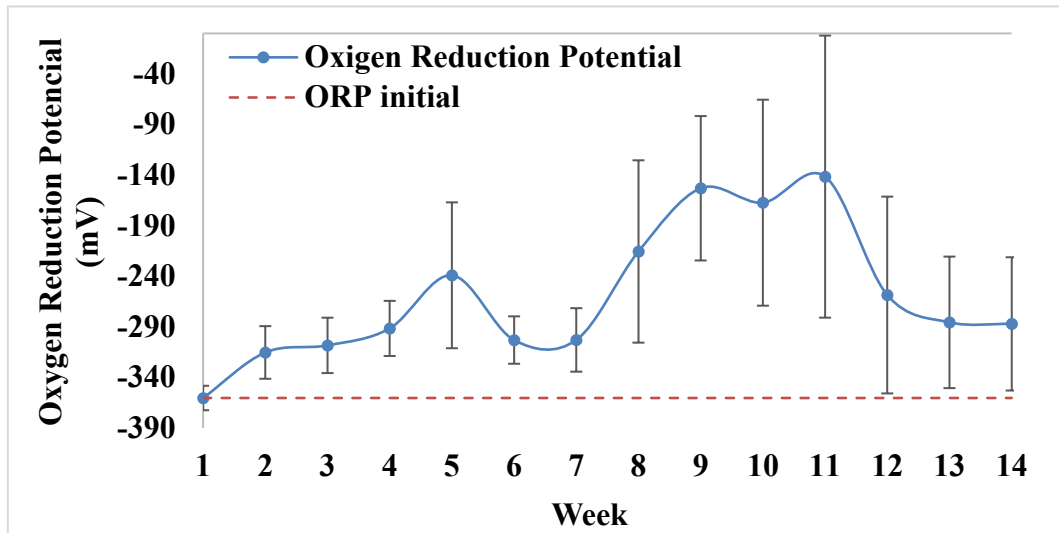


Figure 4. Variation of oxidation-reduction potential (ORP) during anaerobic digestion of buffalo manure.

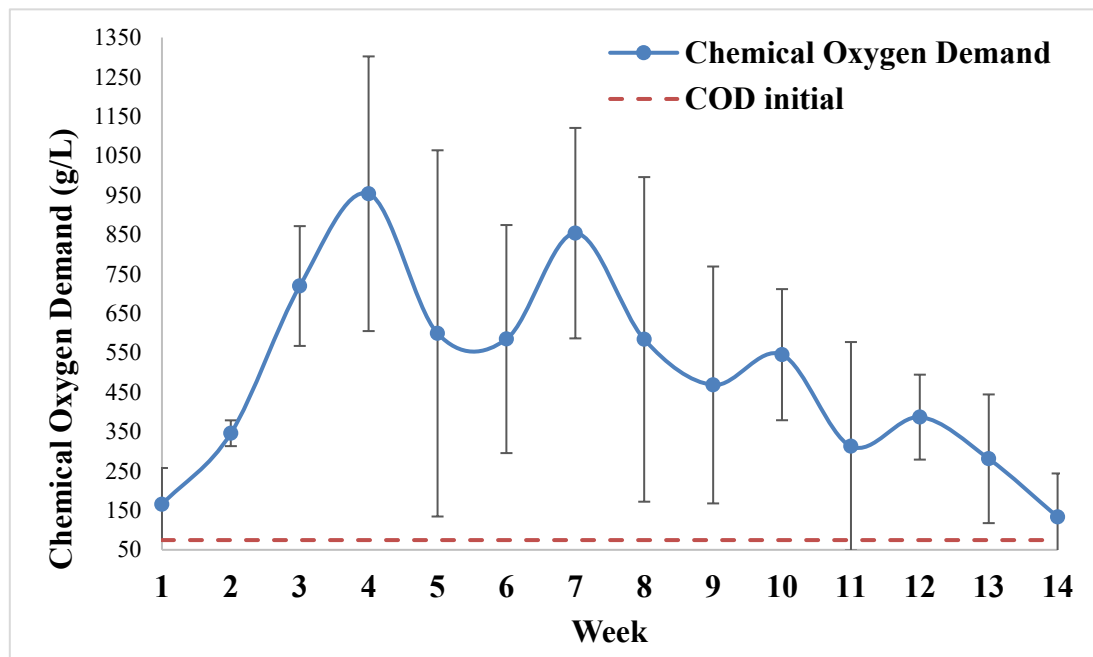


Figure 5. Variation of chemical oxygen demand (COD) during anaerobic digestion of buffalo manure.

