

# Co-digestion of water hyacinth with human faecal matter for optimal biogas production in Kisumu, Kenya

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**Abstract:** The invasion of water hyacinths in Lake Victoria and poor waste management in Kisumu City have worsened pollution, threatened ecosystems and public health. This study explores the anaerobic co-digestion of water hyacinth (WH) and human fecal matter (FM) for biogas production in the ratio. The methodology involved conducting experiments at the Kenya Industrial Research and Training Institute (KIRDI), where different substrate ratios and chemical pretreatments were tested to optimize biogas yield. The WH: FM were mixed in the ratios of 1:0, 0.75:0.25, 0.5:0.5, 0.25:0.75, and 0:1 respectively, while the water hyacinth was pretreated with a 1% sodium hydroxide (NaOH) solution for 3 days. Monte Carlo simulations were applied to evaluate the most effective substrate combination for maximizing biogas production. Gas production was measured, and methane output was analyzed to determine the effects of substrate composition and pretreatment on biogas yield. Results show that pure FM produced the highest gas yields (177.1 ml), while co-digestion with WH improved methane production efficiency, with the 0.25 WH: 0.75 FM ratio yielding 123.1 ml. Chemical pretreatment of WH with 1% NaOH enhanced biodegradability, increasing methane output by 15% compared to untreated WH. Statistical analysis confirmed that the 1g FM had a maximum yield (mean of 71.11 ml). Similarly, this substrate showed high variability (standard deviation of 66.03 ml). These findings present a sustainable waste-to-energy model, contributing to renewable energy solutions and environmental conservation. Meanwhile, the 0.25WH to 0.75 FM showed an elevated output of biogas yield, though it was not the maximum value. The study underscores the potential of co-digestion to address sanitation and ecological challenges in the Lake Victoria region.

**Keywords:** water hyacinth; co-digestion; methane output; waste management; anaerobic digestion

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## 1 Introduction

The invasion of Lake Victoria's water hyacinth (WH) has led to severe environmental and economic challenges, significantly impacting aquatic ecosystems, fishing activities, and water transport (Ayanda et al.,

2020). Despite extensive removal efforts by the county and national governments, the weed remains a persistent problem. Water hyacinth rapidly regenerates, obstructing water bodies and depleting oxygen levels, thereby threatening aquatic life and the livelihoods of communities that depend on the lake. At the same time,

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Kisumu City faces critical challenges in human waste management due to rapid urbanization and inadequate sewage infrastructure. Informal settlements such as Kondele, Otonglo, and Migosi suffer from poor sanitation, with raw sewage frequently discharged into the lake, further deteriorating water quality and public health.

The uncontrolled disposal of human waste and the spread of water hyacinth have exacerbated pollution levels, necessitating sustainable solutions to mitigate their environmental impact (Barua et al., 2019; Kundu et al., 2017). Existing efforts to physically remove water hyacinths lack economic incentives and often result in additional ecological complications. Similarly, Kisumu's inadequate waste disposal systems contribute to the direct seepage of fecal matter into Lake Victoria, causing contamination and increasing the prevalence of waterborne diseases. Addressing these issues requires a strategic approach that removes these pollutants and converts them into valuable resources. Anaerobic co-digestion of water hyacinth and fecal matter (FM) presents a promising solution by producing biogas, a renewable energy source (Bote et al., 2020; Nagy, 2024). Research indicates that co-digestion enhances gas yields compared to mono-digestion due to the complementary biochemical properties of the substrates (Omondi et al., 2019). This study is grounded in Ecological Modernization Theory, which emphasizes the role of technological innovation in achieving both economic development and environmental sustainability. Within this framework, co-digestion of organic waste—specifically water hyacinth and human fecal matter—offers a practical solution for renewable energy generation and improved waste management. Accordingly, the study seeks to determine the optimal conditions for biogas production, focusing on the effects of substrate proportions and chemical pretreatment on yield (Olatunji et al., 2021; Syaichurrozi et al., 2021).

Previous studies, such as Mrosso et al. (2023), have investigated biogas production from water hyacinth and various organic wastes. However, while their research highlights the potential of water hyacinth as a

substrate, it does not extensively explore its co-digestion with human fecal matter. This gap underscores the need for further investigation into optimizing biogas yields through co-digestion of these locally abundant waste streams. For instance, Adelodun et al. (2022) investigated the anaerobic co-digestion of water hyacinth with cow dung and poultry waste under mesophilic conditions, reporting enhanced biogas yields. While their study contributed valuable insights into co-digestion with animal waste, limited research has explored the potential of co-digesting water hyacinth with human fecal matter, particularly in relation to its biochemical characteristics and impact on gas production efficiency. Similarly, Omondi, Ndiba, and Njuru (2019) assessed the co-digestion of water hyacinth with ruminal slaughterhouse waste under varying temperatures, demonstrating improved methane yields under thermophilic conditions. Barua et al. (2019) explored the potential of water hyacinth in combination with various agricultural residues, emphasizing the role of pretreatment in enhancing biodegradability. Similarly, Bote et al. (2020) reviewed advancements in the anaerobic digestion of aquatic weeds, noting the challenges associated with lignocellulosic content. While these studies offer important insights into co-digestion with agricultural or animal-based substrates, limited attention has been given to the co-digestion of water hyacinth with human fecal matter—a locally abundant but underutilized resource (Silva et al., 2021; Bueno et al., 2024; Garcia-Perez et al., 2023).

Anaerobic digestion of biomass has been used in the production of biogas, but other methods of bioenergy production like thermochemical conversion are equally ideal. Thermochemical conversion involves a waste valorization route, which relies on initial heat for process initiation and the subsequent breakdown of biomass into biofuel (Paudel, et al., 2024). While thermochemical conversion remains a promising biofuel energy production method, it is subject to a number of challenges compared to the conventional methods such as anaerobic digestion. Thermochemical conversion

has intricacies due to the initial phase of biomass and feedstock selection and classification. Thermochemical conversion as a heat-induced process has significant limitation compared to anaerobic digestion, which relies on microbial decomposition of biomass. Besides, thermochemical conversion is not ideal for high moisture substrates like the ones utilized in this experiment, thus ruling out its possibility for usage. Although previous studies have explored co-digestion of water hyacinth with animal or agricultural waste (Barua et al., 2019; Ahiati, 2021), limited research has addressed its combination with human faecal matter, despite pressing sanitation and waste management challenges in urban areas like Kisumu. This study aims to fill that gap by determining optimal conditions for biogas generation through co-digestion of water hyacinth and human waste, focusing on substrate ratios and sodium hydroxide pretreatment. Beyond energy production, this approach supports environmental conservation by reducing invasive hyacinths and improving waste disposal (May et al., 2022). It also offers a sustainable alternative to traditional fuels and presents economic opportunities for communities around Lake Victoria (Cioabla and Popescu, 2021).

## 2 Materials and methods

This study was conducted between June and September 2022 in Kisumu County, Kenya, focusing on water hyacinth and human faecal matter as substrates for biogas production. The experimental procedures were carried out at the Kenya Industrial Research and Training Institute (KIRDI) in South C, Nairobi. Water hyacinth samples were collected from Dunga Beach, Kisumu County, a location known for heavy infestation. Human fecal matter was sourced from King's Serenity Apartments in Ongata Rongai and Oloolaiser National School in Ngong. These sites were selected due to the availability of fresh waste, addressing both ecological and urban sanitation concerns.

