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Research Article

Synergistic Biogas Production: Optimizing Methane Yields from Egg-Laying Chicken, Broiler Chicken, and Cow Dung Waste Mixtures.

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Abstract. Anaerobic digestion for biogas production was assessed utilizing egg-laying chicken, broiler chicken and cow dung as co-substrate. This was demonstrated in varying reactors named R1, R2, R3, and R4 containing various proportions of egg-laying chicken, broiler chicken and cow dung with a total solid concentration of 8%. This experiment was carried out for 40 days at a temperature of 25ºC. The volatile solid and chemical oxygen demand for all the reactors were as follows; 29.1, 56.6, 44.7, and 36.6% and 54.5, 89.17, 70.1, and 64.01% respectively. R2 contains 75% egg-laying chicken and 25% cow dung gave the optimal outcomes; a methane yield of 85.4%, and VS reduction of 56.6%.

Keywords: Methane, Cow Dung, COD, VS, TS, C/N ratio, Anaerobic Digestion.

1. Introduction

Anaerobic digestion-derived biogas presents a viable alternative to fossil fuels, offering distinct benefits compared to other bioenergy sources (Iglesias et al., 2021). The majority of anaerobic digestion facilities are concentrated in Europe, accounting for 91% of the total, with a smaller share found in Asia and a limited number in the United States. Among European nations, Germany leads the sector, representing 35% of all anaerobic digestion plants, followed by Denmark, Sweden, Switzerland, and Austria (Verma, 2002). Bio-digestion is a process in which organic matter decomposes anaerobically within a digester, where microorganisms break down glucose, proteins, and lipids to generate biogas in the absence of oxygen. This biogas typically consists of 45–80% methane (CH_4), 27–45% carbon dioxide (CO_2), 0.5–3% nitrogen (N_2), 1–10% hydrogen (H_2), 1% carbon monoxide (CO), and 1% oxygen (O_2), along with trace levels of hydrogen sulfide (H_2S) (Atelge et al., 2020). The composition of the gas is influenced by the type of feedstock used, with animal waste generally producing higher concentrations of CO_2 (Kadam & Panwar, 2017). The quantity of biogas generated per kilogram of feedstock depends on several factors, including digester efficiency, temperature, pH, and the carbon-to-nitrogen (C:N) ratio of the substrate (Isha et al., 2021). Once released into the atmosphere, a methane molecule has approximately 21 times the greenhouse gas effect of a carbon dioxide molecule.

The International Energy Agency reports that global energy-related CO_2 emissions hit an all-time high of 37 gigatons in 2022, reflecting a 1% rise compared to pre-pandemic levels in 2019. Although efforts are being made worldwide to reduce emissions, the energy sector remains a significant driver of climate change. This underscores the pressing need to transition toward cleaner and more sustainable energy solutions (Cozzi et al., 2020). Anaerobic digestion plays a vital role in both sustainable waste management and renewable energy generation. The biogas production process consists of four main stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. During hydrolysis, hydrolytic bacteria break down complex organic matter such as carbohydrates, proteins, and lipids into simpler molecules, includ-

ing soluble sugars, amino acids, and fatty acids. In the next phase, acidogenesis, acidogenic bacteria ferment these compounds into volatile fatty acids, hydrogen, and carbon dioxide. This is followed by acetogenesis, where volatile fatty acids are further converted into acetic acid, along with additional hydrogen and carbon dioxide, by acetogenic bacteria; acetic acid being a key precursor for the final step. Lastly, methanogenesis takes place, during which methanogenic archaea transform acetic acid and hydrogen into methane and carbon dioxide, making this stage crucial for biogas production (Khanal et al., 2021; Kumar et al., 2024).

Egg laying-Chicken, Broiler Chicken, and Cow Dung are all essential feedstocks for anaerobic digestion due to their widespread availability and the large volumes in which they are produced. Leftover food serves as a consistent and nutrient-rich source of organic material for biogas generation, while Cow Dung, abundantly derived from agricultural activities, provides a steady supply of organic matter that enhances biogas yields. Several research (Tang et al., 2019; Zhao et al., 2017) have explored the digestion of Food Waste for energy and fuel production. Nevertheless, the rapid decomposition of readily degradable compounds in leftover foods can lead to excessive volatile fatty acid accumulation, causing acidification as well as limiting the improvement of organic loading rates and biogas output in mono-digestion systems (Panigrahi et al., 2020). This pH decline inhibits methanogenic bacteria, significantly lowering methane production and potentially destabilizing the process (Mu et al., 2020). Therefore, maintaining a balanced nutrient composition is crucial to preventing VFA accumulation and ensuring stable biogas production.

