Optimization of biogas production from co-digestion of sugarcane trash blended with sugarcane vinasse

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Abstract: Biogas, a critical form of biofuel, is produced through the anaerobic digestion of biomass. This process not only provides renewable energy and fertilizer but also reduces greenhouse gas emissions and repurposes agricultural and industrial waste. Co-digesting sugarcane trash with sugarcane vinasse has the potential to enhance biogas production while mitigating environmental impacts. However, the technical feasibility of this blend and its potential biogas yield from pre- and post-treatment processes require further validation. This study aimed to optimize biogas production by co-digesting sugarcane trash and vinasse. Feedstock characterization showed an optimal carbon-to-nitrogen ratio and adequate moisture content, confirming its suitability for anaerobic digestion. Using an orthogonal array design, the study optimized parameters such as mixed ratios, temperature, and retention time. The optimal conditions for biogas production were identified as a 2:1 sugarcane trash-to-vinasse ratio, a temperature of 45°C, and a retention time of 20 days, significantly improving biogas yield. Analysis revealed that nitrogen and hydrogen from vinasse were crucial for enhancing methane production. Proximate analysis confirmed the low ash content and high volatile matter in sugarcane trash, making it ideal for biogas production. The study recommends managing moisture levels, selecting low-sulfur feedstock blends, and sustainability assessments for commercial-scale biogas production.

Keywords: biogas, anaerobic digestion, biofuel, sugarcane vinasse, co-digestion, optimization

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1 Introduction

Sugarcane is one of the world's most significant cash crops, cultivated on approximately 26 million hectares across 115 countries, with a global production of about 1.91 billion tons annually (Adarme et al., 2019). Beyond its primary product, sugarcane generates a range of by-products such as animal feed, bioenergy, biofuels, bioplastics, paper, and fertilizers, primarily derived from residues like bagasse and trash. These residues contain valuable components, including hemicellulose, lignin, proteins, and cellulose, whose composition depends on soil characteristics and the plant's maturity (Purwanta et al., 2022). The in-

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creasing consumption of fossil fuels has raised concerns about environmental degradation and the depletion of energy resources, necessitating a global shift towards renewable energy sources (Colla et al., 2019). Biomass is a prominent renewable and sustainable energy source that can serve as an alternative to fossil fuels (Da Silva et al., 2024).

Among biomass energy options, biogas stands out due to its rapid conversion and lower capital requirements compared to other renewable energy sources. Biogas, derived from the anaerobic digestion of various biomass sources, offers dual benefits: contributing to renewable energy generation and mitigating environmental challenges by repurposing agricultural residues. Sugarcane bagasse, a residue rich in carbohydrates, hemicellulose, and cellulose, is particularly promising for second-generation biofuel production, where enzymatic hydrolysis converts its components into simple sugars (Gong and Lunelli, 2024). Additionally, the sugar industry produces significant amounts of wastewater, such as vinasse, which, if discharged untreated into ecosystems, poses environmental risks due to its high biological and chemical oxygen demand (Evidente and Almendrala, 2022).

Vinasse is generated during the cleaning of equipment such as evaporators, clarifiers, and centrifuges. Sugarcane trash, a non-crushable biomass residue consisting of tops and dried leaves, also holds potential for energy generation. It constitutes approximately one-third of sugarcane's total energy content and remains underutilized. Studies have demonstrated that combining molasses with sugarcane trash enhances the concentration of easily degradable sugars, indicating its suitability as a feedstock for biogas production (Malekzadeh et al., 2020). Against this backdrop, the current study aims to evaluate the feasibility of utilizing liquid sugarcane waste and sugarcane trash (SCT) as substrates for biogas production. The global push for alternative energy sources is driven by economic, environmental, and national security concerns. This has increased demand for sustainable and cost-effective energy solutions utilizing both food-based and non-food-based substrates (Marafon et al., 2020). Sub-Saharan Africa faces significant challenges in energy access, which biomass and wastewater energy generation can address. In countries like Brazil, sugarcane biomass energy has already provided access to renewable energy for approximately 5 million people, demonstrating the potential of biomass for energy security and socio-economic development (Mendes et al., 2023).

The sugar sector plays a vital role in Kenya, employing about 260,000 farmers and 11,700 workers and supporting over 6 million Kenyans directly or indirectly. Expanding sugar production and leveraging its residues for energy production present a significant opportunity for Kenya's socio-economic growth. During the immense production of sugarcane harvests from farmers in Ndhiwa-Sub-County in October yearly, Sukari Industry, produces about 500 tonnes and 200 litres per day of sugarcane trash and sugarcane vinasse respectively. Technological advancements have improved the use of anaerobic digestion (AD) for processing sugarcane vinasse. AD is a resource recovery method that uses sugarcane residues to produce bioenergy and reduce environmental degradation. Recent research has focused on the potential of blending sugarcane trash and vinasse for optimized biogas production (Wongarmat et al., 2022). However, the feasibility and optimization of such processes in Kenya's sugarcane sector remain underexplored, leaving a critical knowledge gap.

Vinasse, an environmentally challenging by-product, can be converted into methane through anaerobic digestion, offering a dual benefit of waste management and renewable energy production (Nualsri et al., 2024). Research has shown that pre-treatment techniques like diluted acid hydrolysis improve the efficiency of anaerobic digestion by breaking down complex organic materials into simpler substrates. However, most studies have focused on other sectors, such as coffee and beer industries, rather than the sugarcane sector (Rodrigues et al., 2023). This highlights the need for context-specific studies to optimize the use of sugarcane residues in Kenya. Sugarcane trash and vinasse, when blended, can significantly reduce environmental degradation by generating biogas. Despite the promising potential, the technical feasibility of this approach, particularly concerning pre-treatment and post-treatment processes, remains under-researched. For example, hydrolytic microbes play a critical role in breaking down substrates during anaerobic digestion, releasing enzymes such as lipase, xylanase, and cellulase (Niz et al., 2021). These processes rely on stringent and facultative anaerobes like Clostridium and Streptococci. However, research has not adequately addressed how these processes function within the Kenyan agricultural and sugarcane context.

Optimizing biogas production from sugarcane residues involves analyzing factors such as substrate properties, inoculum ratios, temperature, pH, and retention time. Research suggests that mix ratios, such as 1:1 or 2:1, yield optimal biogas output, but these ratios vary depending on the substrate (Takeda et al., 2022). Studies using the Taguchi approach have demonstrated the value of systematically optimizing these parameters for enhanced biogas production. However, the application of such techniques in Kenya's sugarcane sector is limited. Further, the low sulfur content of hemicellulose hydrolysate (HH) produced during sugarcane processing can reduce sulfur concentrations in vinasse, making it more suitable for biogas production (Adarme et al., 2022). Research has also indicated that vinasse digestate, a partially broken-down substrate, has less microbial growth inhibition and lower phenolic compound content, improving the efficiency of anaerobic digestion (Gbadeyan et al., 2024). However, these findings primarily focus on international contexts, underscoring the need for localized studies in Kenya.

Kenya's sugarcane sector provides a unique opportunity to harness sugarcane trash and vinasse for renewable energy. The sector generates significant biomass and wastewater, which, if effectively utilized, could reduce dependency on fossil fuels and contribute to environmental sustainability. By investigating the feasibility of biogas production from sugarcane trash and vinasse, this study seeks to address a critical gap in renewable energy research in Kenya. This study aims to evaluate the feasibility of utilizing a combination of sugarcane trash and vinasse for biogas production, optimizing the process for maximum energy yield while addressing environmental and energy challenges in Kenya's sugarcane sector.