### 2.1 Sample collection and preparation

Sample collection and preparation followed a

structured approach using two distinct sampling techniques. Water hyacinth was collected through random sampling approximately 40 meters from the lake shore, while human faecal matter was obtained through targeted sampling. A bucket full of water hyacinth was randomly picked from the Dunga beach in Kisumu county; approximately 5 kg of fresh substrate. On the other hand, about 1 kg of raw and wet human faecal water were collected from both institutional and general affluent points. All these samples were packed in the sample bags and placed in optimal conditions to retain their states before the experiments. There were two replicates in the first experimental setup involving characterization of substrates according to their biochemical properties. In this setup, physical and chemical properties of water hyacinth (such as moisture content, crude protein, crude fat, volatile solid, total solid and ash content) were correlated with similar features from institutional faecal matter and the general effluent waste. On the other hand, the second experiment involving substrate ratios had four replicates, each having distinct ratios. In each setup, key ratios of substrates were used to bring out a distinguishing property in every experiment. Finally, the last test on the chemical pre-treatment had two replicates, one untreated and the other pre-treated both at 0.5:0.5 substrate ratio.

Three sets of experiments were carried out for this purpose, given that the parameters under review were distinct and required their specific sets of experiments. Meanwhile, the benchmark of every experimentation was to establish the yield of biogas. In the first experimental analysis, the levels of minerals, crude protein, lipid, and fibre were determined through proximate analysis. Generally, the sampled substrates adopted certain procedures before classification in the three experimental setups. First and foremost, the water hyacinth biomass was allowed to dry at room temperature ( $25^{\circ}\text{C}\pm 2^{\circ}\text{C}$ ) for about a week and then was chopped into pieces (3-5 mm long). Once dry, the chopped biomass was placed in a crucible and crushed by a motor to finer particles. These samples were stored in a refrigerator at  $4^{\circ}\text{C}$ , while awaiting further

analysis and characterization. The human faecal matter, once collected from the designated points above, was put in a 5-liter container and sealed for transportation to KIRDI. A measured weight of 1 kg drawn from the three samples of waste sludge was dried in an oven at 105°C for 7 hours. The dried samples were weighed

again, and their weight was determined to calculate the moisture content in the sludge. Finally, the dried human waste was crushed, and the two samples packed in a vacuum-sealed bag, and stored in a refrigerator at 4°C. The samples awaited characterization and further co-fermentation in the subsequent experiments.



Figure 1 The picture of a moisture oven

Figure 1 shows a moisture oven used in drying the substrates for further experimental tests and analysis. At the experimental stage, different rates and quantities of substrates were used to realize specific results. In each experimental setup, 2g of dried substrates was mixed with an inoculant to attain 400 ml volume. The mixing was done in a 1,000 ml glass bottles (digester), hermetically sealed, and fitted with outlets that allowed pressure measurements. In this case, a headspace of 600 ml was created, which effectively allowed gas and liquid sampling at each stage of experimentation. All the hermetically sealed bottles were subjected to leak tests by pressurizing them with nitrogen and observing pressure drops over a period of one night. Each digester, ascertained to be leak proof was put in a water bath maintained at a temperature of 37°C. These digesters were stirred and pressure of nitrogen, carbon dioxide and methane was noted on the pressure gauge. The change in pressure in each digester was noted and analyzed using gas

chromatography. Later, the pressure in the bottles was released into the pressure gauge column. All changes in pressure were noted and converted into ml of biogas produced. The measurements of the gas were taken after every 6 days, but the interval increased to 8 days as the methane production declined.

## 2.2 Proximate and ultimate analysis

Proximate analysis was conducted to determine moisture content, ash content, crude fiber, and fixed carbon using standard procedures and corresponding equations to ensure accurate substrate characterization. Moisture content was determined using the ASTM-D3173 standard, and calculated by drying 5 g of each sample in an oven at 105°C for seven hours, with the percentage determined by measuring the weight loss using Equation 5, which calculates moisture as the difference between the weight of the wet and dry samples (Ahiati, 2021). Ash content was determined by placing 2 g of dried samples in a muffle furnace at 500°C for five hours and applying ASTM E1755-

01(2020), which subtracts the weight of the empty dish from the weight of the dish with ash to obtain the percentage (Obileke et al., 2020). Crude fiber content was established by sequential acid and alkali digestion, followed by furnace ignition, with fiber calculated using Equation 2, which compares the weight of the crucible before and after ashing (Tan et al., 2021). Fixed carbon was derived indirectly using Equation 1, where the percentages of moisture, ash, and volatile matter were subtracted from 100 to determine the fixed carbon content (Rabii et al., 2019). Additionally, ultimate analysis was conducted using a CHN analyzer to determine the elemental composition of carbon, hydrogen, oxygen, sulfur, and nitrogen, which provided critical insights into the biodegradability and biogas potential of the substrates. The percentage of protein was also determined using ASTM D5373 code, which calculates protein content based on the titration of nitrogen using the formula: % Protein = (Titer of sample – Titer of blank) × 6.25 × 0.02 × 7, where 6.25 is the nitrogen-to-protein conversion factor, 0.02 is the acid normality, and 7 is the gram equivalent of nitrogen (Manigandan et al., 2023).

$$FC(\%) = 100 - (\text{Moisture Content}\% + \text{Ash Content}\% + \text{Volatile Matter}\%) \quad (1)$$

$$\text{Fiber Content} = \frac{(\text{Wt of crucible+Residue}) - (\text{Wt of Crucible+ash}) \times 100}{\text{Wt of sample}} \quad (2)$$

$$\% \text{ Protein} = (\text{Title of sample} - \text{Titre of Blank}) * 6.25 * 0.02 * 7 \quad (3)$$

$$\text{Ash Content} = \frac{(\text{Dish+Ash}) - (\text{Wt of Dish alone}) \times 100}{\text{Wt of sample}} \quad (4)$$

$$\text{Moisture Content} = (\text{Wt of Sample}(\text{Wt of dish} + \text{sample}) - (\text{Wt of dry matter} + \text{dish}) * 100 \quad (5)$$

## 2.3 Anaerobic digestion conversion procedures

### 2.3.1 Mix ratios

Five substrate ratios of water hyacinth (WH) and faecal matter (FM) were prepared: 100% WH, 75% FM:25% WH, 50% WH:50% FM, 25% WH:75% FM, and 100% FM. Each mixture was weighed and combined with equal volumes of water to form a slurry. These were loaded into airtight 5-litre batch digesters and incubated at ambient temperature for 67 days. Gas volumes were measured regularly using the water displacement method. Digesters were manually

agitated daily, and temperature and Ph were monitored using a Systronics Ph meter to maintain optimal microbial conditions (Ayanda et al., 2020; Li et al., 2022). Biogas production was quantified by measuring the displacement of water in the graduated cylinders, and the volume of biogas was calculated using the expression in Equation 6. This simple yet effective method allowed accurate tracking of biogas output while maintaining the anaerobic integrity of the system. The setup was carefully sealed to prevent gas leakage, and ensured optimal conditions for microbial digestion, consistent with the design standards of similar studies (Ayanda et al., 2020).

$$\text{Biogas Volume (ML)} = V_{\text{displaced}} \quad (6)$$

### 2.3.2 Experimental setup and procedure

The experimental process, as shown in Figure 1, was systematically followed to achieve the study's objectives. Water hyacinth (WH) was collected from Lake Victoria, and human faecal matter was sourced from selected sites. The collected waste was dried, ground, and mixed with water in appropriate proportions to create a homogenous substrate mixture, ensuring uniformity for efficient anaerobic digestion. The mixture was then subjected to anaerobic digestion under controlled conditions, with temperature and pH levels carefully monitored to facilitate optimal microbial activity (Tan et al., 2021; Lukitawesa et al., 2019).

Two primary experimental procedures were conducted. Initially, varying proportions of water hyacinth and human faecal matter (4:0, 3:1, 2:2, 1:3, 0:4) were mixed to form substrate combinations for co-digestion. The prepared mixtures then underwent chemical pretreatment using a 1% NaOH solution to enhance substrate breakdown. Following pretreatment, the substrates were subjected to anaerobic digestion to produce biogas (Obileke et al., 2020). The gas generated was collected, measured, and analyzed using gas chromatography to determine biogas yield and methane concentration. Additionally, human faecal matter was characterized through proximate and ultimate analyses to determine its elemental composition, including nitrogen (N), hydrogen (H),

and carbon (C) (Xiao et al., 2022). This structured sequence provided empirical data for optimizing

biogas production while managing waste effectively (Zhou et al., 2024).

