

Experimental Evaluation of Biogas Potential from Animal Manure, Poultry Litter, and Plant Residues

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Abstract

The present study evaluates the biogas production potential of animal manure, plant leaf waste, poultry bedding (chicken litter), and their co-digested mixture through anaerobic digestion under controlled laboratory conditions. The effects of substrate composition, C: N ratio, biogas quality, methane enrichment, and generation rate were investigated to determine an effective feedstock combination for enhanced renewable energy production. Four experimental sets consisting of individual substrates and a mixed feedstock system were analyzed. The elemental analysis showed significant variation in substrate characteristics, with animal manure, plant leaf waste, and poultry bedding exhibiting C: N ratios of 20.8:1, 38.5:1, and 17.0:1, respectively. The co-digested mixture (E-4) achieved an optimized C: N ratio of 28.3:1, providing favorable conditions for microbial activity and digestion stability. Biogas characterization revealed that feedstock composition significantly affected methane production and fuel quality. The methane content varied from 54.1% to 61.2% for individual substrates, while the E-4 mixture produced the highest methane concentration of 62.7% with lower CO₂ (31.7%) and H₂S (190 ppm) contents. The highest calorific value of 23.6 MJ/m³ was obtained from the mixed substrate. Additionally, E-4 achieved the maximum biogas generation rate of approximately 1.00 m³/kg, demonstrating improved substrate conversion efficiency. The enhanced performance was attributed to the synergistic effect of combining carbon-rich plant residues with nitrogen-containing animal wastes. The findings confirm that anaerobic co-digestion is an effective strategy for agricultural waste management and renewable energy recovery.

Keywords: Anaerobic digestion; Biogas production; Animal manure; Poultry bedding; Plant leaf waste; Co-digestion; C: N ratio; Methane enrichment; Renewable energy;

1. Introduction

The rapid increase in global energy demand, depletion of conventional fossil fuel resources, and growing environmental concerns have encouraged the development of renewable and sustainable energy technologies. Fossil fuels remain the major source of energy worldwide; however, their extensive utilization contributes significantly to greenhouse gas emissions, climate change, and environmental degradation. Therefore, the conversion of renewable biomass resources into clean energy has become an important research area. Among different renewable energy technologies, biogas production through anaerobic digestion (AD) has received considerable attention due to its dual benefits of renewable energy generation and effective organic waste management [1].

Anaerobic digestion is a biological process in which microorganisms convert biodegradable organic materials into biogas under oxygen-free conditions. The produced biogas mainly consists of methane (CH₄) and carbon dioxide (CO₂), where methane acts as the primary energy carrier and can be used for cooking, electricity generation, heating applications, and as an alternative fuel for internal combustion engines. In addition to energy recovery, anaerobic digestion provides an environmentally friendly approach for reducing waste volume, controlling odor emissions, minimizing uncontrolled decomposition, and producing nutrient-rich digestate suitable for agricultural applications [2-3]. Thus, anaerobic digestion represents an effective pathway for integrating waste management with renewable energy production.

Agricultural and livestock sectors generate enormous quantities of organic residues every year, including animal manure, poultry litter, and plant-based agricultural wastes. In many developing and agricultural-based regions, these wastes are commonly disposed of through uncontrolled dumping, open burning, or conventional storage practices, resulting in environmental pollution and greenhouse gas emissions. The improper disposal of animal and poultry wastes can contaminate surface and groundwater resources due to nutrient leaching and may also contribute to the release of ammonia and methane into the atmosphere [4]. Similarly, large quantities of plant residues generated from agricultural activities are often burned in open fields, causing air pollution and loss of valuable biomass resources. Therefore, converting these organic wastes into biogas provides a sustainable solution for reducing environmental impacts while recovering renewable energy [5].