2 Materials and methods

The study was conducted between June and September 2022 in sugarcane growing areas of Ndhiwa Sub-County at the Sukari Industry in Homabay County, Kenya with latitudes 00.81794°S and longitudes 034.37871°E on GPS coordinates. This location was strategically chosen because of its importance in sugarcane farming and processing. Ndhiwa Sub-County has extensive sugarcane cultivation, providing a plentiful supply of sugarcane trash, while the Sukari Industry, a major sugar production facility, generates large quantities of sugarcane vinasse, as a by-product (Wongarmat et al., 2022). The proximity of these two sites allowed for the timely collection of fresh sugarcane trash and sugarcane vinasse, ensuring access to high-quality substrates for experimentation. Furthermore, the research site's relevance aligns with the broader aim of the study, which is to address the environmental and energy challenges posed by sugarcane wastes in the local region.

The process of preparing sugarcane trash involved getting rid of coarse inorganic extraneous components which was done by rinsing the trash under high pressure running water. After that, the washed trash was shredded into smaller pieces about 10 mm long and dried at 80°C for five days to attain an approximate 10% moisture level as a dry base (Volpi et al., 2023). Using a sugarcane miller, the dry trash was pulverized and then run through a 2.0 mm mesh. The ground-up waste was placed into bags, autoclaved and sterilized for 30 minutes at 121°C to promote enzymatic fermentation and hydrolysis. Nine replicates were performed for each experimental condition. The desired blends of sugarcane vinnase to sugarcane trash for the experiments was determined by choosing ratios such as 1:1, 1:2, and 2:1 which were attained after comparing the studies of Albuquerque et al. (2019), Alruqi and Sharma (2023) and Rodrigues et al. (2023) of sugarcane trash and sugarcane vinasse used independently to produce biogas. The appropriate quantities of vinnase and sugarcane trash were determined and combined in a mixing container to ensure a homogeneous mixture. The prepared mixture was stored in airtight containers to maintain its consistency and prevent further contamination before use in the experimental setups. This process ensured a consistent and replicable preparation of sugarcane vinnase mixed with sugarcane trash for various experimental applications.

The physical and chemical properties of sugarcane trash blended with sugarcane vinasse were determined by ultimate and proximate analyses. The ultimate analysis helped in determining the percentages of hydrogen, oxygen, sulphur, carbon, and nitrogen in biomass employing a TrueSpec CHN LECO elemental analyzer. The ASTM D5373 methodology was utilized to analyse 0.05–0.1 g samples on a dry basis. TrueSpec LECO software was used to record results which were obtained from the furnace heated to 950°C, incorporating helium and oxygen (Rodrigues et al., 2023). Fixed carbon, volatile matter, ash, and moisture content were determined by applying proximate analysis, utilizing GA-701 LECO thermogravimetric analyzer. During the process 0.5 kg were independently analysed. Volatile matter was removed at 950°C within inert atmosphere and moisture content at 105°C -110°C. The ash content was obtained by burning the residue in oxygen subjected to above 700°C (González et al., 2022). The Taguchi Experimental design was applied to determine the optimization of biogas with relation to the tested factors and levels. This enabled the identification of the best combination of parameters and levels for biogas yield maximization. The parameters comprised mixed ratio (1:1, 1:2, and 2:1), pH range (at constant value of 6.8), retention time (16, 18, and 22 days), and temperature (25°C, 35°C, and 45°C).

The study employed Taguchi design of experiment (DoE) designs, Taguchi- grey relational analysis (GRA) and ANOVA to examine and investigate the data and also optimize biogas production from the combination of sugarcane trash and sugarcane vinasse. This design choice allows for controlled manipulation of key variables, facilitating the exploration of their impact on the given phenomenon. A range of variables, namely temperature, retention duration, and mix ratio, were thoroughly investigated. The use of the Taguchi design methodology permits a methodical analysis of these variables on several levels.

The analysis of variance (ANOVA) conducted in this experiment aimed to assess the significance of the effects of individual interactions among the parameters on biogas yield which included mixed ratio, temperature and retention time. Mix ratios were explored to ascertain their influence on the carbon-to-nitrogen (C/N) ratio, which is vital for microbial activity and biogas production. Temperature and retention time variations were evaluated to understand their effects on biogas generation (Valmaña García et al., 2019). The process for performing the ultimate analysis is detailed in part two of this paper. The C/N ratio, is an essential metric for assessing the substrate's viability in biogas production. Calorific values were computed utilizing the ultimate analysis data provided, comprising percentages of hydrogen (H), nitrogen (N), carbon (C), sulphur (S), and oxygen (O). The higher heating value (HHV) was estimated using the Dulong formula for solid fuels as shown in Equation 1.

$$\text{Energy Content (MJ/m}^3\text{)} = \text{Element Percentage (\%)} \times \text{HHV of the Element (MJ/m}^3\text{)} \quad (1)$$

Source: Arruda et al. (2025)

The percentage of methane was calculated using formula shown in Equation 2.

$$Methane(\%) = \left(\frac{calorific\ value\ of\ biogas}{39.8}\right) \times 100 \quad (2)$$

Source: Rashidi et al. (2024)

Where: Higher heating value (HHV) of methane =39.8 MJ/m^3

In addition, fixed carbon (FC) was determined using Equation 3:

$$FC = 100\% - (\text{moisture content \%} + \text{ash content \%} + \text{volatile matter \%}) \quad (3)$$

Source: Rodrigues et al. (2023)

The Equation 4 calculates the signal-to-noise (S/N) ratios.

$$S/N(dB)=10log(y^2) \quad (4)$$

Where: y = is the biogas yield (m^3); Source: Wongarmat et al. (2022).

The experimental biogas setup began with substrate collection and preparation, after which the organic material was introduced into the system through the feeding hopper—a slot located on the barrel lid. The substrate passed into the inlet pipe, which guided it directly into the main digester chamber, a sealed barrel where anaerobic digestion took place. Inside this chamber, a curved aperture located 80 cm above the digester base helped regulate the maximum substrate level and created space for biogas buildup. The substrate, once in the chamber, underwent microbial decomposition under controlled conditions. To maintain an optimal digestion temperature, the system used a temperature control mechanism. This included electric immersion heaters placed inside a water tank, powered by a power supply and regulated by a thermostat. The heated water was circulated around the digester by a centrifugal pump driven by an electric motor, with flow controlled by a gate valve and managed through inlet and outlet pipes.

Real-time temperature monitoring in the experimental setup was achieved using thermocouple wires embedded within the system, which transmitted data to a control unit equipped with Proportional-Integral-Derivative (PID) and Programmable Logic Controllers (PLCs). These controllers automatically regulated the immersion heaters to maintain the desired temperature conditions. System operations were continuously monitored through a data logger connected to a computer, while a power logger recorded energy consumption. A communication system (Com) facilitated seamless data transfer between monitoring components. Biogas generated in the digester chamber exited via a biogas output slot, which was connected to a 12-inch plastic pipe and a flexible tube, directing the gas into an expansion chamber for temporary storage. A dedicated outlet allowed excess gas to be safely flared. For biogas volume measurement, the collected gas was channeled into a graduated cylinder that was partially filled with water and inverted in a water-filled container. As the gas entered the cylinder, it displaced the water into a bucket. The displaced water volume corresponded precisely to the amount of biogas produced, enabling accurate measurement. This water displacement method is a reliable and commonly used approach in laboratory-scale biogas studies, ensuring consistent and precise gas quantification. The process completed the biogas generation, monitoring, collection, and measurement cycle.