The variability observed between weeks 4 and 11 could be attributed to internal reactor hydrodynamics, particularly depth differences among the four recirculation lines (ranging from 0.8 m at sampling point 1 to 1.10 m at point 4). These differences resulted in lower COD concentrations at points 1–2 and higher concentrations at points 3–4, as illustrated in Figure 6.

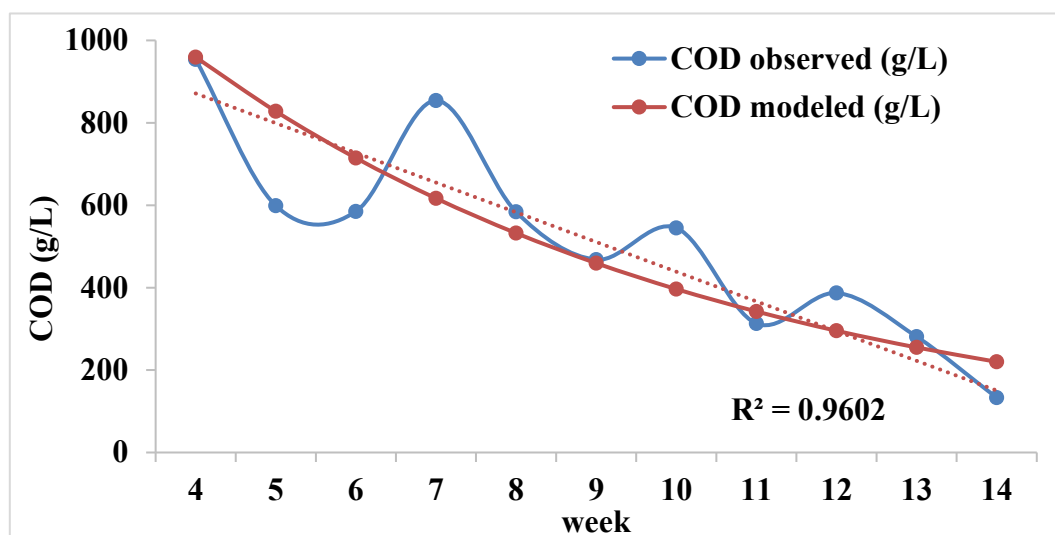


Figure 6. Observed and modeled COD profile during anaerobic digestion of buffalo manure (first-order kinetics).

This pattern indicated organic matter consumption by microbial consortia and the conversion of soluble compounds into biogas, reflecting the progressive efficiency of anaerobic digestion. First-order kinetics analysis yielded a degradation constant (k) of 0.147 week^{-1} and an average degradation rate of $82.1 \text{ g L}^{-1} \text{ week}^{-1}$, confirming that the system followed first-order degradation kinetics, as expressed by the following equation:

$$COD_t = COD_0 * e^{-0.147t} \quad (10)$$

where COD_0 represents the initial concentration at the beginning of the degradation phase and t is the relative time in weeks from the maximum value. This kinetic model accurately describes the progressive removal of organic load and confirms the high operational efficiency of the anaerobic digester, which achieved a COD removal efficiency of 86.6%.

An inverse relationship between pH and ORP was observed: Initial pH near 7.86 corresponded to strongly reducing conditions (-360 mV), whereas a decrease in pH to ~ 7.8 shifted ORP to $\sim -180 \text{ mV}$ (weeks 9–11), reflecting the transition from acidogenic to methanogenic conditions and biochemical stabilization of the system.

In contrast, the relationship between total dissolved solids (TDS) and chemical oxygen demand (COD) exhibited a non-linear pattern. The maximum COD value (954 g L^{-1}) coincided with intermediate TDS concentrations ($3,331 \text{ mg L}^{-1}$), whereas the highest TDS value ($3,448 \text{ mg L}^{-1}$) occurred after COD had declined. This observation suggested that the persistence of dissolved salts is primarily related to inorganic solubilization processes rather than to the concentration of biodegradable organic matter.

Table 2. Stoichiometric results: Chemical formula and theoretical biogas composition.

Calculated conditions	Chemical formulas	Theoretical composition of biogas	%
Considering sulfur and water	$C_{190.38}H_{70.68}O_{39.80}N_{7.97}S_1$	CH_4	47.83
		CO_2	47.64
		NH_3	4.04
		H_2S	0.49

The stoichiometric chemical formulas derived from the elemental composition and the corresponding theoretical biogas composition are summarized in Table 2. These results provide an estimate of the potential methane and carbon dioxide yields expected from the anaerobic degradation of buffalo manure under tropical conditions.

