

Biogas production from water lilies, food waste, and sludge: substrate characterization and process performance

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ABSTRACT

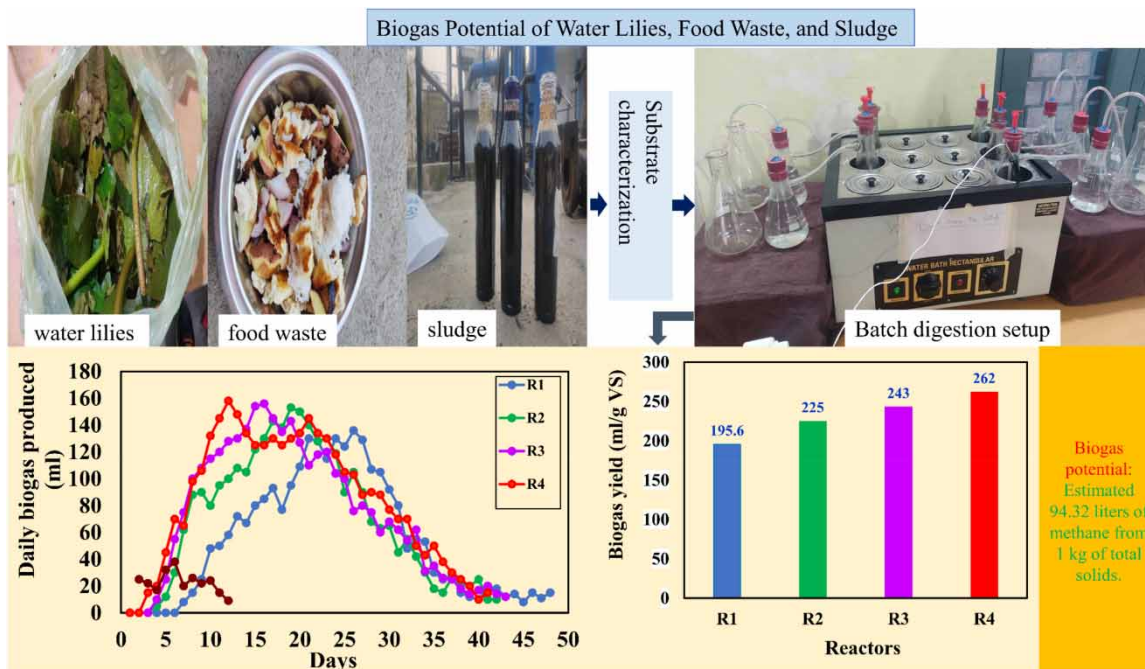
The potential of water lilies, food waste, and sludge as substrates for biogas production through anaerobic digestion was investigated. We thoroughly characterized these substrates and found that water lilies had a pH of 6.4, total solids (TS) of 18.42%, volatile solids (VS) of 81.46%, and a moisture content of 87%. Food waste exhibited a pH of 7.6, TS of 27.23%, VS of 90.6%, and a moisture content of 75%. Sludge had a pH of 6.5, TS of 6%, VS of 60%, and a moisture content of 95%. Biogas production exhibited variations among the reactors. Reactor 1 reached a cumulative production of 2,527 mL, while Reactor 4 achieved 3,404 mL, with different lag phases. Reactor 4 displayed the highest biogas yield at 262 mL/g VS. Post-digestion tests confirmed efficient digestion, with volatile fatty acids ranging from 140 to 300 mg/L acetic acid and alkalinity levels between 800 and 1,500 mg CaCO₃/L. Our study estimated a significant methane content, with the potential to produce 94.32 L of methane from 1 kg of TS.

Key words: anaerobic digestion, biogas production, food waste, sludge, water lilies

HIGHLIGHTS

- Investigation of water lilies, food waste, and sludge as substrates for biogas production.
- Characterization of substrates based on pH, total solids, volatile solids, and moisture content.
- Evaluation of biogas production dynamics, including daily production, cumulative production, lag phase, and time to peak production, to understand substrate performance and microbial activity.

GRAPHICAL ABSTRACT



1. INTRODUCTION

The rapid global population increase has led to a surge in energy demands (Asif & Muneer 2007). Many countries still heavily rely on conventional energy sources such as coal, petroleum, and natural gas, which not only lead to the emission of greenhouse gases but also suffer from limited availability (Pathak 2020). This reliance has detrimental environmental effects, including rising global temperatures and ecological imbalances (Intergovernmental Panel on Climate Change 2018). The depletion of these resources and the associated environmental damage are worldwide concerns (Christensen & Olhoff 2019). It is crucial to reduce dependence on non-renewable resources and find eco-friendly alternatives (Gielen *et al.* 2019).