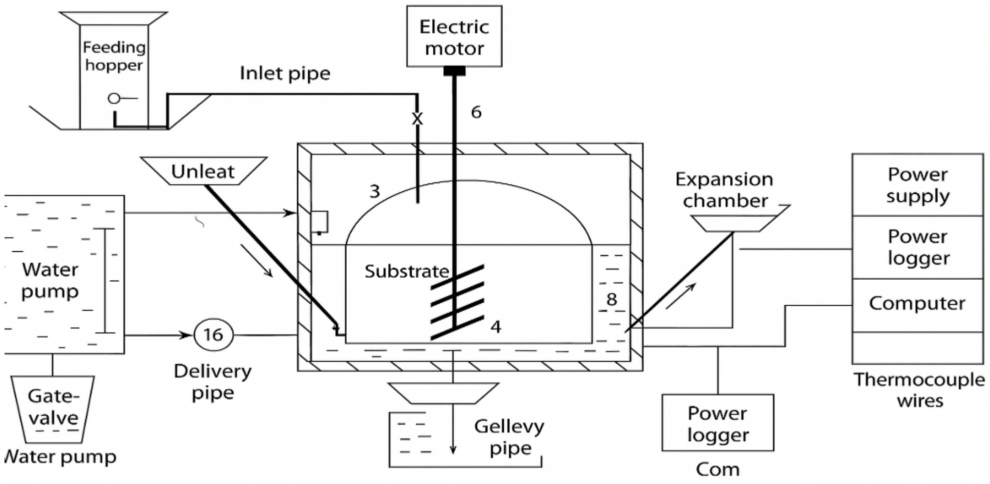

Figure 1 Block diagram of the digester set-up

3 Results and discussion

3.1 Physical and chemical properties of sugarcane trash blended with sugarcane vinasse

The ultimate and proximate analyses helped in determining physical and chemical properties of sugarcane trash blended with sugarcane vinasse. The physical properties analyzed were the moisture content and particle size distribution of the blends. Chemical properties involved the analysis of fixed carbon, volatile matter, hydrogen, Sulphur, oxygen, and nitrogen.

3.1.1: Ultimate analysis

Figure 2 shows that sugarcane vinasse is characterized by a composition of 40.23% carbon (C), 6.12% hydrogen (H), and 1.65% nitrogen (N). The composition of carbon, hydrogen, and nitrogen is consistent with other biomass studies, where carbon typically ranges from 35%-50%, with similar hydrogen and nitrogen values in this study. The results indicate that sugarcane vinasse exhibited a composition possessing a higher nitrogen content of 1.65% compared to sugarcane trash (1.50%), making it a potentially favourable substrate to complement the low nitrogen content in sugarcane trash for biogas production (Silva et al., 2021). Nitrogen is crucial for microbial activity during anaerobic digestion and its presence in sugarcane vinasse could enhance biogas production efficiency. The relatively higher hydrogen content of 6.12% in sugarcane vinasse than 5.12% hydrogen content in sugarcane trash, implies that vinasse is potentially an effective biogas feedstock, as hydrogen is essential for methane formation. Similar results were reported by Kiplagat et al. (2022) that substrates with nitrogen content of above 1.5% and hydrogen content of above 6% enhanced microbial activity and methane production in an assessment of waste biomass and blended bio-resources in biogas production.

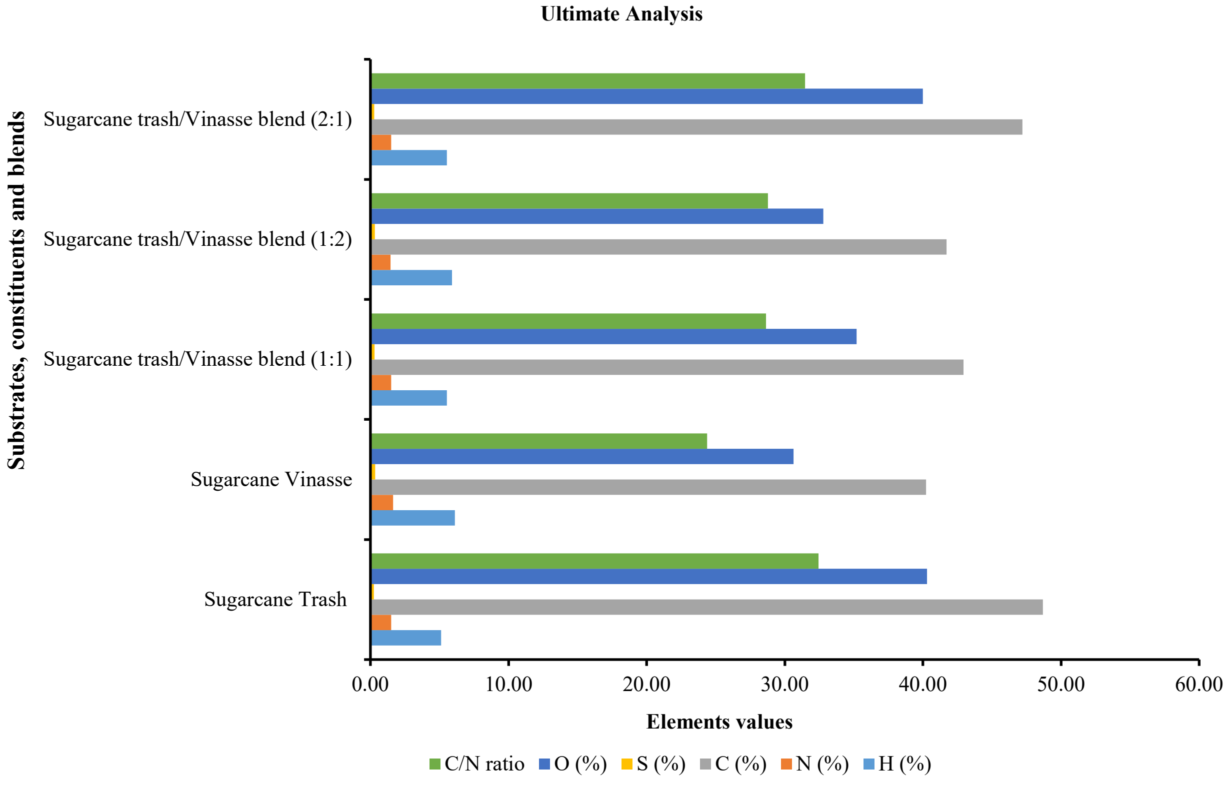

Figure 2 Ultimate analysis of sugarcane trash, vinasse, and their blends

The analysis of sugarcane trash composition revealed a predominant carbon (C) content of 48.68%, crucial for biogas generation. This high carbon content suggested the potential for significant methane production during anaerobic digestion (Tshemese et al., 2023). Moreover, the relatively elevated oxygen content of 40.30% implies the presence of cellulose and hemicellulose, valuable substrates for microbial activity in biogas production. The low sulphur content of 0.25% is also advantageous, reducing the risk of sulphur or its compounds emissions during biogas production hence contributing to environmental sustainability.

Sugarcane vinasse is characterized by a composition that includes 40.23% carbon (C), 6.12% hydrogen (H), 30.64% oxygen (O) ,0.35% Sulphur (S) and 1.65% nitrogen (N). Notably, sugarcane vinasse exhibits a higher nitrogen content compared to sugarcane trash, making it potentially favourable substrate for biogas production (Sitthikitpanya et al., 2024). Nitrogen is an essential element for microbial activity during anaerobic digestion, and its presence in vinasse can enhance the efficiency of the biogas production process. The analysis of the substrates reveals that sugarcane trash has a relatively high C/N ratio of 32.45:1, suggesting slower degradation as a biogas feedstock but offering stable organic matter for soil fertility (Niz et al., 2021). In contrast,vinasse has a much lower C/N ratio of 24.38:1, indicating its potential as a readily available nitrogen source for biogas production and rapid degradation in the biogas reactor. However, Vinasse according to the results also contains high levels of hydrogen and sulphur, which can benefit soil fertility.

The blend of sugarcane trash and sugarcane vinasse in a 1:1 ratio is a combination of carbon-rich nature of sugarcane trash with the higher nitrogen content of sugarcane vinasse. The presence of 35.2% oxygen (O) and a C/N ration of 28.63:1 in the blend offered additional potential as a substrate for biogas production. The significant carbon content of 42.95% suggested the blend's capacity for methane generation during anaerobic digestion (Silva et al., 2021). The findings imply that incorporating both sugarcane trash and sugarcane vinasse in equal proportions provided a balanced feedstock with favourable characteristics for biogas production. Similar results were reported during biomethane production by thermophilic co-digestion of sugarcane vinasse. The 2:1 blend had 5.54% hydrogen, 1.50% nitrogen, 47.2% carbon, 0.28% sulphur, and 40% oxygen, showing a high amount of carbon and a favourable 31.46:1 C/N ratio. The characteristics indicate a favourable balance for microbial digestion, which potentially leads to efficient production of biogas. The moderate amount of nitrogen in the blend minimizes the ammonia formation, whereas the low content of sulphur limits the formation of hydrogen sulphide (H_2S), which negatively affects the biogas yield (Rogeri et al., 2024).