Animal manure is one of the most widely investigated substrates for anaerobic digestion because of its high moisture content, availability, and presence of naturally occurring anaerobic microorganisms. It contains easily degradable organic compounds that support microbial activity during digestion. However, the biogas production potential of animal manure can vary depending on animal type, feeding practices, storage conditions, and physicochemical composition. Although manure provides a stable digestion environment, its relatively low carbon-to-nitrogen (C/N) ratio and moderate organic matter content may restrict methane productivity when used alone. Poultry litter, including chicken bedding waste, is another promising substrate for biogas generation due to its high concentration of organic matter, nitrogen, and energy-rich compounds [6]. The poultry industry produces significant amounts of litter consisting of manure, bedding materials, feathers, and residual feed particles. These components provide a suitable environment for anaerobic microorganisms and can enhance methane production. However, excessive nitrogen content in poultry litter may result in ammonia accumulation during digestion, which can inhibit microbial activity and negatively affect process stability. Therefore, understanding the individual digestion behavior of poultry litter is essential for determining its practical biogas production potential [7].

Plant leaf residues represent an abundant lignocellulosic biomass resource generated from agricultural fields, gardens, and landscaping activities. These residues contain carbohydrates, cellulose, hemicellulose, and lignin, which can serve as substrates for microbial degradation. However, the complex lignocellulosic structure of plant materials often limits hydrolysis, which is considered the rate-limiting step in anaerobic digestion. The biodegradability and methane yield of plant residues depend strongly on their chemical composition, particle size, and pretreatment conditions. Evaluating the performance of plant residues as an individual substrate is important for determining their suitability for renewable energy recovery [8-9].

The efficiency of anaerobic digestion is strongly influenced by substrate characteristics, including total solids (TS), volatile solids (VS), moisture content, organic matter concentration, C/N ratio, pH, and biodegradability. These parameters determine microbial growth, hydrolysis efficiency, and methane formation during digestion. Therefore, the characterization and performance evaluation of individual feedstocks are essential for understanding their inherent biogas generation capability and for designing efficient biogas production systems [10].

Previous research has extensively focused on improving biogas production through co-digestion approaches, where two or more substrates are combined to achieve balanced nutrients, improved C/N ratio, and enhanced microbial activity. Although co-digestion has demonstrated significant advantages, it often makes it difficult to identify the individual contribution and actual biogas potential of each substrate [2, 11]. Limited studies have systematically compared animal manure, poultry litter, and plant residues as separate feedstocks under similar experimental conditions. A clear understanding of the independent performance of these substrates is necessary for selecting appropriate feedstocks for different biogas applications, especially in decentralized rural energy systems where individual waste streams are commonly available.

Despite significant progress in anaerobic digestion research, several knowledge gaps remain regarding the comparative evaluation of individual agricultural waste substrates. Most existing studies emphasize mixed-feedstock digestion, optimization of co-digestion ratios, or improvement of methane yield through external treatments [12]. However, limited attention has been given to evaluating the intrinsic biogas generation characteristics of individual animal manure, poultry litter, and plant residue substrates without the influence of substrate interactions.

The novelty of the present study lies in the experimental investigation and comparative assessment of three different agricultural waste feedstocks under identical anaerobic digestion conditions. This approach enables a direct comparison of substrate-specific biogas production behavior, methane generation potential, and digestion performance. The study provides valuable information regarding the suitability of individual agricultural residues for renewable energy conversion and supports the development of efficient waste-to-energy strategies.

The main aim of this study is to experimentally evaluate the biogas generation potential of individual agricultural waste substrates, including animal manure, poultry litter, and plant residues, through anaerobic digestion. The specific objectives of this research are:

- To analyze the physicochemical characteristics of animal manure, poultry litter, and plant residues relevant to anaerobic digestion performance.
- To experimentally investigate biogas production from each substrate as an individual feedstock.
- To evaluate methane content and overall biogas quality produced from different agricultural wastes.
- To compare the digestion performance and biodegradation behavior of different substrates.
- To identify the most suitable feedstock for renewable biogas production based on gas yield and methane potential.
- To demonstrate the potential of agricultural waste conversion as a sustainable approach for energy recovery and environmental protection.