On the other hand, Cow Manure is among the most abundant organic wastes globally, and its specific properties make it a suitable feedstock for large-scale biogas plants (Abid et al., 2021). Though, its high nitrogen content can lead to excessive ammonia production, which inhibits methanogens and reduces methane yield (Mu et al., 2020). To overcome this challenge and enhance biogas production efficiency in Anaerobic digestion, various strategies have been developed. Biogas can be generated from a wide range of feedstocks, including animal manure, energy crops, industrial waste, and other organic materials. The anaerobic digestion process involves several key biochemical reactions (Mia et al., 2017; Rahman & Al-Muyeed, 2010), which include:

Acetogenic bacteria:

$$\text{Cellulose } C_6H_{12}O_6 \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (1)$$

Methanogenic bacteria:

$$CH_3COOH \rightarrow CH_4 + CO_2,\ CO_2 + H_2 \rightarrow CH_4 \quad (2)$$

The pH level indicates the acidity or alkalinity of a solution, with an optimal range of 6.8 to 7.2 for anaerobic digestion (Ning et al., 2019a; Ning et al., 2019b). If the pH drops below 6.8, the growth of methanogenic microorganisms may be inhibited. Conversely, excessive alkalinity can disrupt crystal structures and negatively impact methane production (Li et al., 2012). High concentrations of certain compounds, such as sodium bicarbonate ($NaHCO_3$), lime (CaO), and sodium carbonate (Na_2CO_3), contribute to increased alkalinity in the system, which can inhibit the digestion process (Li et al., 2017). Additionally, introducing a large quantity of organic material at once—referred to as the organic loading rate can overwhelm methanogens, reducing their ability to break down organic matter effectively (Li, 2016). Adequate stirring is essential to ensure uniform mixing of the slurry, providing microorganisms with sufficient access to nutrients in the digester. Limited agitation can enhance fermentation (Alburquerque et al., 2012), whereas excessive stirring may slow down the digestion rate (Chiu et al., 2013).

2. Method

2.1. Raw Materials

The raw materials utilized in this study were Egg-laying chicken, Broiler chicken and Cow dung. Cow dung was considered as equally inoculum and the substrate in co-digestion and mono-digestion procedures; however, egg-laying chicken and broiler chicken serves as substrate in co-digestion. Cow dung was collected from the Cyprus International University livestock farm. Broiler chicken were scraps or leftover from chickens that were used for the meat production while the egg-laying chicken waste were discarded from eggshells or unused eggs. All the raw material for this experiment stored at 4ºC depending on the day of experiment to stop microbial decay (Oladejo et al., 2020; Xue et al., 2023). On the experimental day, raw material samples were left at room temperature for several hours to stabilize before processing. The cow dung was kept moist and used as co-substrate to maintain the C/N ratio until the experiment

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began, with any foreign materials, such as hay and bulky particles, carefully removed. Meanwhile, the egg-laying chicken and broiler chicken were prepared by eliminating non-biodegradable items like bones and plastic bags. It was then ground into smaller particles to increase surface area, facilitating microbial digestion and improving biogas production.

2.2. Experimental Description

This procedure described how important to know the amount of gas to be generated homogenously. The first setup, Reactor (R1) contains mixture of egg-laying chicken alone. Second setup, Reactor (R2) contains 75% ELC + BC and 25% cow dung. Third setup, Reactor (R3) contains 50% ELC + BC and 50% cow dung and the fourth setup, Reactor (R4) contains 70% ELC and 30% BC (Figure 1). Each digester was securely sealed with a lid containing holes for plastic tubing, allowing biogas to be transferred to a water container by displacement method (Rouf et al., 1999); while preserving anaerobic conditions. To ensure a tight seal, putty was applied, and the biogas transfer tubing was clamped at two points during sampling to prevent both oxygen infiltration and gas leakage.