Comparatively, the 1:2 blend showed a slightly higher composition of hydrogen at 5.9%, lower nitrogen of 1.45%, 41.71% carbon, 32.80% oxygen, 28.77:1 C/N ratio, and a higher amount of sulphur at 0.32%. The lower carbon content and C/N ratio compared to 2:1 which has a C/N ratio of 31.36:1 potentially limits its potential for biogas production, since a lower carbon content may hinder an anaerobic action even if the C/N ration falls with the recommended range of 20:1 to 30:1 (Adarme et al., 2019). Nonetheless, the contents of nitrogen and sulphur fall within manageable levels, implying that blend 1:2 is viable for biogas yield. The slightly higher sulphur content in this blend compared to 2:1 blend shows a potential increase in H_2S formation, which demand adequate gas sweetening costs to reduce corrosions (Agarwal et al, 2022). The calorific values were determined using Equation 1 based on the elemental composition.

As presented in Figure 3, the sugarcane trash/vinasse (1:1) blend showed a calorific value of 22.99 $MJkg^{-1}$, indicating a moderate energy content conducive for biogas production, with a pH value of 7.0, also falling within the optimal pH range of 6.5-8.5 for efficient biogas production (Rahman et al., 2019). The moisture content of the (1:1) blend measures 55%, deemed acceptable for anaerobic digestion, since the acceptable range lies between 50%-70%, as recommended by Meng et al. (2020). However, it was crucial to manage moisture levels adequately to avoid lower gas yields and prolonged retention times. Based on Figure 3 results, the 1:2 blend of sugarcane trash and sugarcane vinasse exhibited a calorific value of 22.09 $MJkg^{-1}$ and a pH of 6.9. The high moisture content of 58% lies within the recommended range of optimum moisture level (Purwanta et al., 2022). This moisture content is higher than 55% in the 1:1 blend. These properties collectively suggested that the 1:2 blends might not be as effective a feedstock for biogas production as the blend 1:1. The low calorific value and pH implied that this blend was suboptimal for biogas production, with the high moisture content potentially leading to reduced gas yields and extended retention times, as per the study by Malekzadeh et al. (2020). From Figure 3, the 2:1 blend, comprising a higher proportion of sugarcane trash demonstrated a high calorific value of 23.38 MJ kg^{-1}. The blend has a low pH of 7.1. These two conditions make this blend the most favorable for biogas production, as compared to lends 1:1 and 1:2.

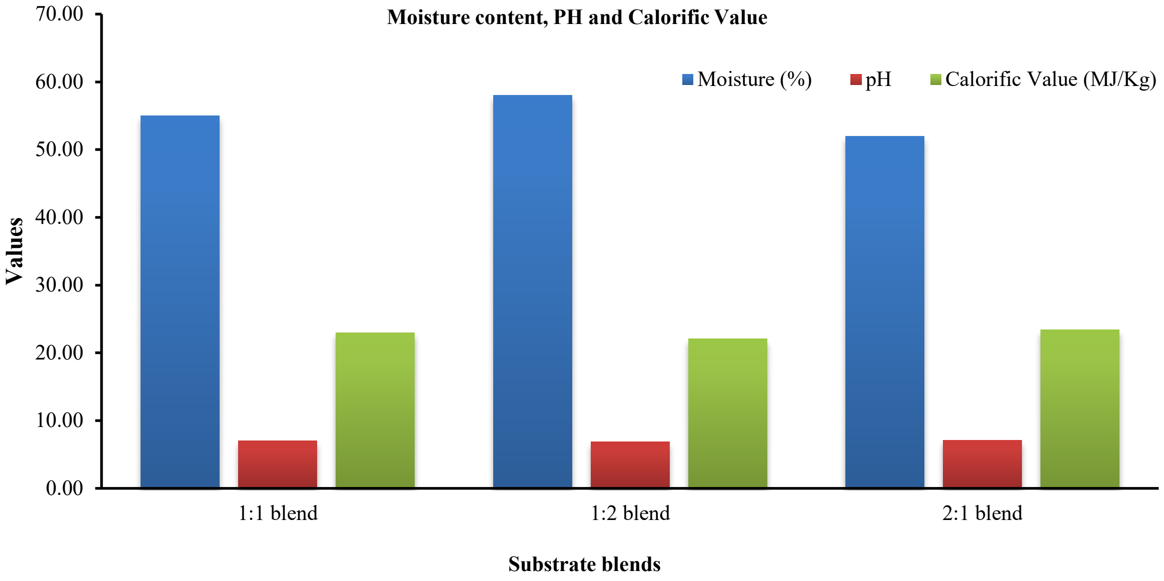

Figure 3 Characteristics of the blends based on moisture content, pH and Calorific

3.1.2 C/N Ratio

The C/N ratio lies a significance in assessing feedstock's suitability for biogas production. A high C/N ratio could impede microbial digestion by providing insufficient nitrogen for microbial metabolism, potentially leading to the accumulation of undigested organic matter and the production of volatile fatty acids (VFAs), which could lower pH and hinder microbial activity. Conversely, a low C/N ratio might result in ammonia formation, which is detrimental to microorganisms, thereby reducing biogas production efficiency. The preferred C/N ratio range lies between 20:1 and 30: 1 (Kunatsa and Xia, 2022). The C/N ratios are presented in Table 1.

Table 1 Results from ultimate analysis of sugarcane trash, vinasse and blends

Substrate	H (%)	N (%)	C (%)	S (%)	O (%)	C/N ratio
Sugarcane trash	5.12	1.50	48.68	0.25	40.30	32.45:1
Sugarcane vinasse	6.12	1.65	40.23	0.35	30.64	24.38:1
Sugarcane trash/vinasse blend (1:1)	5.54	1.50	42.95	0.30	35.20	28.63:1
Sugarcane trash/vinasse blend (1:2)	5.9	1.45	41.71	0.32	32.80	28.77:1
Sugarcane trash/vinasse blend (2:1)	5.54	1.50	47.2	0.28	40.00	31.46:1

From Table 1, the analysis of substrates revealed that sugarcane trash possessed a relatively high C/N ratio of 32.45:1, implying slower degradation as a biogas feedstock but offering stable organic matter beneficial for soil fertility (Kiplagat et al., 2022). In contrast, vinasse exhibited a much lower C/N ratio of 24.38:1, indicating its potential as a readily available nitrogen source for biogas production and rapid degradation in the biogas reactor. However, vinasse also contained elevated levels of 6.12 hydrogen and 0.35 sulphur, which could contribute to soil fertility. The findings on the C/N ratio of sugarcane trash and vinasse align with other research in the field of biogas production and soil fertility. For example, Rowan et al. (2022) reported that substrates with high C/N ratios, like sugarcane trash, degrade more slowly but enhance soil organic matter, supporting long-term soil fertility. In contrast, studies by Marafon et al. (2020) found that substrates with lower C/N ratios, similar to vinasse, facilitate faster biogas production due to increased nitrogen availability, enhancing reactor performance. However, high hydrogen and sulphur levels in vinasse, as observed in this study, align with findings that suggest potential impacts on soil nutrient balance and biogas quality.