The proliferation of aquatic weeds, especially water lilies, in lakes poses a pressing environmental issue, negatively impacting biodiversity and the visual appeal of water bodies (Yan *et al.* 2017). Water lilies are aquatic angiosperms in the Family Nymphaeaceae (Sunian 2004). Although water lilies are essential ornamental plants that enhance the beauty of ponds and lakes, they have significant adverse effects on lake water and ecology. Water lily leaf beetles have been found to increase leaf senescence and reduce leaf density, potentially affecting other aquatic species (Stenberg & Stenberg 2012). Invasive plants, including water lilies, displace native species, reduce ecosystem services, and cause economic losses (Hassan & Nawchoo 2020). Eutrophication is a significant problem in the famous Dal Lake in Kashmir Valley, India (Kahn 2000), leading to the rapid growth of aquatic weeds, including water lilies. Hence, there is growing concern among various communities about the need for the proper utilization and conservation of these weeds.

Additionally, the increasing population, industrialization, urbanization, and changing lifestyles have resulted in a rise in municipal solid waste generation (Gardner 2013). Proper management of municipal solid waste is a significant challenge in Kashmir Valley, India (Mushtaq *et al.* 2020a, 2020b, 2022; Mir *et al.* 2023). Worldwide, one-third of daily food production is wasted (Bond *et al.* 2013). Managing sewage treatment plant sludge has also become a significant challenge, with large quantities of raw sludge generated daily (Cieřlik *et al.* 2015).

As a result, there is a growing need to adopt scientifically sound approaches to organic waste management and renewable energy production (Rorat & Kacprzak 2017). The wastes are currently repurposed as animal feed or used for fertilizer production. While these wastes are currently repurposed as animal feed or used for fertilizer production, most of the generated waste is typically managed through landfilling, incineration, composting, or anaerobic digestion (AD) (Mukherjee *et al.* 2020). Landfilling food waste leads to greenhouse gas emissions and soil and groundwater contamination through percolation. Incineration and composting are not effective options for food waste due to their high moisture content. These improper

waste disposal methods have adverse environmental effects and pose health risks, including the formation of leachate, noxious odors, methane emissions, and contamination of surrounding water and air (Mukherjee *et al.* 2020).

In contrast, AD is a suitable solution for organic waste disposal, producing biogas rich in methane and digested slurry, which can be utilized as fertilizer (Holm-Nielsen *et al.* 2009). The efficiency of biogas generation depends on the chemical composition and biodegradability of the substrate used (Odedina *et al.* 2017). Water lilies, due to their high moisture content and biodegradable organic matter, can serve as a valuable component in biogas generation through AD, particularly when mixed with other suitable organic materials. While they may not be rich in cellulose, hemicellulose, or lignin, their nutrient content and adaptability to anaerobic conditions make them a feasible feedstock for this purpose (Junluthin *et al.* 2021).

Taking the foregoing discussion into account, this study introduces a novel approach by exploring diverse substrates, including water lilies, food waste, and sludge, through AD. The primary objectives are to (1) characterize the composition and properties of these substrates, including pH, total solids (TS), volatile solids (VS), and moisture content, (2) assess the suitability of these substrates for biogas production through pre-digestion tests, considering parameters such as TS, VS, pH, alkalinity, and volatile fatty acids (VFAs), and (3) analyze biogas production dynamics, encompassing daily and cumulative biogas generation, peak production periods, and biogas yield per gram of VS, to determine the biogas generation potential of each substrate.

2. MATERIALS AND METHODS

2.1. Collection of substrates

The water lilies were collected from Dal Lake in Kashmir at different randomly selected sites. They were cleaned, and only the stems and leaves were used for analysis. Food waste, consisting of fruits, vegetables, bread, and rice, was sourced from local markets and hostel messes. Sludge was collected from the Srinagar Hazratbal sewage treatment plant. Once the substrates were obtained from their respective sources, a comprehensive characterization was then conducted to analyze their properties.