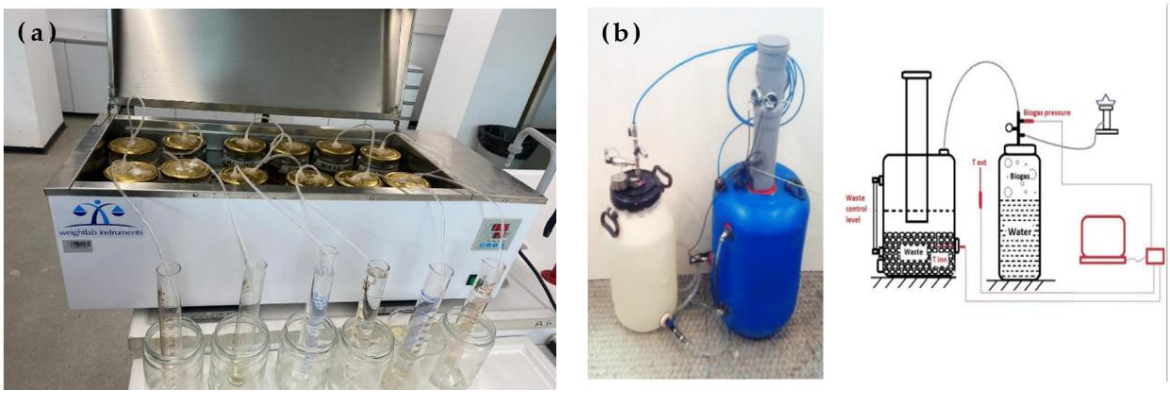

Figure 1. (a) Experimental method consisting of a batch reactor, (b) Schematic design determining biogas production

Retention time influences biogas production by affecting the extent of substrate degradation; while longer retention generally increases gas yield, excessively prolonged durations may lower process efficiency (Kumar et al., 2024). In both mono-digestion and co-digestion phases, with a retention time of 20 days, the digesters were placed in a temperature-controlled warm water bath to improve heat transfer more effectively than an incubator setup. The water bath temperature was regulated within the anticipated levels 30 – 35 °C, utilizing a heating element as well as a digital thermostat regulator.

2.3. Physiochemical Characteristics

Anaerobic digestion stability and biogas production were unswervingly impacted based on the properties of the materials in the reactors when analyzed. Several parameters were considered such as total solids (TS), volatile solids (VS), moisture content (MC), and using the 2540 G standard method, the ash content was measured (APHA, 1926). The chemical oxygen demand (COD) for every initial raw material was quantified as designated in standard methods (Miner, G., 2006). The standard method protocol was followed to ascertain the pH of the mixture (Mikoda et al., 2018). Triplicate measurements were ensured to check reliability and statistical importance of the outcomes and each reactor condition was prepared twice. One-way ANOVA was carried out utilizing R software on all parameters measured toward assessing significant differences ($p < 0.05$) between the various co-digestion ratios.

3. Result and Discussion

Table 1. shows the varying parameters of the raw materials as utilized in the experiment. R1 has a C/N ratio of 7.7 and therefore, considered insufficient for biogas production optimally (Borja & Banks, 1995), however an addition of cow dung would increase it production rate because cow dung possesses higher C/N ratio (Rouf et al., 2015). R2 has a pH of 8.56. Cow dung also has an excellent buffering measurement and is considered the source of all the microorganisms available for anaerobic digestion (Thangamani, et al., 2010; Singh et al., 2021).

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Table 1. Characteristics of raw material based on reactors R1 (ELC+BC), R2 (75:25), R3 (50:50), R4 (70:30)

Parameter	Reactor (R1)	Reactor (R2)	Reactor (R3)	Reactor (R4)
pH	7.16	8.56	7.72	7.35
TS	71.62	85.60	77.20	73.51
VS	29.10	56.61	44.70	36.62
Moisture	92.50	98.70	92.50	90.10
TN	3.00	4.01	2.30	2.00
TOC	40.01	44.2	35.9	39.22
COD	54.50	89.17	70.12	64.01
C/N Ratio	7.7	6.5	24	8.9

3.1. pH maximum and minimum evaluation of reactors

Figure 2. illustrates the maximum and minimum pH values of the substrate for Run 1, Run 2, Run 3, and Run 4, recorded as 7.16 and 5.10; 7.96 and 5.37; 7.60 and 5.40; and 7.45 and 6.00, respectively. In Runs 1 and 4, the highest pH was observed on the first day, followed by a sharp decline over the next two weeks. This drop can be attributed to the hydrolysis stage within the digester, during which organic matter is broken down into amino acids, fatty acids, and sugars, leading to the accumulation of acidic intermediates. As a result, the pH remained in an acidic range and did not recover, ultimately inhibiting biogas production and negatively affecting digester performance. In an efficiently operating anaerobic digester, microbial communities convert these acidic intermediates into methane and carbon dioxide, which helps restore the pH balance. Nevertheless, in Runs 1 and 4, the pH failed to increase and remained consistently acidic, leading to process inhibition.