Table 1 indicate that blending sugarcane trash and vinasse in a 1:1 ratio result in a C/N ratio of 28.63:1, suggesting balanced carbon and nitrogen levels. This aligns with findings by Rowan et al. (2022) noted optimal biogas production at similar ratios. Further increasing the vinasse ratio to 1:2 resulted in a slightly lower C/N ratio of 28.77:1. This slight decrease in the C/N ratio suggests only a marginal increase in nitrogen availability, which is unlikely to significantly accelerate biogas production rate. However, elevating the proportion of sugarcane trash to 2:1 significantly elevated the C/N ratio to 31.46:1, suggesting reduced nitrogen availability and a potential slowdown in biogas production. Although the 1:1 blend falls within the recommended C/N ratio of 20:1–30:1, the 2:1 blend is preferred due to its slightly higher C/N ratio of 31.46:1, which balances carbon stability with nitrogen availability. Additionally, the presence of favorable elements such as oxygen, hydrogen, and Sulphur in the 2:1 blend further enhances its suitability for efficient biogas production, as noted in previous studies (Meng et al., 2020; Sitthikitpanya et al., 2024).

3.1.3 Methane production

To determine the volume of methane (CH_4) in the biogas generated from the conducted experiments, Equation 2 was applied to calculate the percentages of methane in individual substrates (sugarcane trash/vinasse) and their blends (1:1, 1:2 and 2:1). The percentage of methane estimated from each blend, considering the calorific value of biogas and the standard high heating value of methane was presented in Figure 4.

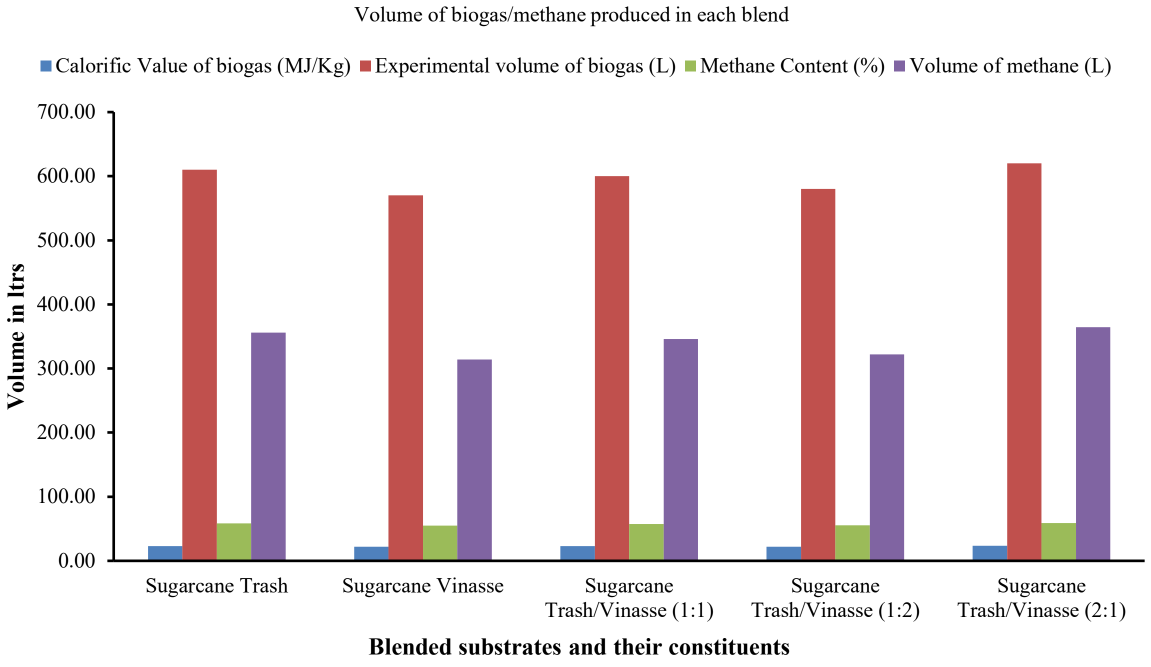

Figure 4 Percentage of Methane, calorific value and volumes of biogas/methane in each blend

According to Figure 4, the blend ratio 2:1 produced the highest methane volume of 364.374 L at 58.77%, which conforms to a recommended biogas yield of 50%-70% methane and this percentage varies as per the feedstock as well as the anaerobic process in place (Volpi et al., 2023). This is followed by a blend ratio of 1:1 at 346.2 L (57.7%). Blend 1:2 produced the lowest methane volume of 322.074 L at 55.53%. Methane volume changes with percentage as it depends on both total biogas volume and methane concentration. Higher methane percentages indicate greater efficiency, resulting in higher methane volumes with consistent biogas output. The results demonstrate the effectiveness of blending sugarcane trash with sugarcane vinasse, specifically in 2:1 blend, in facilitating fermentation process (Silva et al., 2021). In spite of increasing the total amount of biogas yield, this blend enhances the quality of biogas regarding its methane content. The findings are imperative for optimizing biogas yield process, emphasizing the quest for choosing feedstock blends that maximizes the calorific quality and quantity of methane in the biogas produced (Mendes et al., 2023).

3.1.5 Proximate analysis

In this study, proximate analysis was performed on sugarcane trash, sugarcane vinasse, and blends of sugar trash and sugarcane vinasse at ratios 1:1, 1:2, and 2:1.

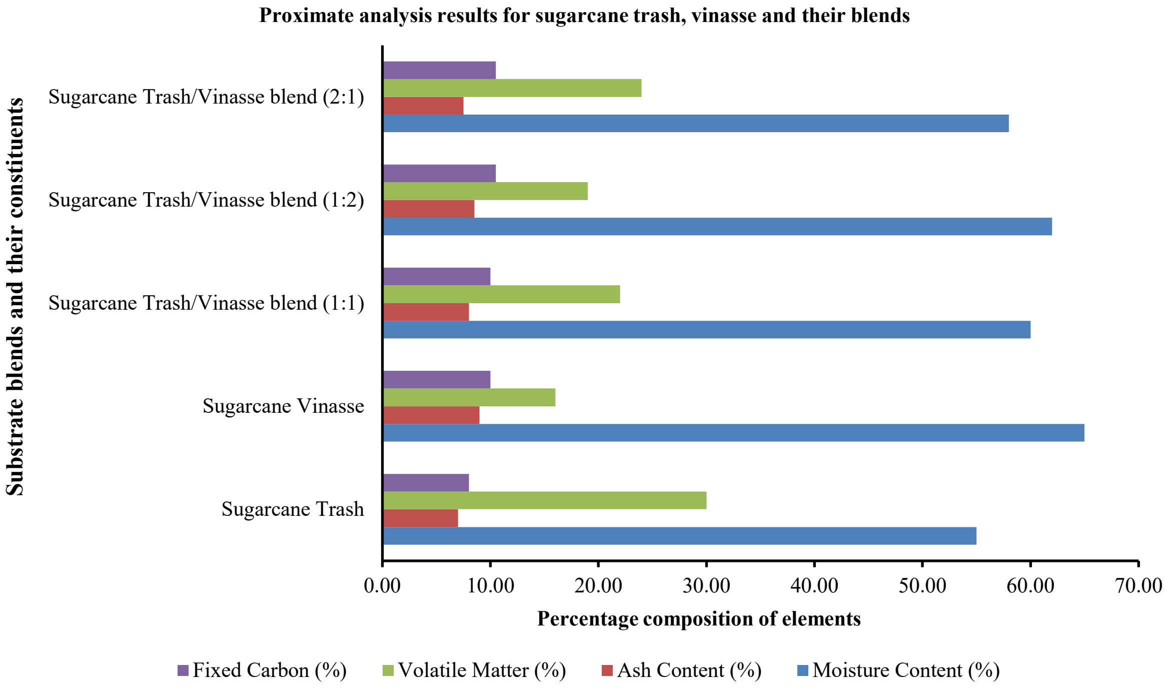

Figure 5 Proximate Analysis results for sugarcane trash, vinasse and blends

The proximate analysis of sugarcane trash revealed a composition of 8% fixed carbon, 30% volatile matter, 7% ash, and 55% moisture (Figure 5). Fixed carbon as calculated using Equation 3, serves as a crucial component in biomass materials for biogas production, as it provides a carbon source for the microorganisms involved in organic matter breakdown (Singh et al., 2021). The relatively low fixed carbon content in sugarcane trash suggests its limitation as a viable standalone feedstock for biogas production. Volatile matter also plays a significant role in biogas production, as it offers energy for the microorganisms involved in organic matter decomposition. The substantial volatile matter content in sugarcane trash (30%) indicated a high potential for aromatic hydrocarbon production, enhancing its suitability for biogas production. The ash content of biomass materials could influence biogas production efficiency. With a low ash content of 7%, sugarcane trash appeared to be a relatively clean feedstock for biogas production, compared to other substrates, though it falls within the recommended range of 5%-15% (Rowan et al., 2022). However, high ash content could lead to increased waste generation and negatively impact biogas quality (Silva et al., 2021). In the proximate analysis of sugarcane vinasse, it was found to contain 10% fixed carbon, 16% volatile matter, 9% ash, and 65% moisture. Compared to sugarcane trash, sugarcane vinasse exhibited a high fixed carbon content, suggesting its greater effectiveness as a biogas production feedstock, indicating its potential for biogas production. However, the high ash content of sugarcane vinasse might negatively impact biogas production efficiency by increasing waste generation and diminishing biogas quality (Takeda et al., 2022).