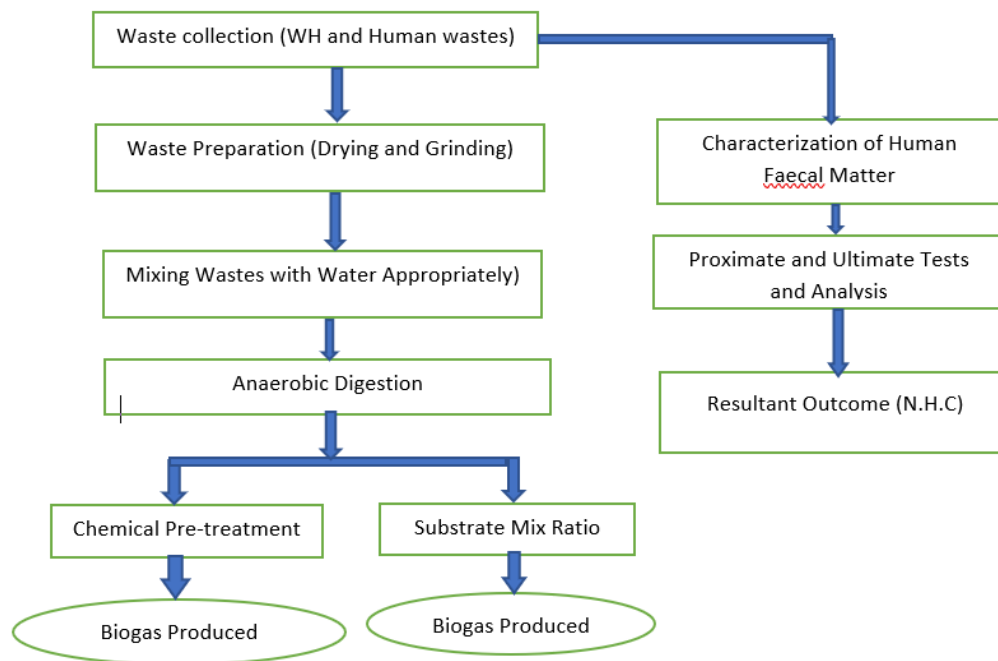


Figure 2 Experimental setup

### 2.3.3 Chemical pretreatment

To improve the digestibility of lignocellulosic biomass, a chemical pretreatment procedure was conducted (Deepanraj et al., 2017). Water hyacinth was pretreated by soaking in a 1% sodium hydroxide (NaOH) solution for three days to disrupt the lignin structure and enhance microbial access to cellulose. After pretreatment, the samples were oven-dried and co-digested with faecal matter. Equal amounts (100 g) of the treated water hyacinth and faecal matter were combined with 500 ml of distilled water and subjected to anaerobic digestion under mesophilic conditions in airtight batch digesters. Methane content in the produced biogas was determined using gas chromatography (CHEMITO). The chemical pretreatment was expected to improve methane yield by increasing substrate biodegradability, as supported by previous findings (Barua, et al 2019; Manigandan et al., 2023; Kulabako et al., 2025; Zhao et al., 2022).

### 2.4 Optimization procedures

The optimization of biogas production was integrated into the anaerobic digestion experiments. Optimization efforts focused on identifying the most effective substrate mixing ratio and evaluating the effect of chemical pretreatment. The empirical data

collected from gas yield and methane concentration were subjected to statistical analysis using Minitab software. Analytical methods included analysis of variance (ANOVA) and response surface methodology (RSM) to assess the interaction effects between substrate composition and pretreatment. These analyses aimed to determine the combination of factors that produced the highest biogas and methane yields, following methods applied in similar optimization studies (Zhou et al., 2024).

The optimization results offer valuable insights into the ideal substrate mixture that enhances biomethane output by maximizing the components most critical to microbial digestion. To achieve this, a mathematical optimization expression was applied, as shown in Equation 7, with the objective of maximizing the sum of volatile solids (Vs), crude protein (Cp), and total solids (Ts). These parameters were chosen due to their respective influence on biogas yield—volatile solids contribute directly to gas production, crude protein supports microbial activity during anaerobic digestion, and total solids reflect the overall energy density of the substrate:

$$\text{Objective: max (Vs + Cp + Ts)} \quad (7)$$

### 2.5 Data collection and analysis

Data collection and analysis procedures were designed to ensure accuracy and reproducibility. Daily biogas volumes were recorded based on water displacement measurements. Methane concentration in the gas samples was analyzed using a gas chromatograph (CHEMITO). Concurrently, pH levels of the digesters were measured daily with a Systronics pH meter to monitor environmental stability within each reactor. All the collected data were compiled and statistically analyzed using Minitab. This involved regression modeling, trend analysis, and optimization to interpret the influence of each treatment. Results were graphically represented using line and bar charts to compare the performance of treated and untreated samples, and to visualize daily and cumulative gas production under different substrate combinations.

### 3 Results and discussion

This section presents and interprets the findings obtained from the characterization of water hyacinth and human fecal matter, their subsequent anaerobic digestion, and the evaluation of biogas and methane yields under varying substrate ratios and treatment conditions. The results are analyzed to understand the influence of substrate composition, pretreatment, and operational parameters on biogas production. Emphasis is placed on key biochemical properties—such as moisture content, ash, crude protein, volatile solids, and total solids—which directly affect microbial digestion efficiency and gas yield. Optimization outcomes are also discussed to identify the most effective substrate blend for enhancing methane output, providing empirical support for sustainable biogas generation from locally available organic waste (Dang et al., 2017; Ilo et al., 2021).

#### 3.1 Characterization of substrates

##### 3.1.1 Proximate composition of water hyacinth and faecal matter

Figure 2 presents a comparative analysis of the proportions of various components found in water hyacinth, institutional fecal waste, and effluent fecal waste. Each parameter is illustrated with three distinct bars, allowing for a detailed examination of the

compositional variability among the different substrates. The figure reveals that water hyacinth possesses the highest moisture content at 90.28%, followed by effluent fecal waste at 80.74%, and institutional fecal waste at 80.13%. This high moisture content in water hyacinth suggests a lower energy density, potentially making it less efficient as a sole feedstock in biogas production. In contrast, the fecal waste samples contain significantly higher levels of crude protein—18.62% for institutional and 19.96% for effluent waste—compared to 12.39% in water hyacinth. Elevated protein levels are beneficial for microbial activity during anaerobic digestion. Moreover, the ash content is notably higher in the faecal matter samples, indicating a greater fraction of inorganic, non-biodegradable material.

When comparing these findings with those of other researchers, the results are largely consistent. For instance, Kowthaman et al. (2021) observed that fecal waste tends to have superior nitrogen content due to its higher protein levels, which supports a more robust microbial community during digestion. The current study affirms this observation and emphasizes the necessity of substrate optimization. While water hyacinth is abundant and biodegradable, its excessive moisture can reduce the digester's efficiency. Thus, blending it with fecal matter could create a more balanced substrate, improving biogas yield and process stability through complementary nutrient profiles and moisture correction.

##### 3.1.2 Component concentration of substrates

Figure 3 presents a box plot comparing the distribution of proximate components—such as moisture content, ash, and protein levels—across three substrates: water hyacinth (WH), effluent waste (AW), and institutional waste (IW). The median values and interquartile ranges (IQRs) provide insight into the compositional variability of each substrate. Water hyacinth exhibits a median value of approximately 30, with a broad IQR, suggesting high variability and a significant moisture content that could impact energy yield. Effluent waste has a higher median, close to 60,

and also displays a wide IQR with several outliers, indicating nutrient richness but inconsistent composition. Institutional waste falls just below a median of 50 and shows a slightly narrower IQR compared to AW, suggesting more uniform characteristics, though variability still exists, particularly in ash content and nutrient concentrations. These differences are crucial in evaluating substrate behavior during anaerobic digestion for biogas production.