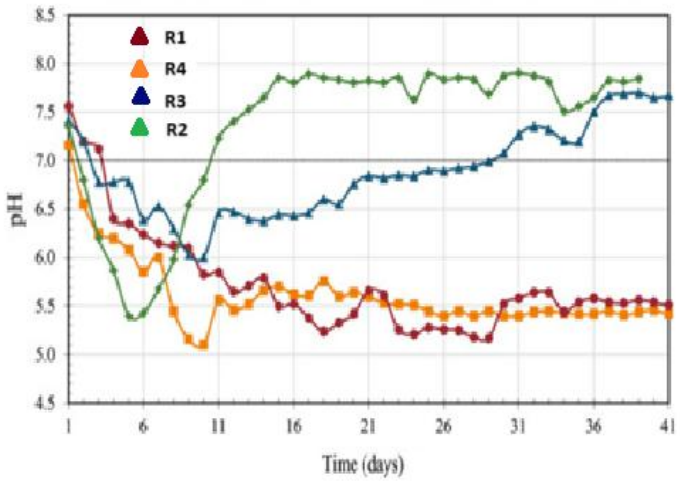

Figure 2. Day to day pH difference in all the reactors

3.2 Increasing biogas generation in all reactors

Figure 3. shows the cumulative biogas production in the various reactors. Reactor R2 demonstrated the highest production of methane gas than the others notwithstanding that R3 had more cow dung in it. It is imperative to know that methane gas production from mixture of ELC + BC manures from anaerobic digestion with an addition of cow dung yields higher gas production. Reactor R1 stopped the production of biogas after week two as well as in Reactor R4 where the digester was unable to generate more biogas after week three.

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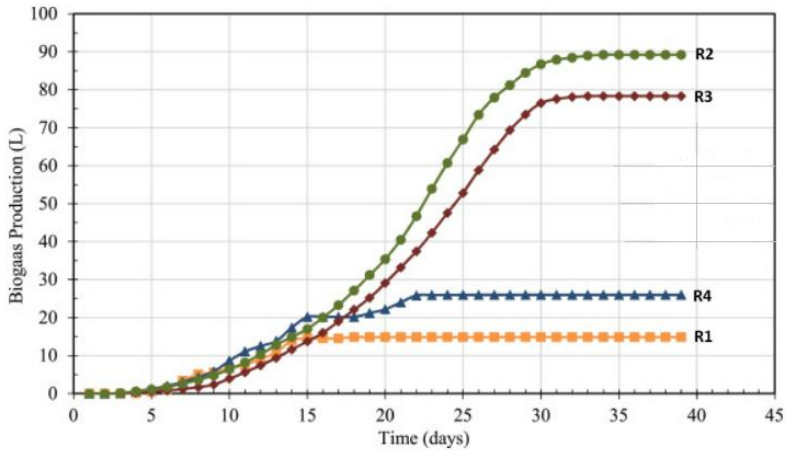

Figure 3. Cumulative biogas production in all four reactors.

Day-to-day gas production was monitored in reactors containing varying amounts of the raw material ((75%ELC+BC) +(25CD)) mixtures. The daily gas measurements were used to identify the peak production period in relation to the initial volatile solid concentration. As illustrated in Figure 4, peak gas generation occurred sooner when the initial VS concentration was higher. Conversely, reactors with lower VS concentrations required a longer duration to reach peak gas production.