The findings of this research contribute to a better understanding of individual biomass feedstock behavior during anaerobic digestion and provide valuable guidance for the selection and utilization of agricultural wastes in future biogas production systems.

2. Materials and Methods

This study was conducted to experimentally evaluate the biogas production potential of animal manure, plant leaf waste, and poultry bedding (chicken litter) through anaerobic digestion under controlled laboratory conditions. The experimental methodology consisted of feedstock collection and preparation, substrate characterization, anaerobic reactor operation, biogas measurement, gas composition analysis, and evaluation of digestion performance. Both individual substrates and a selected co-digested feedstock combination were investigated to understand the effect of substrate characteristics and composition on biogas generation, methane production, and overall process stability.

2.1 Experimental Design

A laboratory-scale batch anaerobic digestion system was employed to investigate the biogas production potential of different agricultural and livestock waste substrates. Four experimental conditions were considered, including three individual feedstocks and one co-digestion mixture, as presented in Table 1. The individual substrate experiments were performed to determine the inherent biogas generation capacity of animal manure, plant leaf waste, and poultry bedding. Furthermore, the mixed substrate condition was evaluated to examine the combined influence of different feedstocks on anaerobic digestion performance. The experimental feedstock combinations used in this study are summarized in Table 1.

Table 1. Experimental feedstock combinations used for anaerobic digestion

Experimental set	Animal manure	Plant leaf waste	Poultry Bedding (Chicken Litter)
E-1	100%	-	-
E-2	-	100%	-
E-3	-	-	100%
E-4	35%	30%	35%

2.2 Feedstock Collection and Preparation

The selected substrates, including animal manure, plant leaf residues, and poultry bedding (chicken litter), also shown in figure 1, were obtained from nearby agricultural and livestock facilities. Prior to digestion, all collected materials were subjected to manual sorting to eliminate unwanted contaminants such as foreign particles and non-degradable materials. The cleaned feedstocks were then processed by mechanical size reduction to achieve a particle size of approximately 3–8 mm, which enhanced substrate uniformity and improved microbial accessibility during anaerobic digestion [13].

Animal manure was prepared by mixing with water at a 1:1 ratio (w/w) to obtain a suitable slurry consistency for reactor feeding. Due to the presence of naturally occurring microbial communities, the prepared manure slurry also contributed as an active biological source for the anaerobic digestion process. The plant leaf residues were processed separately due to their lignocellulosic structure, which can limit microbial degradation. A mild alkaline treatment was applied using a 1% NaOH solution to facilitate partial breakdown of complex organic components and improve substrate digestibility. After treatment, the leaf materials were washed to remove remaining alkali residues, dried, and converted into slurry before use in the digestion experiments. Poultry bedding was subjected to preliminary drying and soaking before digestion to improve handling characteristics and reduce the possibility of ammonia-related inhibition caused by its elevated nitrogen content. The treated poultry litter was mixed with water to obtain a uniform slurry suitable for anaerobic digestion. All prepared substrates were adjusted to similar operating conditions, maintaining the total solids concentration within the range of 10–15%. For the co-digestion experiment (E-4), the individual substrate slurries were combined according to the selected composition of 35% animal manure, 30% plant leaf waste, and 35% poultry bedding. The mixture was thoroughly blended to ensure homogeneous distribution of substrate components before being introduced into the anaerobic digestion reactor.