The findings of this study align with earlier research, particularly that of Kowthaman et al. (2021), who emphasized the importance of compositional balance in substrate selection for optimal biogas output. The variability observed in water hyacinth and effluent waste underlines the challenges of using them as sole substrates, due to fluctuations in nutrient and moisture levels that may disrupt microbial activity. By contrast, institutional waste appears to offer a more stable composition, supporting more consistent biogas yields. These insights reinforce the importance of co-digestion strategies that blend substrates with

complementary characteristics, ensuring stability and efficiency in anaerobic digestion processes.

### 3.1.3 Comparison of different waste types

Figure 4 presents the mean values and variability of key proximate composition parameters for three substrates: water hyacinth, effluent waste, and institutional waste. Red markers indicate the average composition levels—16.18 units for water hyacinth, 19.18 units for effluent waste, and 21.18 units for institutional waste—while the vertical lines denote the extent of variation around these means. The data reveal that institutional waste has the highest mean value, particularly in terms of crude protein and total solids, both critical components for efficient biogas production. Effluent waste follows closely, with moderate nutrient concentrations, while water hyacinth registers the lowest average values and greater variability. These trends suggest that institutional waste may offer more reliable substrate properties, whereas the broader variation in water hyacinth highlights its less predictable nature for digestion processes.

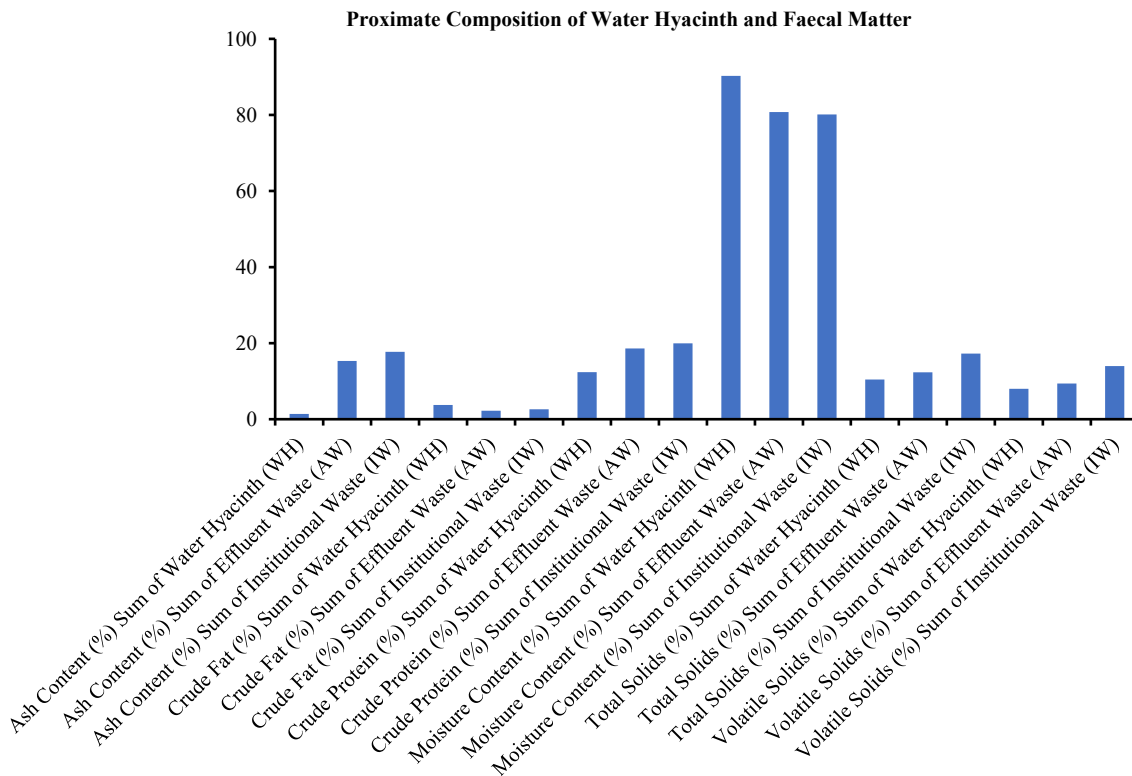


Figure 3 Proximate composition showing ash content, moisture content, crude protein, volatile solids, and total solids for water hyacinth (WH), general affluent waste (AW) and the institutional waste(IW) respectively