2.2. Preparation of inoculum

In this study, the inoculum for AD was prepared using sludge obtained from a wastewater treatment plant in sample collection bottles, and excess water was drained. The concentrated sludge was then filled into flasks, which were tightly sealed with aluminum foils to create an anaerobic environment conducive to the growth of anaerobic bacteria. These sealed flasks were placed in a bacteriological incubator set at a temperature of 30 °C to promote microbial growth. Throughout the incubation period, parameters such as dissolved oxygen (DO), TS, VS, biochemical oxygen demand (BOD), and pH were regularly monitored using standard methods following Moradi *et al.* (2023). The monitored parameters were consistently assessed during the entire incubation period to ensure the suitability of the inoculum for subsequent experiments.

2.3. Anaerobic digestion

In the laboratory-scale investigation, five 500 mL Buchner flasks were used as batch reactors following Ali *et al.* (2018). The reactors were tightly sealed with rubber corks to create anaerobic conditions, and a small pipe was inserted for sample collection of pH and temperature. Biogas was collected through one outlet using the water displacement method. The study was conducted at a mesophilic temperature of 35 °C, maintained by submerging the reactors in a water bath. Biogas was measured by the water displacement method. Precise temperature control and the water displacement method were essential for the experiment's success.

2.4. Substrate proportioning

In the first reactor, only water lilies were added as the substrate to evaluate the yield of biogas from the mono-digestion. The amount of water lilies added was 14 g of TS. In reactors, 2, 3, and 4, a mixture of food waste and sludge was added based on the percentage of TS as shown in Table 1. After adding the predetermined amount of substrates to each reactor, water was added to make a slurry of 150 mL in each reactor, ensuring a consistent substrate-to-water ratio. A total of 150 mL of inoculum was added to all four reactors, maintaining a substrate-to-inoculum ratio of 1:1. Reactor 5 was used as a control, where only 300 mL of inoculum was added without any additional substrate.

Table 1 | Substrate proportioning

Reactor	Water lilies: sludge: food waste (% total solids)	Water lilies (g)	Food waste (g)	Sludge (g)	Inoculum (mL)
Reactor 1 (R1)	100:0:0	14	–	–	150
Reactor 2 (R2)	80:10:10	11.2	1.4	1.4	150
Reactor 3 (R3)	70:15:15	9.8	2.1	2.1	150
Reactor 4 (R4)	60:20:20	8.4	2.8	2.8	150
Reactor 5 (R5)	Inoculum (control)	–	–	–	300

2.5. Biogas potential tests

The substrates were mixed in a fixed proportion, and pre-digestion tests were conducted to measure TS, VS, pH, alkalinity, and VFAs. TS, VS, and alkalinity were analyzed according to the Indian standard code 3025:1983 Part 17, Part 18, and Part 23, respectively. VFA analysis was done using the direct titration method as mentioned in [Feng *et al.* \(2019\)](#). The reactors were submerged in a water bath at a constant temperature of 35°C. Manual stirring of the reactors was performed twice daily to ensure uniform distribution. Biogas production was measured daily using the water displacement method. pH and temperature were monitored daily using a pH meter and thermometer, respectively. Post-digestion tests were conducted after the digestion phase. Data on biogas production, pH, and temperature observations were recorded and analyzed to assess the efficiency of the AD process.

3. RESULTS AND DISCUSSION

3.1. Characterization of substrates

[Figure 1](#) represents the images of collected water lilies, food waste, and sludge. The properties of the substrates, namely water lilies, food waste, and sludge, were thoroughly characterized to analyze their composition and characteristics. The results are presented in [Table 2](#).

The characterization analysis provided valuable insights into the composition of the substrates and their suitability for the AD process. [Table 2](#) shows that food waste has the highest potential for biogas production due to its high VS content, while

**Figure 1** | Snapshots of collected substrates (a) water lilies, (b) food waste, and (c) sludge.

Table 2 | Substrate characterization

Substrate	pH	Total solids (%)	Volatile solids (%)	Moisture content (%)
Water lilies	6.4	18.42	81.46	87
Food waste	7.6	27.23	90.6	75
Sludge	6.5	6	60	95

water lilies also offer good potential. Sludge, despite having a lower TS content, still contains a significant amount of VS, making it a viable substrate for AD as per [Makhura *et al.* \(2020\)](#).