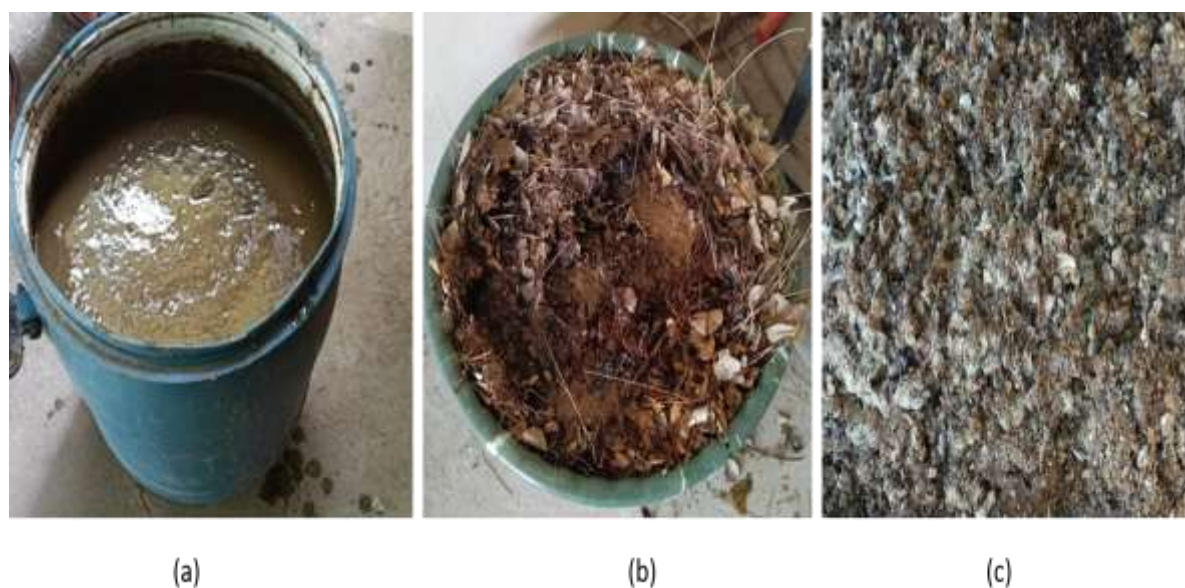


Fig. 1. Photograph of (a) animal manure, (b) plant leaf waste, and (c) chicken litter

2.4 Biogas Measurement and Characterization

The elemental composition of the substrates was determined using CHNS elemental analysis to quantify carbon, hydrogen, nitrogen, and sulfur contents. The obtained carbon and nitrogen values were used to calculate the C:N ratio, which is an important parameter influencing microbial activity and methane generation during anaerobic digestion. The composition and quality of the produced biogas were analyzed by measuring methane (CH_4), carbon dioxide (CO_2), and hydrogen sulfide (H_2S) concentrations. Gas chromatography was employed for accurate determination of methane concentration and evaluation of biogas fuel quality. The energy potential of the generated biogas was assessed through calorific value measurement using a bomb calorimeter. In addition, gas pressure and flow rate were periodically monitored to evaluate reactor stability, gas production behavior, and operational efficiency.

3. Results and Discussion

The present section describes the experimental findings obtained from the anaerobic digestion of animal manure, plant leaf waste, and poultry bedding under controlled laboratory conditions. The results are systematically analyzed and discussed to understand the influence of individual substrate characteristics and co-digestion effects on biogas production performance. The obtained experimental data are interpreted based on biogas generation behavior, methane production potential, substrate degradation efficiency, and overall digestion stability.

3.1 Carbon-to-Nitrogen (C) Ratio Analysis

The carbon-to-nitrogen (C) ratio of different experimental substrates was evaluated using CHNS elemental analysis to determine their suitability for anaerobic digestion and biogas production. The C:N ratio is a critical factor that influences microbial growth, substrate decomposition, and methane formation during the digestion process. Carbon acts as the primary energy source for microorganisms, whereas nitrogen supports microbial cell development and enzyme production. Therefore, an appropriate balance between carbon and nitrogen is essential for maintaining stable anaerobic digestion conditions. Table 2. represent the C:N ratio of different experimental feedstocks.