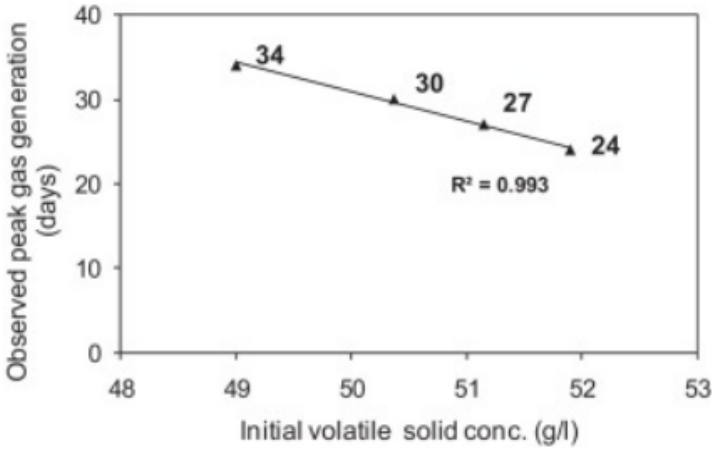

Figure 4. Initial volatile solid concentration with highest gas production time.

3.3 Evaluated COD and VS removal

A significant factor in determining the degradation of organic matter in an anaerobic digestion system is based on the amount of COD and VS removed. The removal of these two components underlines the effectiveness of the AD system and the biochemical processes that enhance the breakdown of organic matter and production of methane gas through the following processes hydrolysis, acidogenesis, acetogenesis as well as methanogenesis (Abbas et al., 2023; Wang et al., 2019). Figure 5. Shows that the highest VS removal by percent was achieved in reactor R2 at 56.6% and reactor R3 recorded the closest highest value at 44.7%. Furthermore, reactor R2 recorded the highest removal of COD at 89.1% reduction level while R1 demonstrated the least at 54.5% COD removal as well as 26.1% VS removal. These outcomes signify the potency of the reactors regarding the biogas production. This study therefore proves that the factors that influence the production of biogas could be the optimal removal of organic matter and by so doing increases the biogas production (Jo et al., 2024).

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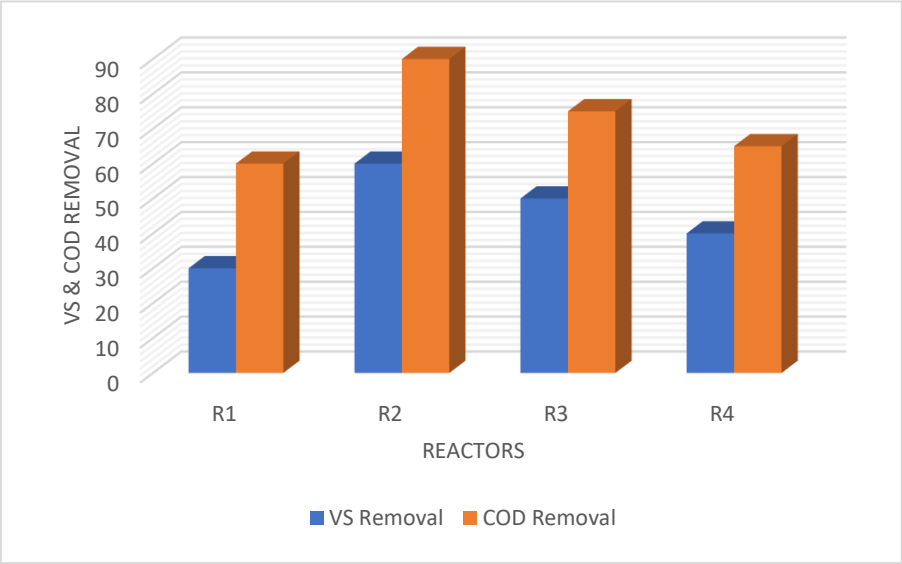

Figure 5. COD and VS removal levels from all reactors (R1, R2, R3, and R4)

3.4 COD reduction effect on biogas production

Anaerobic digestion process reduced the COD in the system significantly. This reduction may be cause by any substrate in the system due to reduction in pollutant load. Figure 6. Shows the cumulative biogas production and COD reduction. COD reduction values recorded from all reactors are as follows; R1 54.50, R2 89.17, R3 70.12, and R4 64.01%. In R2 the highest COD reduction occurred which however, may have resulted in the maximum quantity of gas produced. This COD reduction supports Rahman & Al-Muyeed, (2010).