The sugarcane trash has a moisture content of between 45%-55% on a wet basis, with the best moisture content range varying from 50%-80%, as recommended by Rashidi et al. (2024). The relatively higher moisture content (65%) in sugarcane vinasse could render it a less efficient feedstock for biogas production compared to sugarcane trash. In this study, proximate analysis was extended to sugarcane trash (trash) blended with sugarcane vinasse in various ratios to evaluate their physical and chemical properties for biogas production. The ratios of sugarcane trash to vinasse tested were 1:1, 1:2 and 2:1. As shown in Figure 5, the proximate analysis revealed that the physical and chemical properties of the blended materials varied depending on the ratio of sugarcane trash to vinasse utilized. When blended in a 1:1 ratio, the material comprised 10% fixed carbon, 22% volatile matter, 8% ash, and 60% moisture. The relatively high moisture content in this blend could potentially impede the efficiency of the biogas production process (Parliament of Kenya, 2023). Blending in a ratio of 1:2 (sugarcane trash: vinasse) resulted in a material containing 10.5% fixed carbon, 19% volatile matter, 8.5% ash, and 62 % moisture. The notably high fixed carbon content suggested that this blend serves as a more efficient feedstock for biogas production compared to the 1:1 blend. Moreover, the lower ash and moisture contents could further enhance biogas production efficacy.

From Figure 5, in 2:1 blend (sugarcane trash to vinasse ratio), the material exhibited 10.5% fixed carbon, 24% volatile matter, 7.5% ash, and 58% moisture. While the relatively high fixed carbon content indicated the potential for biogas production, the lower volatile matter content compared to other blends suggests it might be less effective as a biogas production feedstock. Prior studies on proximate analyses yielded diverse findings regarding different blends. Wongarmat et al. (2022) investigated the impact of fixed carbon and moisture content on biogas production, concluding that substrates with higher fixed carbon and lower moisture content tend to yield more biogas. Similarly, Zongo et al. (2023) explored the influence of volatile matter and ash content on biogas production efficiency, finding that substrates with lower volatile matter and ash content were more favourable for biogas production. Rashidi et al. (2024) examined the biogas potential of various biomass blends, observing that blends with high volatile matter and low moisture content were associated with higher biogas production rates.

Comparing these previous studies, sugarcane trash exhibits relatively low fixed carbon (8%) and moisture content (55%), aligning with the findings of Wongarmat et al. (2022) favouring a lower moisture content for biogas production. Conversely, sugarcane vinasse boasts higher fixed carbon (10%) and lower moisture content (65%) compared to sugarcane trash, making it more suitable for biogas production. The 1:1 blend of sugarcane trash/vinasse demonstrates moderate volatile matter (22) and moisture content (60%), consistent with Rashidi et al. (2024) findings supporting a balanced blend (1:1) for biogas production. The 1:2 blend of sugarcane trash/vinasse presents the higher fixed carbon (10.5%) and a moderate moisture content (62%), aligning with the favourable characteristics identified in Paulose and Kaparaju (2021). Similarly, the 2:1 blend shows highest fixed carbon (10.5%) and a relatively lower moisture content (58%), consistent with findings from Rahman et al. (2019). Considering the proximate analysis data and comparisons with the referenced studies, the 2:1 blend of sugarcane trash/vinasse appears to hold the most promising cadre for optimal biogas production due to its high fixed carbon content, and relatively low moisture levels, attributing to an efficient biogas yield according to the referenced studies (Meng et al., 2020).

3.2 Optimization of biogas production from sugarcane trash blended with sugarcane vinasse

3.2.1 Taguchi method

The Taguchi method was employed to optimize biogas production, utilizing mixed ratio, temperature and retention time at mixed ratios (1:1,1:2,2:1), temperatures (25^0C, 35^0C and 45^0C) and retention times (18, 20 and 22 days). The volume of biogas yield and S/N ratios were ascertained for each combination of parameters and levels using Equation 4.

Table 2 presents the outcomes of biogas production across various experimental conditions designed using the Taguchi L9 orthogonal array. Among the nine trials, Experiments 2, 4, and 9 recorded the highest biogas yields, generating 795 L, 787 L, and 766 L, respectively. These experiments also exhibited high signal-to-noise (S/N) ratios—58.01 dB for Experiment 2, 57.92 dB for Experiment 4, and 57.85 dB for Experiment 9—indicating consistent and stable performance. Notably, Experiment 2, which involved a 2:1 mix ratio, 35°C temperature, and a 20-day retention time, produced the best results. This suggests that a higher proportion of sugarcane trash in the feedstock blend significantly improves microbial digestion and gas production. The performance of these optimal trials underscores the importance of carefully selecting operational parameters to maximize biogas yield.

Notably, certain experiments with identical input parameters yielded differing biogas volumes, pointing to other influencing factors beyond the set variables. For instance, Experiments 1, 6, and 8 all used a 1:1 mix ratio, maintained at 30°C with a pH of 6.8 and an 18-day retention time, yet their respective yields varied: 698 L, 720 L, and 668 L. Similarly, Experiments 3, 5, and 7 shared the same conditions—a 1:2 mix ratio, 45°C temperature, pH 6.8, and 22-day retention—but resulted in outputs of 750 L, 728 L, and 700 L, respectively. These discrepancies likely arise from minor experimental inconsistencies, such as variations in substrate particle size, inoculum activity, or digester sealing and agitation. Such variations highlight the sensitivity of anaerobic digestion to small deviations and reinforce the need for rigorous standardization and control in future trials to enhance reliability and reproducibility.

The 2:1 mixing ratio resulted in the highest mean biogas yield of 782 L, indicating that this substrate combination is the most effective for biogas generation (Garcia-Perez et al., 2023). Temperature also played a crucial role, with 35°C producing the optimal yield of 782 L, while significantly lower outputs were recorded at 30°C (696 L) and 45°C (725 L). For retention time, 20 days yielded the highest biogas output at 782 L, with 18 days (696 L) and 22 days (725 L) producing comparatively lower volumes. These results confirm that the combination of a 2:1 mixing ratio, 35°C temperature, and 20-day retention time is the most favorable condition for maximizing biogas production in this study (Fuess et al., 2021). Furthermore, the Signal-to-Noise (S/N) ratio analysis presented in Figure 7 and Table 4 reinforces these findings, showing that higher yields correlate with higher S/N ratios, which reflect improved process efficiency, stability, and reduced production variability (Paulose & Kaparaju, 2021).

Figure 7 indicates that a 2:1 mixed ratio provides the highest mean S/N ratio of 57.95 dB, denoting the most preferred condition for minimizing noise and maximizing signals in biogas production. This ratio is more effective compared to 1:2 (57.25 dB) and 1:1 (56.80 dB). The 35°C temperature yields the highest S/N ratio (57.95 dB), implying optimal efficiency as well as stability in the biogas production process. However, the 30°C and 45°C temperatures proved less effective, as shown by lower S/N ratios of 56.85 dB and 57.25 dB, respectively. Besides, a retention time of 20 days results in the highest S/N ratio of 57.95 dB, indicating an optimal duration for maintaining process stability (Freitas et al., 2023). Retention times of 18days - 22 days lead to lower S/N ratios of 56.85 dB and 57.25 dB respectively, showing less optimal conditions for biogas yield.