Table 2. Carbon, nitrogen content, and estimated C:N ratio of different experimental feedstocks

Experiments	Carbon Content (%)	Nitrogen Content (%)	Estimated C: N Ratio (Approx.)
E-1	38.5	1.85	20.8:1
E-2	46.2	1.20	38.5:1
E-3	35.1	2.10	17.0:1
E-4	41.2	1.55	28.3:1

The estimated carbon and nitrogen contents along with the corresponding C:N ratios of all experimental sets are presented in Table 2. The results indicate that the individual feedstocks exhibited significant variation in their elemental composition due to differences in their organic structure and nutrient characteristics.

The animal manure substrate (E-1) showed a carbon content of 38.5% and nitrogen content of 1.85%, resulting in an estimated C:N ratio of 20.8:1. This value indicates that animal manure provides a relatively balanced nutrient environment for anaerobic microorganisms. The presence of naturally available microorganisms and moderate nitrogen concentration makes manure a suitable substrate for biogas production.

The plant leaf waste substrate (E-2) exhibited the highest carbon content (46.2%) and the lowest nitrogen content (1.20%), resulting in a higher C: N ratio of 38.5:1. The elevated C:N ratio can be attributed to the presence of carbon-rich lignocellulosic components such as cellulose and hemicellulose in plant residues. Although these carbon compounds provide a significant energy source, insufficient nitrogen availability may restrict microbial growth and reduce digestion efficiency if used alone [14].

The poultry bedding substrate (E-3) showed a carbon content of 35.1% and nitrogen content of 2.10%, producing a lower C:N ratio of 17.0:1. The lower ratio was associated with the higher nitrogen concentration present in poultry litter. Although nitrogen is necessary for microbial activity, excessive nitrogen levels may lead to ammonia accumulation during anaerobic digestion, which can negatively affect methanogenic microorganisms and reduce methane production.

The co-digested substrate combination (E-4), containing 35% animal manure, 30% plant leaf waste, and 35% poultry bedding, demonstrated a carbon content of 41.2% and nitrogen content of 1.55%, resulting in a C:N ratio of 28.3:1. This value represents a more balanced nutrient composition compared with individual substrates. The combination of carbon-rich plant residues with nitrogen-containing animal and poultry wastes improved the overall C balance, creating

favorable conditions for microbial activity and methane generation. Overall, the results indicate that the mixed substrate (E-4) achieved a C:N ratio within the generally recommended range for anaerobic digestion, which supports effective microbial conversion of organic matter into biogas. The improved nutrient balance of the co-digested feedstock is expected to contribute to enhanced biogas production and digestion stability compared with individual substrates [15].

3.2 Biogas Quality Analysis

The compositional characteristics and fuel quality parameters of biogas produced from different experimental feedstocks (E-1 to E-4) are presented in Table 3. The analyzed parameters include methane (CH₄), carbon dioxide (CO₂), hydrogen sulfide (H₂S), and calorific value (CV), which are important indicators for evaluating the suitability of biogas for energy applications. The variation in gas composition among different experimental sets demonstrates the influence of substrate characteristics and nutrient composition on anaerobic digestion efficiency.

The methane concentration is considered the most important quality parameter because methane directly contributes to the energy content of biogas. The animal manure substrate (E-1) produced biogas with a methane content of 58.6%, along with 39.5% CO₂ and 380 ppm H₂S, resulting in a calorific value of 20.9 MJ/m³. The obtained methane concentration indicates effective microbial conversion of organic matter present in animal manure. However, the comparatively higher CO₂ content suggests that a portion of the degraded organic matter remained in the form of intermediate carbon compounds rather than being completely converted into methane [16,19].

The plant leaf waste substrate (E-2) showed the lowest methane concentration (54.1%) among all experimental conditions, with a higher CO₂ content of 44.2% and a calorific value of 19.3 MJ/m³. The lower methane production can be attributed to the complex lignocellulosic structure of plant residues, which restricts hydrolysis and slows microbial degradation. The presence of cellulose, hemicellulose, and lignin components limits the accessibility of organic matter to anaerobic microorganisms, resulting in reduced methane enrichment.