3.2. Pre-digestion tests

The laboratory anaerobic batch digestion setup used in this analysis is shown in [Figure 2](#). During the pre-digestion phase, the substrates underwent analysis to evaluate their potential for biogas production. The results, presented in [Table 3](#), highlight key parameters relevant to the digestion process.

The pre-digestion results demonstrated favorable conditions for the subsequent AD process. The TS concentration ranged from 5.42 to 5.76%, with VS ranging from 72.61 to 75%. The pH values were adjusted to a neutral range (7.06–7.23), creating a suitable environment for anaerobic microorganisms ([Makhura *et al.* 2020](#)). The alkalinity levels, ranging from 1,800 to 3,000 mg CaCO₃/L, indicated sufficient buffering capacity. The initial concentration of VFAs (such as acetic acid) was within the recommended limit of 150–300 mg/L, suggesting the feasibility of biogas generation from the selected substrates ([Feng *et al.* 2019](#)).

3.3. Biogas production

The daily biogas production during the digestion period exhibited variations among the reactors. In Reactor 1 (R1), biogas production continued until day 48 before ceasing. Similarly, in Reactor 4 (R4), biogas production stopped on day 41. The

**Figure 2** | Laboratory anaerobic batch digestion setup.**Table 3** | Pre-digestion test summary

Reactor	Total solids (%)	Volatile solids (%)	pH	Alkalinity (mg CaCO ₃ /L)	Volatile fatty acids (mg/L) as acetic acid
1	5.42	75	7.14	2,200	200
2	5.50	73.44	7.15	2,500	150
3	5.67	73.1	7.22	2,400	250
4	5.76	72.61	7.23	3,000	170
5	4	60	7.06	1,800	300

daily biogas production variation is depicted in Figure 3, providing valuable insights into the dynamics of biogas generation. The lag phase and duration of biogas production varied among the reactors. R1 had a lag phase of 7 days, while R2, R3, and R4 had lag phases of 4, 4, and 3 days, respectively (Figure 3). These variations can be attributed to differences in substrate composition and microbial activity (Hegde & Trabold 2019). The longer lag phase in R1 suggests that water lilies require more time to establish a stable anaerobic environment for efficient digestion due to its high carbon and fiber contents, including cellulose, hemicelluloses, pectin, and lignin. In contrast, R4 had a shorter lag phase of 2 days, possibly due to the presence of readily fermentable substrates from food waste and sludge (Goswami *et al.* 2016). Cumulative biogas production varied among the reactors (Figure 3). R1 accumulated a total of 2,527 mL of biogas, while R2 reached 2927 mL. R3 and R4 demonstrated further increases, reaching 3,164 and 3,404 mL, respectively. In comparison, R5 yielded insignificant biogas production compared to the other four reactors. Peak biogas production occurred at different time points. R1 reached its peak on the 26th day, with a maximum of 136 mL/day. R2 peaked on the 19th day, reaching 153 mL/day. R3 had its peak on the 16th day, with a maximum of 156 mL/day. R4 exhibited the highest production in the second week, reaching 158 mL/day on the 12th day. These variations can be attributed to differences in substrate degradation rates, microbial activity, and the availability of fermentable compounds (Alam *et al.* 2022). R4, with a shorter time to peak production, suggests a higher proportion of easily fermentable substrates, facilitating faster biogas generation.

3.4. Cumulative biogas production

The cumulative biogas production over the retention time varied among the reactors as shown in Figure 4. R1, containing only water lilies as the substrate, accumulated a total of 2,527 mL of biogas. R2, with a mixture of water lilies, food waste, and sludge, exhibited a higher cumulative biogas production of 2,927 mL. R3 and R4, which had varying proportions of water lilies, food waste, and sludge, demonstrated further increases in cumulative biogas production, reaching 3,164 and 3,404 mL, respectively. This observation highlights the collective importance of these selected substrates (Panizio *et al.* 2020). While R5 yields insignificant biogas compared to the other four reactors.

3.5. pH variation and retention time

The pH values in all reactors exhibited a drop in the initial week of the digestion process, reaching around 5 or 5.5. However, after the first 2 weeks, a noticeable shift occurred, and the pH began to rise back toward neutrality, stabilizing around 7. The retention time, referring to the duration that the substrate remains in the AD reactors, varied among the different reactors. R1 had the longest retention time of 26 days, followed by R2 with 19 days, R3 with 16 days, and R4 with the shortest retention time of 12 days. The variation in retention time can be attributed to the composition of substrates and their degradation rates (Panizio *et al.* 2020).