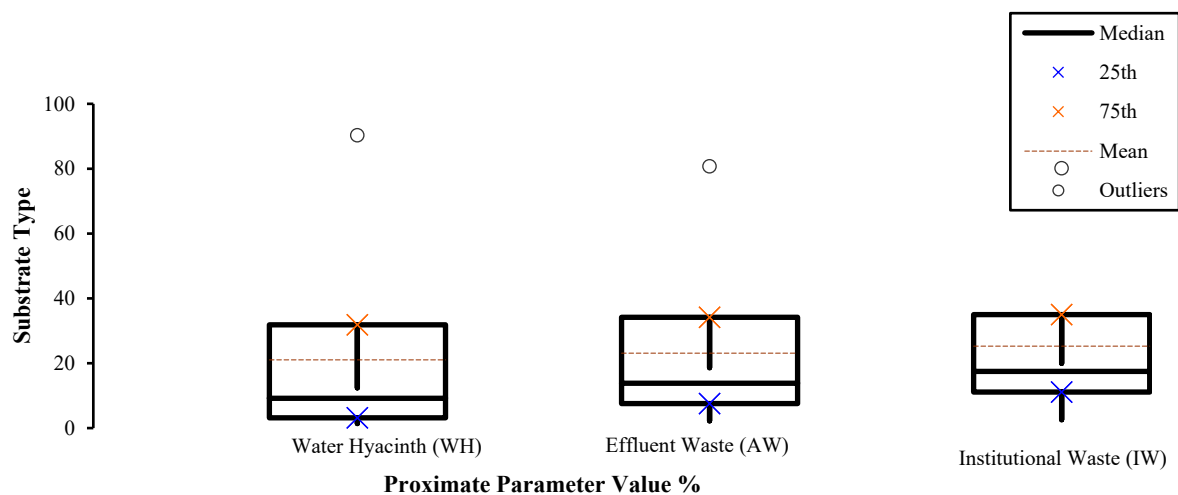


Figure 3 Proximate composition of substrates

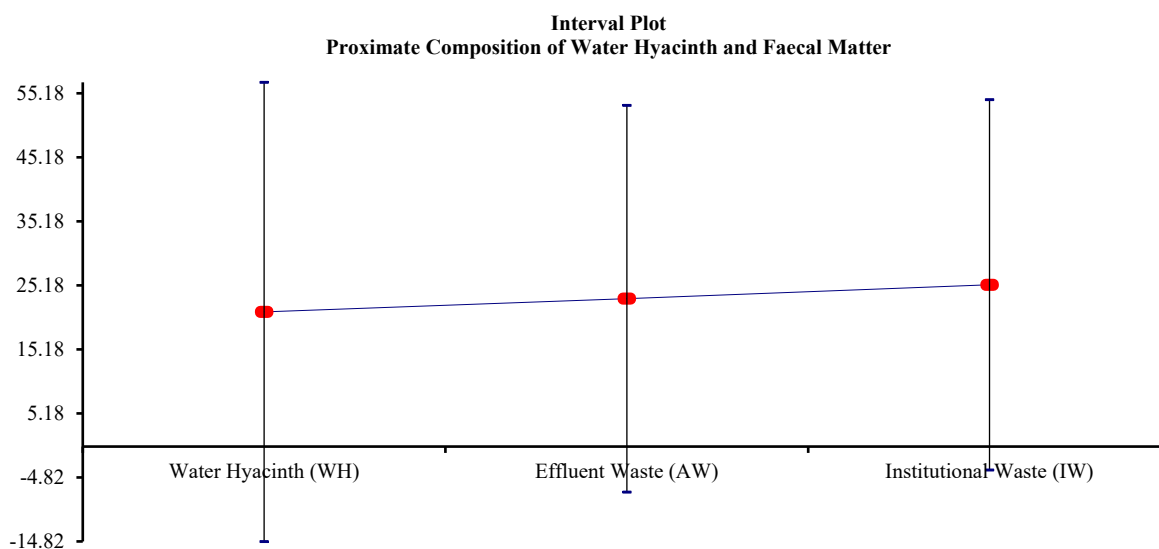


Figure 4 Proximate Composition of Water Hyacinth and Faecal Matter

Table 1 Optimized proximate composition of different substrate combinations

Combination	Crude fat (%)	Ash content (%)	Moisture content (%)	Crude protein (%)	Volatile solids (%)	Total solids (%)
IW + AW	6.40	10.17	83.58	17.96	8.18	23.00
WH + IW	3.20	12.22	82.01	15.36	9.78	20.93
WH + AW	4.99	11.70	82.81	17.11	8.60	22.99
WH + IW + AW	4.21	10.23	83.71	16.29	8.87	22.25

These findings are consistent with those reported by Kowthaman et al. (2021), who emphasize that substrates with higher protein and solid content yield better biogas outcomes. The relatively stable composition of institutional waste supports its potential as a primary substrate, particularly due to its consistent nutrient profile which fosters stable microbial activity during anaerobic digestion. In contrast, the variability in water hyacinth's composition underscores the need for careful preprocessing or co-digestion strategies to mitigate

fluctuations in feedstock quality. This comparative analysis strengthens the argument for optimizing substrate mixtures by balancing high-energy components with more stable materials, thereby enhancing the efficiency and predictability of biogas generation systems.

### 3.1.4 Substrate combination analysis

Monte Carlo simulations determined the most practical combination of water hyacinth (WH), Effluent waste (AW), and institutional waste (IW) based on their proximate compositions. Table 1

presents the weighted combinations and their corresponding compositional values.

The experimental results indicate that the IW + AW combination is the most effective for biogas production. It had the lowest ash content (10.17%)—slightly lower only in the three-substrate mix (10.23%)—alongside the highest crude protein (17.96%) and total solids (23.00%). This optimal balance of nutrients supports efficient microbial digestion, enhances methane production, and allows higher organic loading without excessive dilution. Low ash levels reduce inert residue accumulation, while high protein content provides sufficient nitrogen for microbial growth and activity.

Other combinations, especially those involving water hyacinth (WH), showed mixed outcomes. WH + IW had the highest volatile solids (9.78%), suggesting more biodegradable material, but its low protein (15.36%) and total solids (20.93%) could limit productivity. WH + AW showed a more balanced profile with moderate protein (17.11%) and total solids (22.99%). The three-substrate mix offered high moisture (83.71%) and low ash content (10.23%) but had intermediate protein (16.29%) and solids (22.25%). Although WH improves digester stability through moisture, its lignocellulosic nature limits biodegradability. These findings underscore the need for substrate combinations that balance organic content and nutrient availability. Co-digesting faecal matter with water hyacinth presents a sustainable, high-yield biogas solution.

### 3.2 The effect of mix ratio on biogas production

This section presents the experimental results examining how different mix ratios of water hyacinth (WH) and faecal matter (FM) influenced biogas production over a 67-day anaerobic digestion period. The findings reveal significant variations in both production rates and cumulative biogas yields across different substrate combinations.

#### 3.2.1 Effect of substrate mix ratios on biogas production

Figure 5 illustrates biogas production trends over a 67-day anaerobic digestion period using varying ratios

of water hyacinth (WH) and faecal matter (FM). The data demonstrate the substantial impact of substrate composition on both the rate and total volume of biogas generated. The 100% FM mix (line 5) produced the highest biogas yield, reaching peak production around days 40–45. This performance is attributed to FM's high nitrogen content (17.96% crude protein) and total solids (23.00%), which create favorable conditions for methanogenic microbial activity. After peak production, the output declined gradually as the readily biodegradable material was depleted.

The 100% WH mix (line 1) produced the lowest biogas volumes, likely due to its lignocellulosic structure, which limits microbial degradation. This supports Zhang et al. (2017), who found that lignocellulosic materials underperform as mono-substrates. The 75% WH:25% FM mix (line 2) showed fluctuating production with minor peaks, indicating slow and inconsistent degradation, consistent with Kowthaman, Selvan, and Kumar, (2021), who observed similar patterns with fibrous residues. The 50:50 WH:FM mix (line 3) maintained moderate but steady biogas output, likely due to a balanced nutrient profile. Adekunle and Okolie (2015) also noted that co-digestion with balanced C: N ratios improves process stability. The 25% WH:75% FM mix (line 4) resembled the 100% FM mix in performance but with slightly lower peaks. It showed a sharp increase in production between days 35–50 before declining. FM-dominant mixtures ( $\geq 75\%$  FM) produced the highest yields, while equal ratios supported more stable microbial activity over time.

#### 3.2.2 Optimal substrate mix ratios for enhanced biogas production and process stability

Figure 6 offers an insightful overview of the cumulative biogas production for various substrate ratios over the study period. It displays the distribution of biogas production for each substrate mix, including 1 g of water hyacinth (WH), different combinations of WH and faecal matter (FM), and pure FM (Nahar et al., 2024). The 1 g FM mixture consistently shows the highest cumulative biogas production, with a wide range, as indicated by the length of the box and the

range of whiskers. This indicates high variability, possibly due to fluctuations in microbial activity and nutrient availability. The median for 1 g FM is higher

than for all other ratios, suggesting it is the most effective substrate for biogas production.