Table 2 Biogas production from experiments conducted as per the Taguchi L_9 orthogonal array design

Experiment No.	Mixed Ratio	Temperature (°C)	pH	Retention Time (Days)	Biogas Yield (L)	S/N Ratio (dB)
1	1:1	30	6.8	18	698	56.88
2	2:1	35	6.8	20	795	58.01
3	1:2	45	6.8	22	750	57.50
4	2:1	35	6.8	20	787	57.92
5	1:2	45	6.8	22	728	57.24
6	1:1	30	6.8	18	720	57.15
7	1:2	45	6.8	22	700	56.90
8	1:1	30	6.8	18	668	56.50
9	2:1	35	6.8	20	766	57.85

Table 3 Mean response for biogas yield

Factor	Levels	Mean Biogas Yield (L)
Mixing Ratio	1:2	725
	1:1	696
	2:1	782
Temperature	30°C	696
	35°C	782
	45°C	725
Retention Time	18 days	696
	20 days	782
	22 days	725

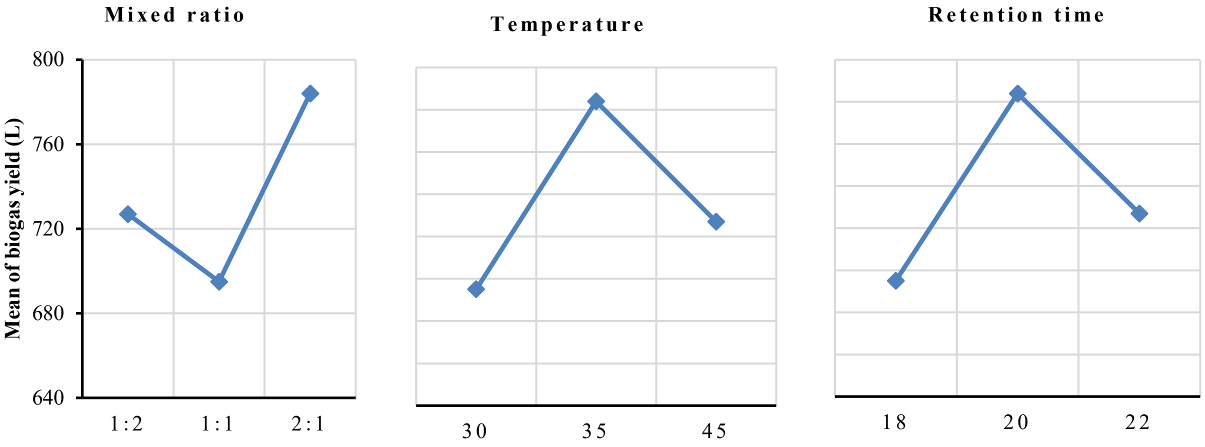

Figure 6 Effect of different parameters on mean biogas production

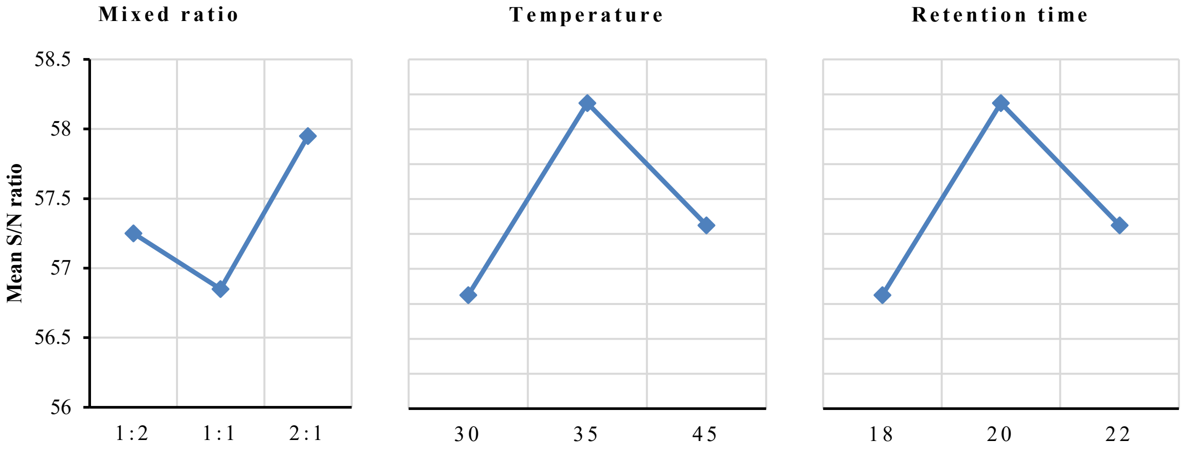

Figure 7 Effects of different factors on mean S/N ratio of biogas production

Table 4 Mean response for signal-to-noise ratios (Larger is better)

Factor	Levels	Mean S/N ratio
Mixed Ratio	1:2	57.25
	1:1	56.85
	2:1	57.95
Temperature	30°C	56.85
	35°C	57.95
	45°C	57.25
Retention Time	18 days	56.85
	20 days	57.95
	22 days	57.25

3.2.2 Analysis of variance

The results revealed that the mixed ratio of sugarcane trash and sugarcane vinasse, temperature and retention time had a significant impact on biogas production (Chai et al., 2022). The statistical comparison of the parameters and their effects on biogas yield is summarized in Tables 5, 6 and 7.

Table 5 ANOVA results for mixed ratio

Source of variation	SS	DF	MS	F-Value	*p*-Value	F crit
Between Groups	68681.21	1	68681.21	33.33	0.00004	4.6
Within Groups	28847.33	14	2060.523			
Total	97528.54	15				

Table 6 ANOVA results for temperature

Source of variation	SS	DF	MS	F-Value	*p*-Value	F crit
Between groups	35226.6	1	35226.6	17.0844	0.001014	4.60
Within groups	28866.82	14	2061.916			
Total	64093.42	15				

Table 7 ANOVA result for retention time

Source of variation	SS	DF	MS	F-Value	*p*-Value	F crit
Between groups	44494.6	1	44494.6	21.503	0.00038	4.60
Within groups	28969.2	14	2069.22			
Total	73463.9	15				