Theoretical biogas production was estimated at 801.5 m³, corresponding to a yield of 2.07 m³ kg⁻¹ VS. However, stoichiometric estimations assume the complete conversion of organic matter, representing an idealized reaction pathway that is not fully achieved under real field operating circumstances; therefore, these calculations are best interpreted as reference estimates to be contrasted with measured biogas production. Figure 7 illustrates the actual average biogas composition measured directly at the reactor outlet and after the first and second purification stages. Methane (CH₄) was the predominant component of the biogas, accounting for 58–60% (v/v) and remaining stable throughout the operational period. These values were slightly lower than those reported by Nahar et al. [20] under semi-batch operation. Carbon dioxide (CO₂) concentrations ranged from 34 to 38% (v/v), showing a slight decrease after filtration, which enhanced the biogas energy fraction. Hydrogen sulfide (H₂S) was detected only in raw gas (~22 ppm, variable) and was removed during the first purification stage.

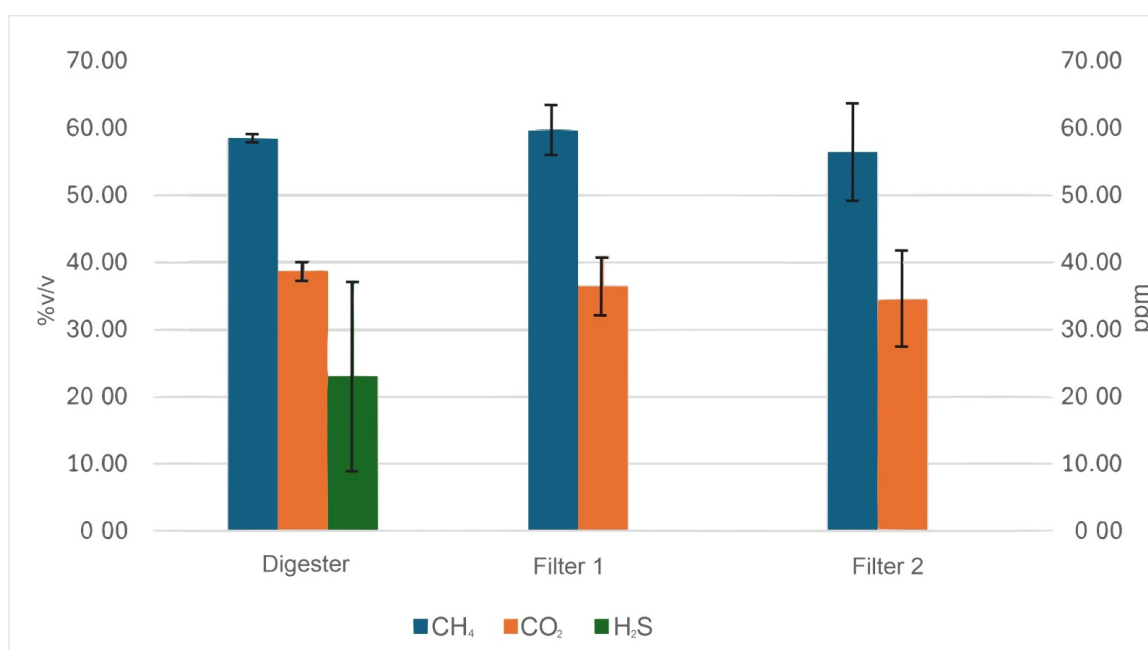


Figure 7. Variation of biogas composition at different purification stages (H₂S concentrations, ppm).

The relatively high standard deviation observed could be attributed to the operation of the system under real field conditions, where fluctuations in ambient temperature are inherent. Overall, the purification system effectively reduced undesirable compounds, particularly H₂S, while ensuring stable biogas quality suitable for direct domestic applications and small-scale energy systems. Calorimetric results are presented in Figure 8.