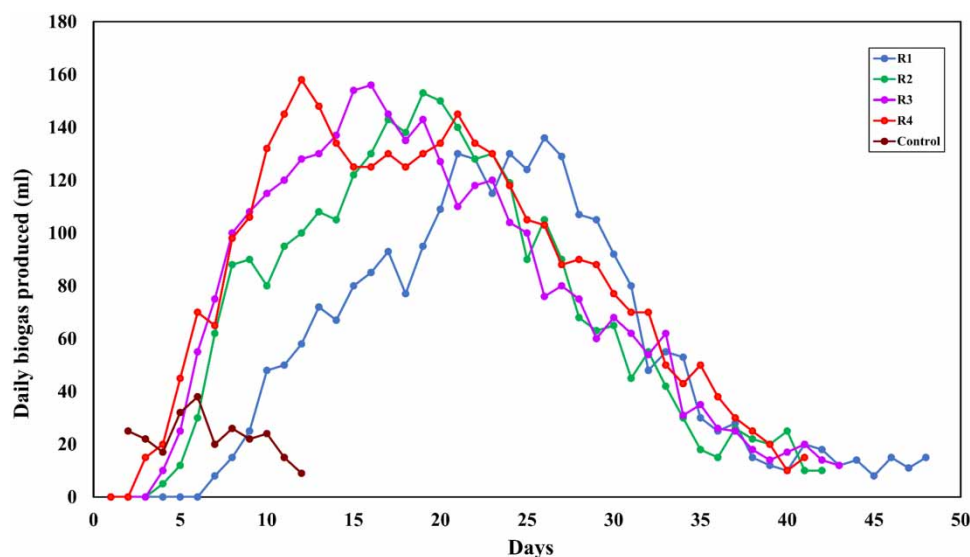


Figure 3 | Daily biogas variations.

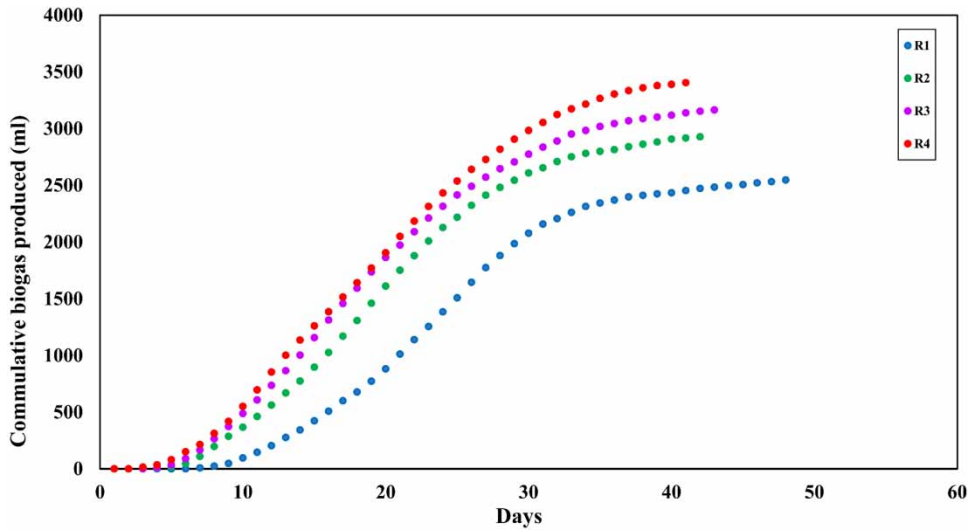


Figure 4 | Cumulative biogas produced by four reactors.

3.6. Total biogas production per gram of VS

Biogas production per gram of VS varied among the reactors. R1 yielded 195.6 mL/g VS, while R2 had a slightly higher yield of 225 mL/g VS. R3 showed a higher production of 243 mL/g VS, indicating more efficient conversion. R4 had the highest yield at 262 mL/g VS as shown in Figure 5. These differences reflect the influence of substrate composition. R1’s lower yield may be due to factors such as lower alkalinity, nutrient deficiency, and higher lignin and cellulose content. In contrast, R4 exhibited a higher yield, suggesting a more favorable substrate composition, nutrient balance, and greater availability of easily degradable organic matter (Goswami *et al.* 2016).