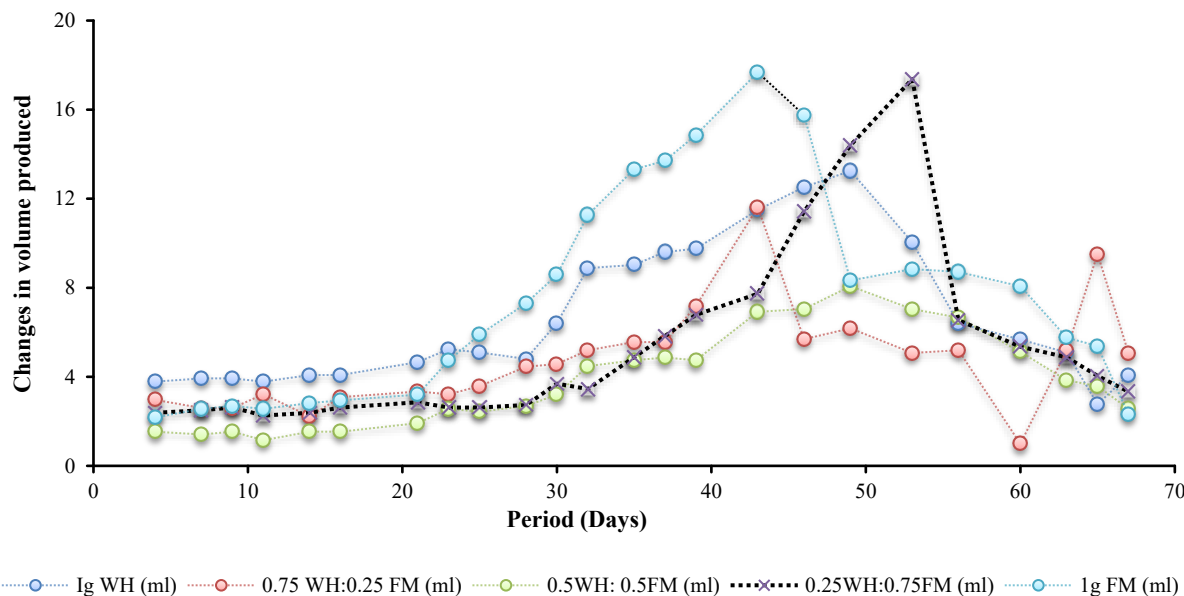


Figure 5 Changes in volume produced for different substrate mixes

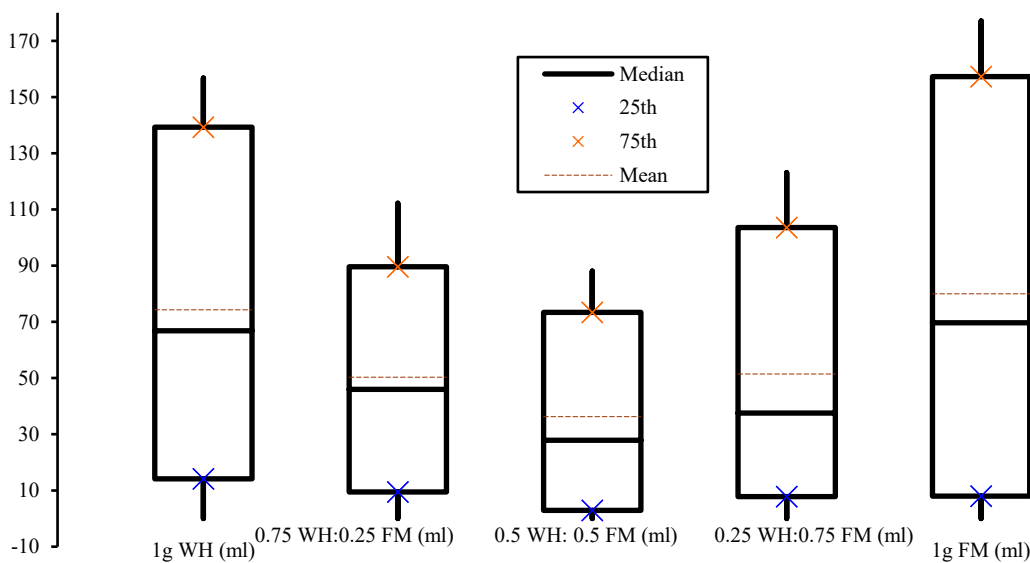


Figure 6 Box plot showing the distribution of cumulative biogas production (ml) from different substrate mix ratios of water hyacinth (WH) and fecal matter (FM) over a 67-day anaerobic digestion period.

The 0.75 WH:0.25 FM mix produced the lowest cumulative biogas, with a lower median and narrower range, indicating reduced efficiency. This is likely due to the dominance of water hyacinth, which has high moisture but limited nutrients for methanogens. The 0.5 WH:0.5 FM and 0.25 WH:0.75 FM mixes showed moderate yet more consistent biogas output, balancing nutrients and enhancing process stability compared to pure WH or highly skewed FM ratios. These findings align with Kumar et al. (2021), who noted that higher

animal manure content in co-digestion yields more biogas, and Zhang et al. (2017), who reported that lignocellulosic materials like WH perform better when combined with nutrient-rich substrates. The wide distribution in pure WH and FM treatments suggests greater variability, supporting Adekunle and Okolie's (2015) conclusion that optimized co-digestion enhances stability. Mixed substrates create a more favorable environment for microbial activity, underscoring the value of balanced ratios in small-

scale anaerobic systems.

### 3.2.3 Variability and mean production trends across substrate combinations in a comparative analysis

The interval plot in Figure 7 illustrates mean cumulative biogas production and variability across different substrate ratios. A U-shaped trend emerges, with the highest output from pure substrates and the lowest from the 50:50 WH:FM mix. Pure fecal matter (1g FM) produced the most biogas (~80 ml), followed

closely by pure water hyacinth (1g WH) at ~74 ml. In contrast, the 0.5 WH:0.5 FM mixture yielded the lowest (~36 ml), suggesting it may not optimize biogas generation. Notably, pure substrates had wider confidence intervals ( $\pm 45$ – $50$  ml), indicating inconsistent performance, while mixed ratios—especially the 0.5 WH:0.5 FM—exhibited narrower intervals ( $\pm 30$  ml), pointing to more stable and predictable biogas output.

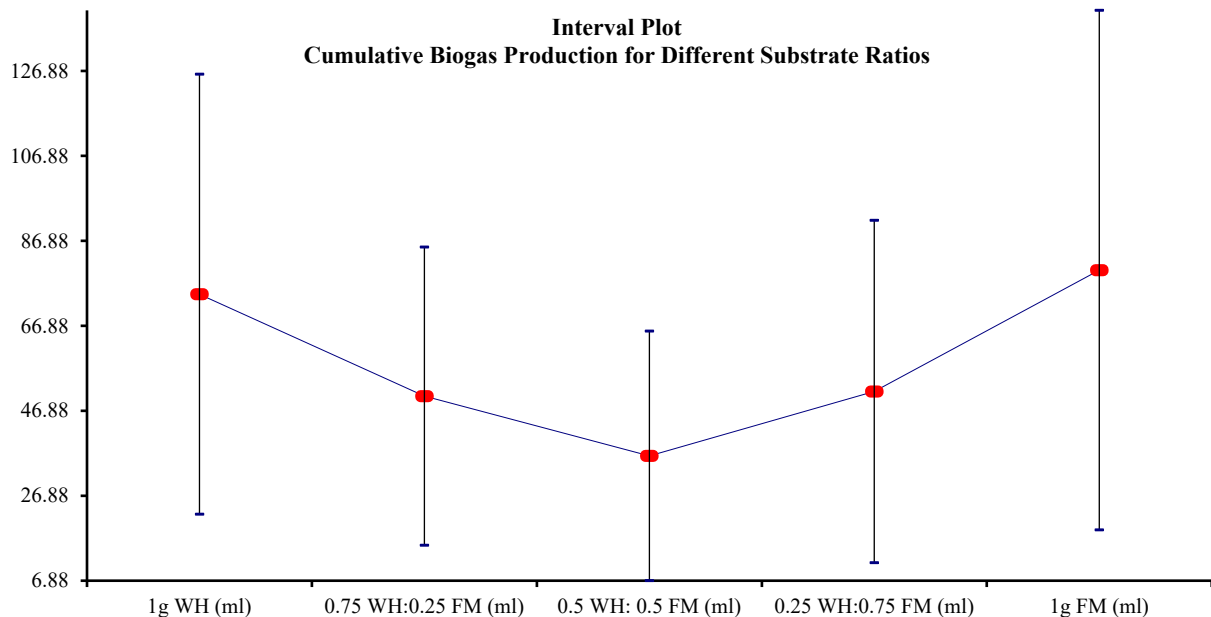


Figure 7 Mean cumulative biogas production with 95% confidence intervals

These findings both support and challenge earlier research. Mao et al. (2019) similarly reported higher peaks from mono-substrates, while our results contrast with Wang et al. (2021), who found balanced ratios more efficient. The variability in pure substrates aligns with Okonkwo et al. (2018), who observed instability in mono-digestion due to C: N imbalance. The stable performance of mixed substrates echoes Jabeen et al. (2020), who emphasized reliability over yield in practical systems. Interestingly, the low output from the 50:50 ratio contradicts the theoretical assumption of optimal C balance, hinting at other factors like microbial surface area or possible antagonistic effects between substrates. These results underscore the complexity of anaerobic co-digestion and emphasize the need for empirical validation when formulating feedstock combinations, particularly with structurally resistant materials like water hyacinth.