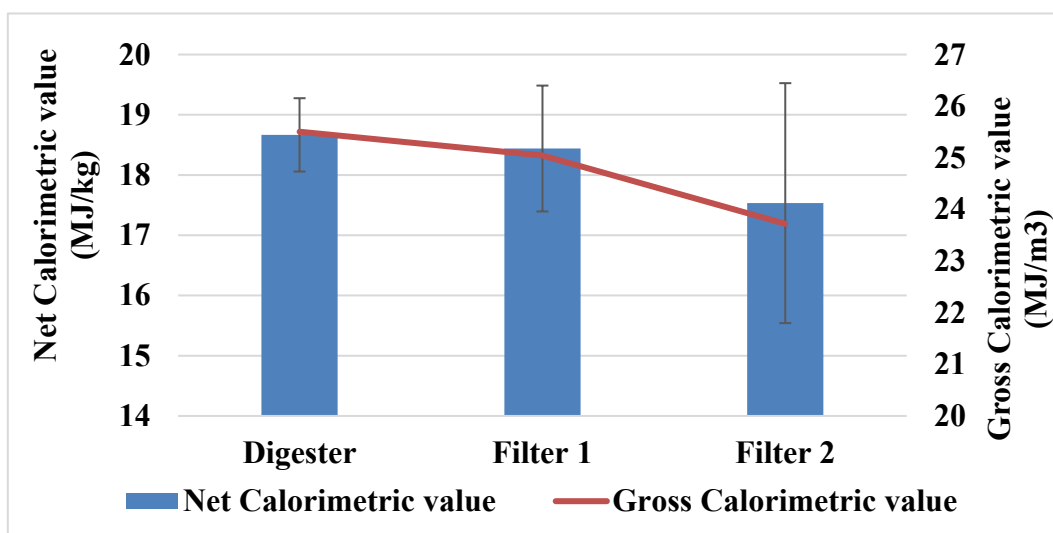


Figure 8. Variation of calorific values at each purification stage of biogas.

The highest calorific values were obtained in raw biogas, prior to purification, with a Net Calorific Value (NCV) of 18.67 MJ/kg and a Gross Calorific Value (GCV) of 25.51 MJ/m³ as reported by the Optima Biogas 7000 analyzer. The energy content decreased by 1.51% after the first purification stage and by 6.53% after the second. The reduction in methane concentration may explain the lower calorific value, along with the removal of other combustible gases such as H₂S, which provides only a minor caloric contribution (<~3%).

These results confirmed the stability of buffalo manure as a substrate under tropical conditions, yielding a higher-quality methane-rich biogas with efficient COD removal and effective H₂S purification. They also confirm the efficiency of purification and the suitability of buffalo manure as a renewable energy feedstock, highlighting its potential as a sustainable bioenergy resource.

4. Conclusions

The valorization of buffalo manure as a substrate for biogas production represents a significant step toward sustainable energy transition, contributing to the diversification of the energy matrix with local, renewable, and low-impact resources. By converting livestock waste into useful energy, this study demonstrates how bioenergy can be integrated into circular economy schemes, closing material and energy cycles while reducing dependence on fossil fuels and associated emissions. The results confirmed stable system performance, with high efficiency in organic matter removal and the production of methane-rich biogas, thereby reinforcing its role as a sustainable bioenergy option for tropical rural contexts and positioning buffalo manure valorization as a replicable model for other tropical regions facing similar challenges in renewable energy adoption. Under full-scale field operation, methane accounted for 58–60% (v/v), H₂S in raw gas (~22 ppm) was removed during purification, and the calorimetric assessment reported an NCV of 18.67 MJ/kg and a GCV of 25.51 MJ/m³.

Beyond its technical contribution, this research underscores the importance of promoting the rational use of agricultural resources through clean technologies adapted to local contexts. Such initiatives can contribute to the achievement of several Sustainable Development Goals (SDGs), particularly SDG 7 (affordable and clean energy), SDG 12 (responsible consumption and production),

and SDG 13 (climate action). This study was designed as a field-scale engineering assessment; therefore, microbial community structure and volatile fatty acids were not evaluated, and a detailed economic feasibility analysis was beyond the scope of the available data. Future efforts should incorporate targeted monitoring (e.g., volatile fatty acids) and microbial community characterization, as well as substrate pretreatment strategies and low-cost automated process control suitable for decentralized tropical applications. In addition, the potential use of digestate as a biofertilizer should be assessed to maximize environmental and economic benefits.

Use of AI tools declaration

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

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Conflict of interest

The authors declare that they have no conflicts of interest.

Author contributions

H. D. F. C. (Undergraduate student): Conceptualization; Methodology; Investigation; Formal analysis; Writing—original draft. J. A. S. O. (Ph.D.): Methodology; Resources; Supervision; Formal analysis; Data curation; Writing—review & editing. J. R. L. C. (Ph.D.): Methodology; Resources; Funding acquisition; Supervision; Formal analysis; Review & editing. A. S. F. (Undergraduate student): Investigation; Data curation (COD analysis). A. D. P. T. (Undergraduate student): Investigation (digester operation).







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Supplementary information

<i>Sample Collection</i>	<i>Digester Loading</i>	<i>Heat testing</i>
		
		
		



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