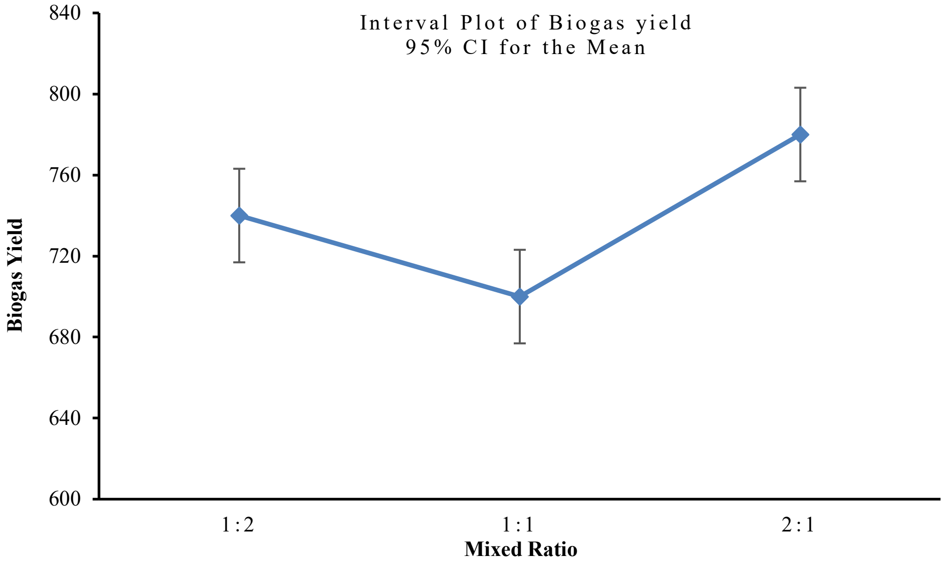

Figure 8 Interval plot of biogas yield vs mixed ratio

The ANOVA results in Table 5 compared the variations in biogas yield between different mixed ratios (1:1, 2:1, and 1:2) and the variation within each group. The largest difference between these two variations (68681.213 vs. 28847.325) indicated that the mixed ratio was the most crucial factor influencing biogas yield as compared to temperature and retention time. The high F-statistic (33.33) and the associated low p-value (0.000048) further confirmed the statistical significance of the mixed ratio's impact on biogas production. These findings suggest that optimizing the proportions of sugarcane trash and vinasse in the feedstock blend could enhance the efficiency of biogas production. The ANOVA analysis established mixed ratio as a pivotal determinant of biogas yield, aligning with the studies to understand the factors influencing the biogas production process and optimizing its output (Bamba et al., 2021). The interval plot in Figure 8 demonstrates the impact of different mixed ratios of sugarcane trash and vinasse on biogas yield. The biogas yield was highest in blend 2:1 with a biogas yield of (795L). It decreased to 750L at a 1:2 ratios and dropped further to 720L at a 1:21ratios. This trend suggests that increasing the proportion of sugarcane trash enhanced biogas yield up to a certain threshold, beyond which a higher vinasse ratio may reduce the yield.

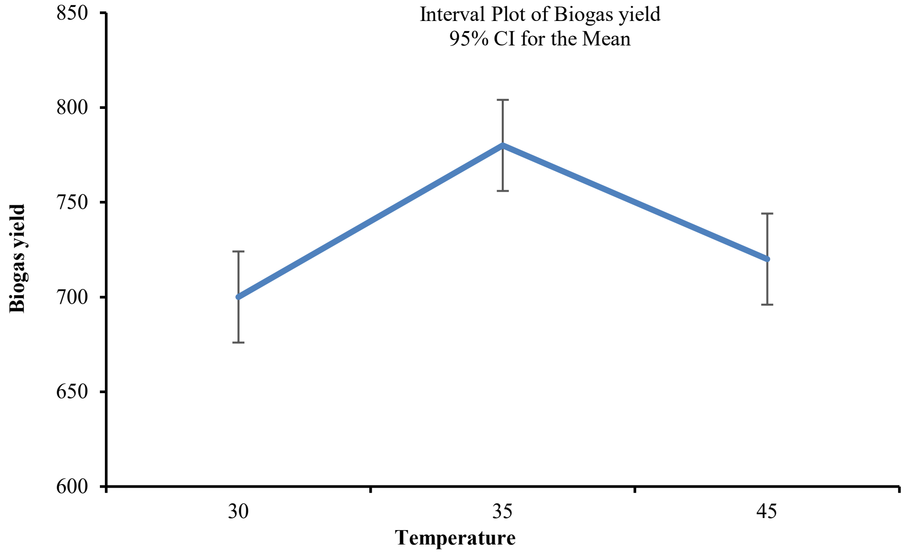

Figure 9 Interval plot of Biogas yield vs temperature

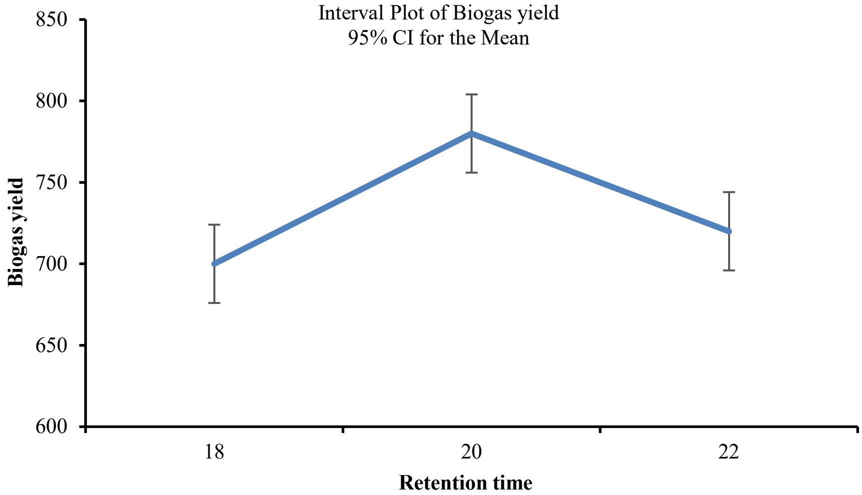

Figure 10 Interval plot of biogas yield vs retention time

The interval plot on Figure 9 illustrates the relationship between temperature and biogas yield, with the highest yield occurring at 35°C. The 95% confidence intervals show that while yields at 30°C and 45°C are lower, there is some overlap, indicating variability (Arruda et al., 2025). The optimal temperature for maximum biogas production appears to be around 35°C. The ANOVA analysis in Table 3.4 revealed a significant impact of temperature on biogas yield and the temperatures tested comprised 30°C, 35°C, and 45°C. The large difference between the "Between Groups" variation (35226.598) and the "Within Groups" variation (28866.822) for different temperature levels (35°C and 45°C) emphasized the crucial role of temperature in influencing biogas production. The high F-statistic (17.08) and the associated low *p*-value (0.0010) confirmed the statistical significance of temperature's effect on biogas yield. This finding supports the importance of precisely controlling and optimizing temperature conditions to augment biogas production efficiency, as suggested by previous researches (Arotingo et al., 2023).

ANOVA results in Figure 10 shows that retention time significantly impacts biogas yield. The interval plot reinforces the statistical results by demonstrating a peak in biogas production at a retention time of 20 days of retention, followed by a decline at 22 days. The confidence intervals depict significant increase in biogas yield when extending retention from 18 to 20 days

(Alruqi and Sharma, 2023). However, the intervals between 20- and 22-days overlap, denoting that the decrease, while observable, may be statistically insignificant. This denotes diminishing returns from extending retention time beyond 20 days, pointing to 20 days as the optimal period for maximizing biogas output (Albuquerque et al., 2019).

The statistical ANOVA results in Tables 5, 6 and 7 indicate that retention time significantly affects biogas yield. The "Between Groups" variation (44494.629) represents the differences in biogas yield associated with different retention times (20 days and 30 days), while the "Within Groups" variation (28969.197) accounts for the variability within each group. The substantial difference between these two values indicates that retention time is indeed a significant factor influencing biogas production. The *F*-statistic of 21.50 is the ratio of these variations. A high *F*-value suggests that the differences between groups are much greater than the variations within groups, highlighting the importance of retention time in determining biogas yield. The associated *p*-value (0.00038) is significantly lower than the common significance level of 0.05, demonstrating strong statistical significance (Misra et al., 2023). ANOVA analysis suggests that all the three parameters significantly impact the biogas yield following their respective *p*-values in Tables 5,6 and 7. The extremely low p-values indicate that these factors are highly influential, with mixed ratio (0.00004) having the strongest effect, followed by retention time (0.0038) and temperature (0.001014).

4 Conclusion

The study analyzed the chemical and physical properties of sugarcane trash and vinasse to optimize biogas production through co-digestion. Proximate and ultimate analyses provided insights into substrate composition, revealing differences in the blends. Optimization involved assessing key parameters like temperature, retention time, and mix ratio using the Taguchi method and ANOVA to identify their impact on biogas yield. The mix ratio was found to be the most significant factor, followed by retention time. Optimal conditions for the highest biogas output of 795 liters were determined to be at a temperature of 35°C, a retention time of 20 days, and a mix ratio of 2:1. ANOVA results underscored the importance of these conditions, and the study recommended maintaining moisture levels within 50%-80% to enhance microbial activity. To improve environmental sustainability, blends with lower sulfur content were suggested to minimize emissions.

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