The poultry bedding substrate (E-3) exhibited improved biogas quality compared with individual manure and plant residue digestion, producing 61.2% methane and 36.6% CO₂, with a calorific value of 22.1 MJ/m³. The higher methane content may be associated with the presence of easily biodegradable organic compounds and higher nutrient availability in poultry litter. However, the H₂S concentration (450 ppm) was higher than that of other substrates due to the presence of sulfur-containing compounds and protein-rich materials in poultry waste [17].

The co-digestion mixture (E-4), consisting of animal manure, plant leaf waste, and poultry bedding, demonstrated the highest overall biogas quality performance. This combination produced 62.7% methane, the lowest CO₂ concentration (31.7%), and a reduced H₂S concentration of 190 ppm compared with individual poultry bedding digestion. The calorific value of E-4 was also highest (23.6 MJ/m³), indicating improved fuel characteristics. The enhanced performance of the mixed substrate can be attributed to improved nutrient balance, optimized C: N ratio, and synergistic interactions among different feedstocks, which promoted microbial activity and improved conversion of organic matter into methane. Overall, the results indicate that substrate composition significantly affects biogas quality and energy potential. Among all experimental conditions, the co-digestion system (E-4) provided better methane enrichment, lower impurity content, and higher calorific value, demonstrating its suitability for renewable energy generation compared with individual substrate digestion [18-20].

Table 3. Biogas composition and quality parameters of different experimental feedstocks

Mixture	CH ₄ (%)	CO ₂ (%)	H ₂ S (ppm)	CV (MJ/m ³)
E-1	58.6	39.5	380	20.9
E-2	54.1	44.2	180	19.3
E-3	61.2	36.6	450	22.1
E-4	62.7	31.7	190	23.6

3.3 Biogas Production Rate

The biogas generation rate obtained from different experimental feedstocks (E-1 to E-4) is presented in Fig. 2. The results demonstrate a clear variation in biogas production potential depending on the substrate composition. The