3.7. Post-digestion tests

After the digestion process, post-digestion tests were conducted, and the results are presented in Table 4. The concentration of VFAs ranged from 140 to 300 mg/L as acetic acid. These levels indicate that the digestion process did not experience

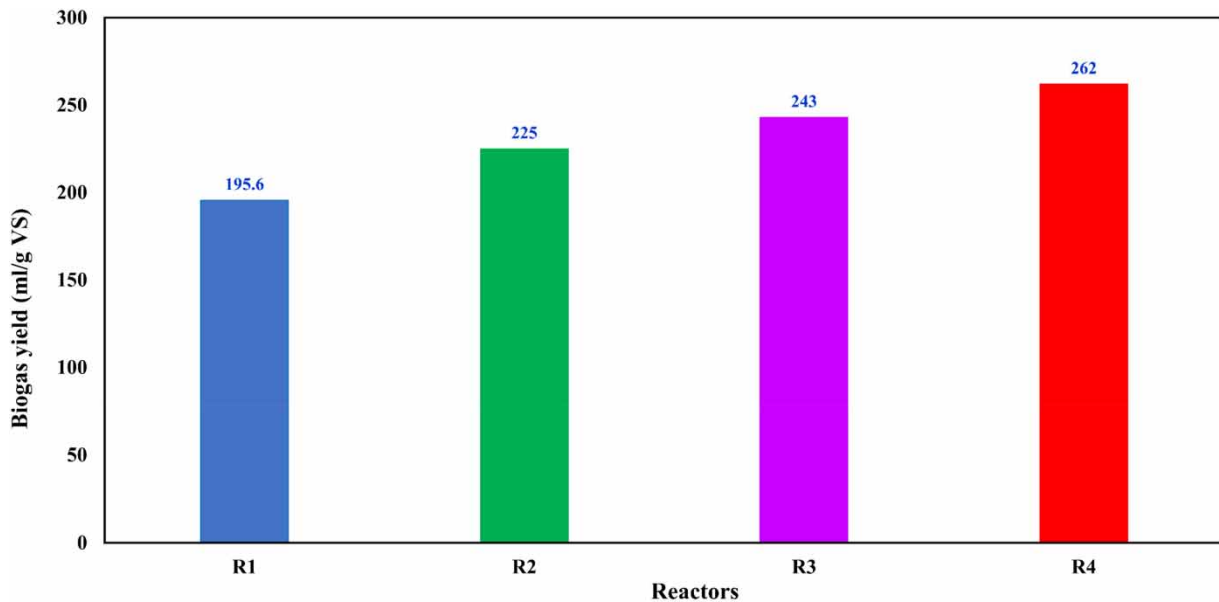


Figure 5 | Biogas yield of different reactors.

Table 4 | Post-digestion test results

Reactor	Total solids (%)	Volatile solids (%)	pH	Alkalinity (mg CaCO ₃ /L)	Volatile fatty acids as acetic acid (mg/L)
1	3.53	41.22	7.5	1,500	300
2	3.4	32.92	7.23	1,800	120
3	3.37	30.12	7.6	1,300	150
4	3.3	27.27	7.14	1,200	270
5	4	3.46	7.34	800	140

excessive acid accumulation that could have hindered the process. Additionally, the alkalinity of all the reactors ranged from 600 to 1,200 mg as CaCO₃. These post-digestion test results affirm the successful and efficient completion of the digestion process, with no signs of acidification or alkalinity depletion (Panizio *et al.* 2020).

3.8. TS reduction and VS removal efficiency

During the AD process, the reactors displayed varying levels of TS reduction as shown in Figure 6. R1 exhibited a reduction of 31.57%, indicating a significant decomposition of organic matter within the substrate as per Panizio *et al.* (2020). The highest reduction in TS was observed in R4, with a remarkable reduction of 42.61% thus reducing the volume of final digestate. R1 exhibited a removal efficiency of 45%. R4 exhibited the highest removal efficiency at 62.39%. The removal efficiency indicates the extent of organic matter degradation during AD. R1's lower efficiency may be due to the presence of recalcitrant compounds like lignin and cellulose in the water lilies substrate according to (Goswami *et al.* 2016). In contrast, R4 benefited from a balanced C/N ratio and positive synergistic effects between co-digested substrates, leading to enhanced organic matter degradation. Overall, optimizing operating conditions, including pH, alkalinity, and C/N ratio, promotes microbial activity and improves process efficiency.