### 3.2.4 Biogas production variability across different

substrate mix ratios

Statistical analysis of biogas yield across different substrate mix ratios revealed notable differences in both production levels and consistency. Table 2 presents descriptive statistics for biogas generation from combinations of water hyacinth (WH) and fecal matter (FM). Pure substrates yielded the highest mean outputs, with 1g FM producing 71.11 ml and 1g WH 67.12 ml. However, these high yields came with substantial variability—standard deviations were 66.03 ml and 55.34 ml, respectively. In contrast, mixed ratios exhibited lower but more stable production, with the 0.5 WH:0.5 FM mix showing the lowest mean yield (31.57 ml) but also the smallest standard deviation (31.30 ml). Confidence intervals echoed this pattern, as pure substrates had wider 95% intervals (26.67 ml for FM, 22.35 ml for WH), while the balanced mix showed a narrower interval (12.64 ml), indicating more consistent performance.

**Table 2 Descriptive statistics of biogas production (ml) from different substrate mix ratios of water hyacinth (WH) and fecal matter (FM)**

Parameters	1g WH	0.75 WH:0.25 FM	0.5 WH:0.5 FM	0.25 WH:0.75 FM	1g FM
Mean	67.12	45.44	31.57	45.18	71.11
Standard Deviation	55.34	37.30	31.30	42.97	66.03
Median	52.61	36.82	20.67	28.83	48.78
Confidence Level (95%)	22.35	15.07	12.64	17.35	26.67

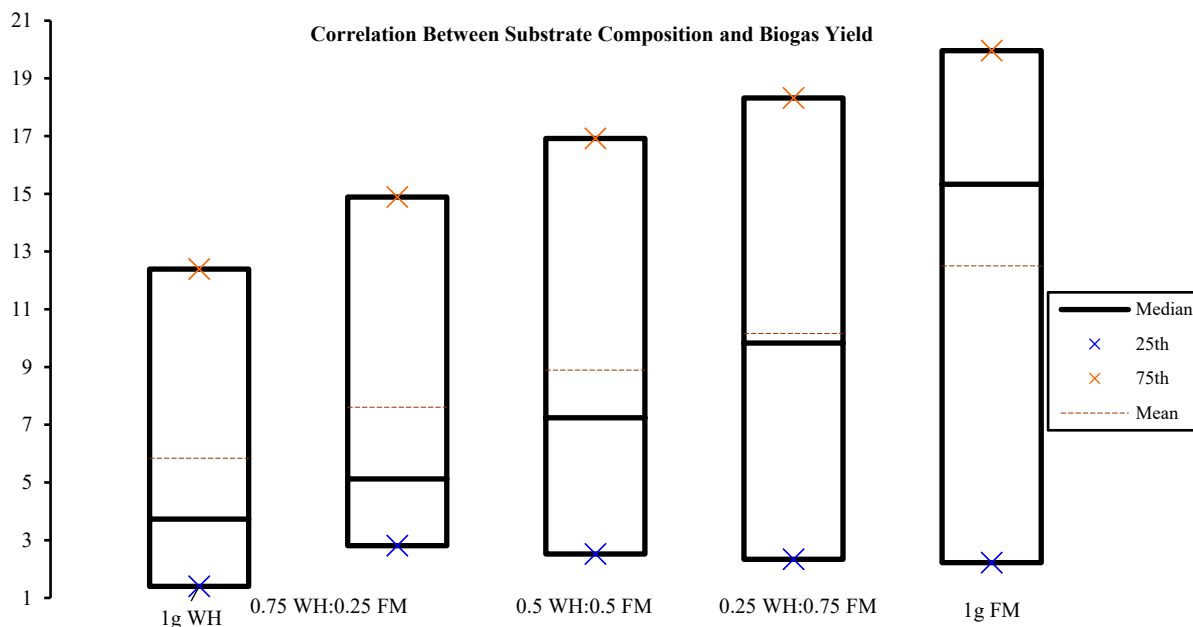


Figure 8 Box plot showing the correlation between substrate composition and biogas yield across different mix ratios of water hyacinth (WH) and fecal matter (FM).

The results from this study differ from previous research on co-digestion of lignocellulosic materials and nitrogen-rich substrates. While Hassan et al. (2021) found that optimal biogas production occurs at balanced carbon ratios, typically seen in 50:50 mixtures, our findings indicate that pure substrates may produce higher biogas volumes, albeit with greater variability. This difference may be attributed to the specific properties of water hyacinth, which contains complex carbohydrates that can limit microbial access unless pretreated. The greater stability observed in the 0.5 WH:0.5 FM mixture supports Li et al. (2020) suggestion that co-digestion can enhance stability by providing complementary nutrients, even if it does not yield the highest output. Furthermore, the variability observed in pure FM production is consistent with Chen et al. (2019), who noted that nitrogen-rich substrates alone can cause ammonia buildup and pH imbalances, disrupting methane production. These findings underline the important balance between maximizing biogas

production and maintaining stability in anaerobic digestion systems, which is especially crucial for rural or small-scale applications where consistent and predictable performance may be prioritized over maximizing high, but variable, yields.

### 3.2.5 Impact of biogas production rate on substrate composition

Figure 8 illustrates the relationship between substrate composition and biogas yield across different mix ratios of water hyacinth (WH) and fecal matter (FM). The box plot reveals a trend of increasing biogas production with higher proportions of fecal matter. Pure fecal matter (1g FM) yielded the highest median biogas production (15.4 ml) with a wide interquartile range (2-20 ml), indicating substantial yet variable production. In contrast, pure water hyacinth (1g WH) showed the lowest median yield (3.8 ml) with a narrower spread. Mixed substrates followed a graduated pattern: 0.75 WH:0.25 FM (5.1 ml), 0.5 WH:0.5 FM (7.3 ml), and 0.25 WH:0.75 FM (9.8 ml), correlating directly with the nutritional profiles,

particularly crude protein and fat content, which are significantly higher in FM (17.96% and 6.40%) than in WH (15.36% and 3.20%). The wider variability in FM-dominant mixtures suggests more dynamic microbial activity due to the greater availability of readily digestible nutrients.

These findings align with but expand upon previous research in anaerobic co-digestion. Contrary to Rahman et al. (2017), who reported optimal performance at 70:30 lignocellulosic ratios, our results show a direct correlation between increasing FM content and biogas yield. This discrepancy may be due to the recalcitrance of water hyacinth's structure, which

contains 35% cellulose and 18% lignin, impeding microbial access without pretreatment. Our results support Dharmaraj et al. (2023), who found positive correlations between nitrogen-rich feedstocks and methane production. However, the increased variability in FM-dominant mixtures contradicts Zhao et al. (2021), suggesting that balanced ratios (0.5 WH:0.5 FM) may offer more consistent production in practical applications where operational stability is prioritized. 50:50 ratio contradicts the theoretical assumption of optimal C balance, hinting at other factors like microbial surface area or possible antagonistic effects between substrates.