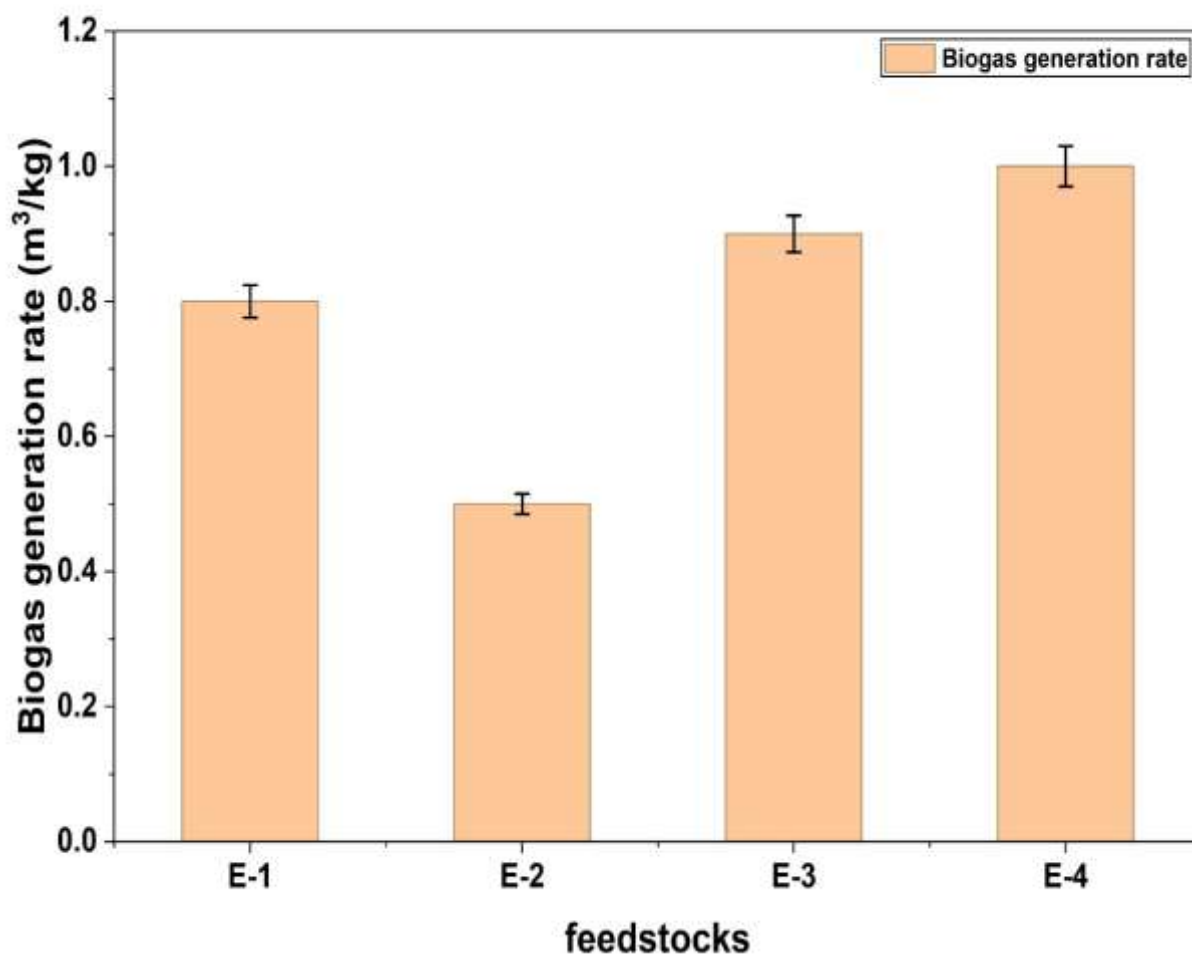
observed differences are mainly attributed to the variation in organic matter content, biodegradability, nutrient balance, and microbial activity associated with each feedstock. The animal manure substrate (E-1) exhibited a biogas generation rate of approximately 0.80 m³/kg. The moderate gas production from animal manure can be attributed to its favorable moisture content, availability of biodegradable organic compounds, and presence of naturally occurring anaerobic microorganisms. However, the relatively lower yield compared with mixed digestion indicates that individual manure digestion may have limitations due to its nutrient composition and carbon availability [19].

The plant leaf waste substrate (E-2) showed the lowest biogas generation rate of approximately 0.50 m³/kg. This reduced performance can be associated with the complex lignocellulosic structure of plant residues, which restricts hydrolysis and slows down microbial degradation. The presence of cellulose, hemicellulose, and lignin reduces the accessibility of organic components, resulting in lower conversion efficiency during anaerobic digestion [19].

The poultry bedding substrate (E-3) demonstrated an improved biogas generation rate of approximately 0.90 m³/kg. The higher gas production compared with animal manure and plant residues can be related to the presence of easily degradable organic matter and higher nutrient availability. Poultry litter provides a suitable substrate for microbial growth; however, excessive nitrogen content may influence digestion performance due to possible ammonia accumulation [20].

The co-digestion mixture (E-4), consisting of animal manure, plant leaf waste, and poultry bedding, achieved the highest biogas generation rate of approximately 1.00 m³/kg. The enhanced performance of E-4 is attributed to the synergistic effect of combining different substrates, which improved the overall nutrient balance and provided a more suitable C: N ratio for anaerobic microorganisms [19]. The combination of carbon-rich plant residues with nitrogen-containing animal and poultry wastes promoted efficient organic matter degradation and increased biogas production.

Overall, Fig. 2 indicates that co-digestion significantly improved biogas generation compared with individual substrate digestion. The higher yield obtained from E-4 confirms that appropriate substrate combination can enhance microbial activity, improve digestion stability, and maximize renewable energy recovery from agricultural wastes [20].