3.9. Biogas potential analysis

Based on the obtained maximum biogas yield of 262 mL/g of VS, calculations were made on the biogas potential from 1 kg of TS of the mixture as shown in Table 5. With a TS content of 1 kg and a VS fraction of 72%, as was in the case of R4, we can expect a biogas yield of 188.64 L. Assuming a methane content of 50%, the total methane production would be 94.32 L. These

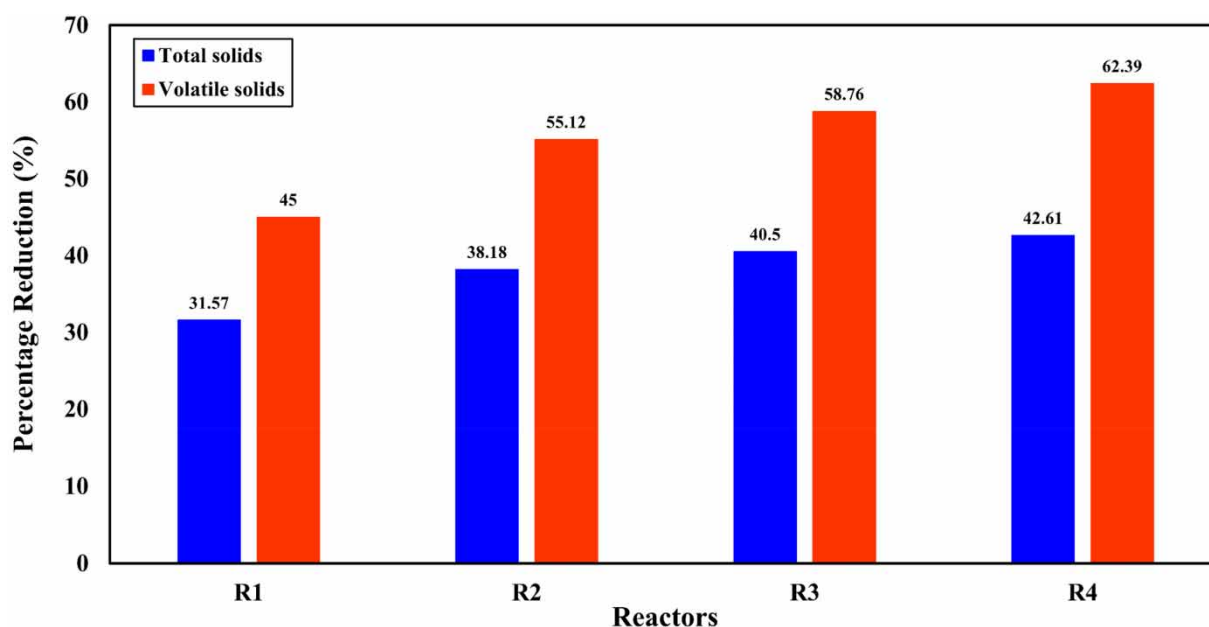
**Figure 6** | Total and volatile solids reduction.

Table 5 | Biogas potential from the current study

Assuming the total solids content of substrates used	1 kg
The concentration of volatile solids in the mixture	72%
Amount of volatile solids in 1 kg of mixture	720 g
Amount of biogas derived from the experiment	262 mL/g VS
The total amount of biogas from 1 kg of total solids	188.64 L
Assuming methane content	50%
The total amount of methane from 1 kg of total solids	94.32 L

results indicate the significant biogas generation potential of the analyzed mixture (Lee *et al.* 2019), demonstrating its suitability for renewable energy production and contributing to waste management efforts.

4. CONCLUSION

The results of this study demonstrate the potential of water lilies, food waste, and sludge as substrates for biogas production through AD. The characterization analysis provided insights into the composition and properties of the substrates. The pre-digestion tests confirmed favorable conditions for biogas production, including appropriate pH, volatile fatty acid concentrations, and alkalinity levels. The biogas production results revealed variations in daily production, cumulative production, lag phase, and time to peak production among the reactors, indicating differences in substrate composition and microbial activity. The post-digestion tests confirmed the successful completion of the digestion process. The estimated methane content highlights the substantial potential of the produced biogas as a renewable energy source. Overall, this study contributes to the understanding of suitable substrates and their dynamics during AD, providing valuable insights for optimizing biogas production systems and promoting sustainable waste management practices.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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