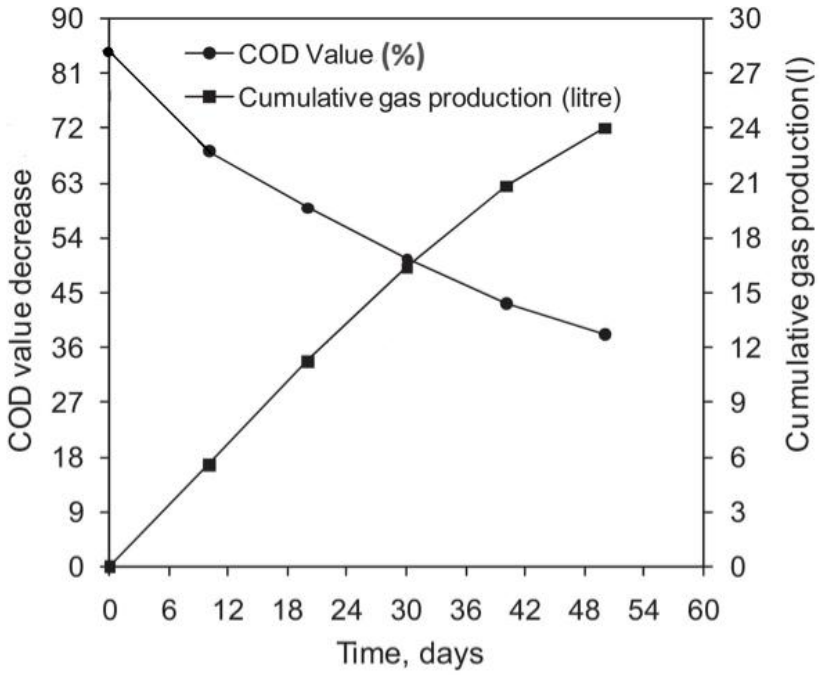

Figure 6. Reactor R2 COD reduction and cumulative gas production.

3.5 Outcome of Cow Dung on biogas generation

Figure 7. demonstrate the result of various percentage of cow dung utilized in varying reactors. Although, the initial pH of egg-laying chicken was high, cow dung was considered to be a buffer in the reactors. Reactor R1 had no cow dung which is considered the major reason for minimum production of gas in the reactor just for 4 days since more

time was needed to digest the egg-laying chicken in the absence of cow dung. Data from all the reactors shows that gas production was optimal at 25% of cow dung present in reactor R2. Cow dung has a C/N ratio value of 24 while egg-laying chicken has 7.7. However, cow dung contains less nitrogen than egg-laying chicken but when cow dung was mixed with egg-laying chicken the C/N ratio increased. Increased cow dung raised the C/N ratio and therefore, reduces the protein composition. Nonetheless, anaerobic digestion is considered not favorable in such conditions.

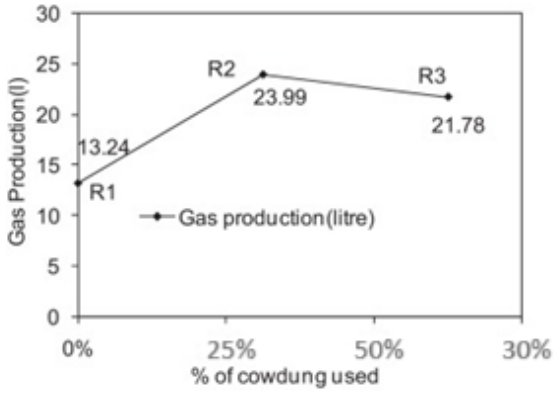

Figure 7. Cow dung production outcome.

3.6 Biogas Generation yield with VS % destruction on all reactors.

Table 2. Gas production yield with VS % destruction on all reactors.

Reactors	VS Reduction (%)	Gas Production Yield (l/g)
R1	29.10	0.59
R2	56.61	0.910
R3	44.70	0.902
R4	36.62	0.70

Conclusion

This research investigated the optimization of biogas production in an anaerobic co-digestion system using egg-laying chicken, broiler chicken and cow dung. The elimination efficacies for COD and VS were significant, with COD reduction extending between 54.5% and 89.1%, while VS removal varied from 29.10% to 56.61%. However, the reactor R2 demonstrated the maximum production of biogas as well as elimination efficacies. The respective degradation efficacies were recorded at 29.10, 56.61, 44.70, and 36.62%. Considering the efficiency of volatile solids degradation and the specific gas production relative to the volatile solid feed, it was established that egg-laying chicken is well-suited for anaerobic digestion when combined with cow dung in a specific ratio to achieve an optimal biogas yield. The ideal mixture for maximum biogas production was found to be 75% egg-laying chicken and 25% cow dung, as observed in the R2 reactor.

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