After completion of the biogas generation process, the raw biogas collected from all experimental mixtures was subjected to a three-step purification treatment to improve its fuel characteristics. The upgrading process was designed to remove major contaminants, including carbon dioxide (CO_2), moisture (H_2O), and hydrogen sulfide (H_2S), which can reduce combustion efficiency and affect equipment performance. The purification process resulted in significant improvement in biogas quality by increasing the methane concentration. Following the upgrading treatment, the purified biogas achieved a methane content of approximately 94.5% CH_4 , indicating a high-quality gaseous fuel with characteristics similar to conventional natural gas. The enhanced methane enrichment increased the energy density and improved the suitability of the upgraded biogas for practical energy applications [19-20]. The purified gas showed a density of approximately 0.671 kg/m^3 and an octane number of nearly 115, demonstrating favorable combustion properties and high resistance to knocking during engine operation. The obtained results confirm that effective biogas purification and upgrading can transform raw biogas into a cleaner and higher-value fuel. The improved methane concentration and combustion characteristics highlight the potential of upgraded biogas as an alternative renewable fuel for power generation and transportation applications [20].

4. Conclusion

The present study evaluated the biogas generation potential of animal manure, plant leaf waste, poultry bedding, and their co-digested mixture through anaerobic digestion under controlled laboratory conditions. The effect of substrate composition, C: N balance, biogas quality parameters, and generation rate was investigated to identify an effective feedstock combination for improved renewable energy production. The major conclusions obtained from this study are summarized as follows:

- The elemental analysis confirmed that substrate composition significantly influenced the C:N ratio and digestion performance. Animal manure (E-1) exhibited a balanced C: N ratio of 20.8:1, whereas plant leaf waste (E-2) showed a higher C: N ratio of 38.5:1 due to its carbon-rich lignocellulosic structure. Poultry bedding (E-3) exhibited a lower C:N ratio of 17.0:1 because of its higher nitrogen content.

- The co-digestion mixture (E-4) achieved an optimized C: N ratio of 28.3:1, which falls within the suitable range for anaerobic digestion. The balanced carbon and nitrogen availability enhanced microbial growth, improved organic matter degradation, and supported stable methane production.
- Biogas quality analysis revealed that feedstock characteristics directly affected methane enrichment and fuel properties. The methane concentration of individual substrates varied from 54.1% to 61.2%, whereas the co-digested substrate (E-4) produced the highest methane concentration of 62.7%.
- The co-digestion approach improved overall biogas quality by reducing undesirable gas components. The E-4 mixture showed the lowest CO₂ concentration (31.7%) and reduced H₂S content (190 ppm) compared with individual poultry bedding digestion, indicating better gas quality and improved suitability for energy applications.
- The calorific value of biogas was strongly influenced by methane concentration. The highest calorific value was obtained for E-4 (23.6 MJ/m³), demonstrating enhanced energy recovery potential compared with individual feedstocks.
- The biogas generation rate results confirmed the advantage of substrate mixing. Plant leaf waste (E-2) showed the lowest biogas generation rate (0.50 m³/kg) due to its resistant lignocellulosic structure, while the co-digested mixture (E-4) achieved the highest generation rate of approximately 1.00 m³/kg.
- Overall, the results demonstrate that anaerobic co-digestion of animal manure, plant leaf waste, and poultry bedding provides improved digestion stability, enhanced methane production, better biogas quality, and higher energy recovery compared with single-substrate digestion. The optimized feedstock combination represents an effective strategy for agricultural waste management and sustainable renewable energy production.

Future studies can investigate the influence of different mixing ratios of animal manure, plant residues, and poultry bedding to further optimize biogas yield and methane enrichment. Long-term continuous digestion experiments using semi-continuous reactors can be conducted to evaluate the practical stability and scalability of the optimized substrate mixture.

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Pankaj Kumar Srivastava: Writing–review, conceptualization, & editing, Supervision.

Prashant Singh Chauhan: Writing–original draft, Review & editing, Supervision.

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