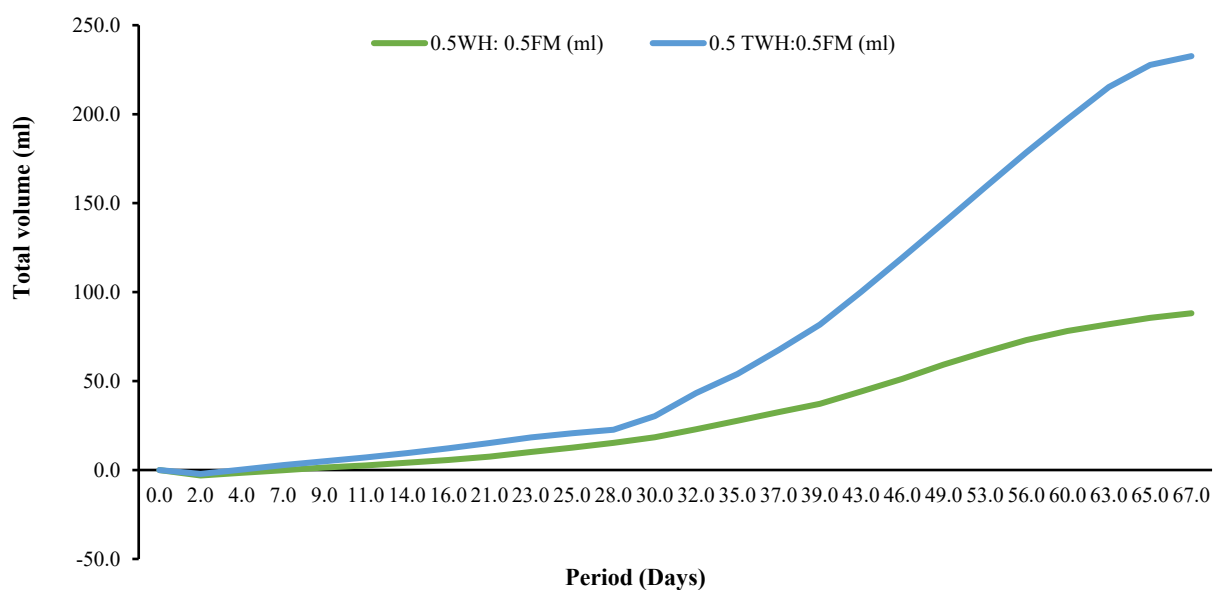


Figure 9 Volume of biogas (ml) produced before and after chemical treatment against period (days)

### 3.3 Effect of chemical pre-treatment on a volume of biogas produced

Investigating the effect of chemical pre-treatment on the volume of biogas produced from a specific mix ratio of water hyacinth (WH) and human faecal matter (FM) consists of pre-treated and untreated samples. The goal of chemical pre-treatment is to enhance the digestibility of organic materials and improve biogas yield. Methane content in biogas is a critical indicator of biogas quality and energy potential. Throughout the observation period, the volume of methane in the biogas closely mirrors the trend observed in total biogas production.

The results suggest that, under the conditions of this study, chemical pre-treatment significantly

impacts the volume of biogas produced from the 0.50 WH: 0.50 FM mix ratio. The consistent increase in biogas production over time indicates effective anaerobic digestion of the organic material with additional chemical interventions. Therefore, this result provides valuable insights into the effect of chemical pre-treatment on biogas production from a specific mix ratio of water hyacinth and human faecal matter. The results suggest that, in this study, chemical pre-treatment significantly influenced biogas production, as evidenced by the consistent increase in biogas volume over time. Without pre-treatment, the total biogas produced by the 67<sup>th</sup> day was 62.08 ml. However, the total biogas produced for the same period after pre-treatment was 232.6 ml (about a 200%

jump in the biogas yield). This important finding aligns with the evidence presented by Sarto et al. (2019), who found that after pre-treatment, total biogas production increased by 131.45% compared to the untreated sample. Wadchasit et al. (2020) give a possible explanation for this phenomenon. The researchers

The descriptive statistics offer valuable insights into the volume of biogas produced from two different mix ratios: 0.50 WH: 0.50 FM and 0.50 TWH (Treated water hyacinth): 0.50 FM. These statistics provide a comprehensive overview of each mix ratio's central tendency, variability, and confidence intervals associated with biogas production.

**Table 3 Mean, STD deviation, Median, Standard Error, and confidence level in various treated and untreated mix proportions**

	0.50 WH: 0.50 FM	0.50 TWH: 0.50 FM
Mean	31.56460542	75.2440798
Standard Error	6.138833011	15.85180255
Median	20.66777342	36.74576321
Standard Deviation	31.30202931	80.82865054
Confidence Level (95.0%)	12.64316326	32.64739849

The mean represents the average volume of biogas produced for each mix ratio. For the 0.50 WH: 0.50 FM mix ratio, the mean biogas production is 31.56 ml, while for the 0.50 TWH: 0.50 FM mix ratio, the mean biogas production is notably higher at 75.24 ml. The higher mean for the 0.50 TWH: 0.50 FM mix ratio suggests that adding chemical treatment (TWH) positively impacts biogas production compared to the untreated mix. On the other hand, the standard error measures the variability of biogas production estimates around the mean for each mix ratio. A lower standard error indicates greater precision in estimating the true mean biogas production. In this case, the standard error is 6.14 ml for the 0.50 WH: 0.50 FM mix ratio and 15.85 ml for the 0.50 TWH: 0.50 FM mix ratio. The lower standard error for the 0.50 WH: 0.50 FM mix ratio suggests higher precision in estimating biogas production than the treated mix ratio.

The median represents the middle value of the biogas production dataset for each mixing ratio. Unlike the mean, which outliers can influence, the median provides a robust measure of central tendency. The median biogas production is 20.67 ml for the 0.50 WH: 0.50 FM mix ratio and 36.75 ml for the 0.50 TWH: 0.50 FM mix ratio. The higher median for the treated

conducted a study examining how pre-treatment affects biogas production. They found that with pre-treatment, the substrate is subjected to a change in lignin and cellulose composition whereby some lignin structures are removed, leading to more accessibility by microorganisms that result in improved digestion.

mix ratio further supports the notion that chemical treatment enhanced biogas production compared to the untreated mix. In addition, the standard deviation quantifies the spread of biogas production values around the mean for each mix ratio. A higher standard deviation indicates more significant variability in biogas production within the dataset. The standard deviation is 31.30 mL for the 0.50 WH: 0.50 FM mix ratio and notably higher at 80.83 ml for the 0.50 TWH: 0.50 FM mix ratio. The higher standard deviation for the treated mix ratio suggests more significant variability in biogas production compared to the untreated mix.

#### 4 Conclusion

This research confirms that the anaerobic co-digestion of water hyacinth (WH) and human faecal matter (FM) offers an effective solution for both biogas production and waste management, particularly in Kisumu City, where pollution from invasive water hyacinth and poor sanitation significantly affects the environment and public health (Cioablă and Popescu, 2021). The study found that pure FM produced the highest biogas yield at 177.1 ml, while combining FM with WH enhanced methane production, with the 0.25

WH: 0.75 FM ratio yielding 123.1 mL of biogas. Additionally, statistical analysis showed that this same ratio (0.25 WH: 0.75 FM) produced a relatively high mean value of 45.18 ml in the mix substrates, whereas pure FM (1g) showed higher variability, as indicated by a standard deviation of 66.03 mL and also the highest mean value of 71.11 mL. Furthermore, pre-treating water hyacinth with a 1% NaOH solution improved its biodegradability, resulting in a 15% increase in methane output compared to untreated WH—highlighting the effectiveness of alkali pretreatment in enhancing lignocellulosic breakdown. These findings support co-digestion as a sustainable waste-to-energy strategy capable of addressing sanitation challenges and ecological degradation in Lake Victoria, while also providing renewable energy. Continued research into pretreatment techniques and scaling up implementation could yield greater environmental and socio-economic gains.

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## Appendix

### Limitations of study and Future recommendations

While this research exhibited a great deal of strengths in the co-digestion of water hyacinth and fecal human matter, certain challenges and limitations were inherent during the experimentation stage. First and foremost, the volatility of the human waste made waste collection and preparation a great deal of challenge. Besides, there was a problem about the sampling procedure, where the waste could not have been as random as desired. This experimentation was subject to operational limitations due to the shortage of labs with the capacity to effectively handle human waste. In future, there should be dedicated laborites well equipped with the necessary tools to handle human waste and other substrates.

### Nomenclature

**WH** – Water Hyacinth

**FM** – Faecal Matter (also spelled Fecal Matter in the abstract)

**KIRDI** – Kenya Industrial Research and Training Institute

**NaOH** – Sodium Hydroxide

**ml** – milliliters

**TWH**-Treated Water Hyacinth

**CHN** – Carbon, Hydrogen, Nitrogen (as in CHN analyzer)

**FC** – Fixed Carbon

**°C** – Degrees Celsius (used with oven temperatures, not a traditional abbreviation but a standard unit)