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Enhancing biogas production from organic solid wastes through selective pretreatment and semi-continuous anaerobic co-digestion

Zaidun Naji Abudi[1*], Alkhafaji R. Abood[2], Samiyaa Yaseen Mohammed[3], Nagham Rajaa[3]

[1] Department of Environmental Engineering, College of Engineering, Mustansiriyah University, Baghdad, 00964, Iraq
[2] Department of Civil Engineering, College of Engineering, ThiQar University, Nasiriyah, 00964, Iraq
[3] Department of Highway and Transportation Engineering, College of Engineering, Mustansiriyah University, Baghdad, 00964, Iraq
* Corresponding author's e-mail: zaidun.naji77@uomustansiriyah.edu.iq

ABSTRACT

This work investigated how substrate-specific pretreatment, operating temperature, and organic loading rate (OLR) influenced the performance of semi-continuous anaerobic co-digestion using the organic fraction of municipal solid waste (OFMSW), thickened waste activated sludge (TWAS), and rice straw (RS). Four laboratory-scale reactors were operated under mesophilic (35 °C) and thermophilic (55 °C) conditions using untreated or selectively pretreated co-substrates. TWAS was subjected to thermo-alkaline pretreatment, whereas RS was pretreated with hydrogen peroxide (H_2O_2). The reactors were operated at OLRs of 2, 3, and 4 g VS L^{-1} d^{-1}, corresponding to hydraulic retention times of 16, 11, and 8 d, respectively. Thermophilic digestion combined with substrate-specific pretreatment showed the highest overall biogas production performance. At the highest organic loading rate (4 g VS L^{-1} d^{-1}), Reactor C produced the maximum volumetric biogas yield (2.13 ± 0.12 m^3 m^{-3} d^{-1}) while maintaining a COD removal efficiency of 73%. It also produced the highest final cumulative biogas volume of 31,886 mL, representing increases of 18.6%, 25.0%, and 23.2% compared with Reactors A, B, and D, respectively. Stepwise increases in OLR caused short-term fluctuations in pH and volatile fatty acid concentrations. However, these transient responses were consistent with microbial acclimation and did not indicate sustained process inhibition. A preliminary energy assessment further indicated that the incremental recoverable energy associated with pretreatment-enhanced biogas production was substantially greater under thermophilic than mesophilic conditions. Overall, integrating substrate-specific pretreatment with thermophilic semi-continuous co-digestion improved organic matter conversion while sustaining biogas production under progressively increasing organic loading conditions.

Keywords: anaerobic co-digestion, biogas production, organic waste valorization, pretreatment techniques, organic loading rate, renewable bioenergy.

INTRODUCTION

Anaerobic digestion (AD) is widely recognized as an effective approach for the stabilization of organic wastes and the simultaneous recovery of renewable energy in the form of biogas. Compared with conventional waste-management options, including landfilling, incineration, and composting, AD provides significant environmental and energy-related benefits (Mousania et al., 2024). Based on substrate-feeding patterns, anaerobic digestion systems can generally be operated in batch, semi-continuous, or continuous modes (Lima et al., 2025). Batch digestion involves a single initial feeding of substrate and inoculum, whereas semi-continuous systems receive substrate intermittently according to the adopted operating and loading conditions

(Ezieke et al., 2024, Kwakye, 2025). Continuous stirred-tank reactors receive a continuous substrate inflow with simultaneous withdrawal of an equivalent digestate volume, allowing relatively stable long-term operation (Ezieke et al., 2024). Semi-continuous and continuous systems are particularly useful for evaluating reactor loading capacity, microbial adaptation, and process stability under changing operational conditions (Abudi et al., 2020, Abudi et al., 2016, Abudi et al., 2016, Hmaissia et al., 2025, Zainal et al., 2022)

Anaerobic co-digestion has received increasing attention because the simultaneous treatment of complementary organic substrates can overcome some limitations associated with mono-digestion. Co-digestion can improve nutrient balance and carbon-to-nitrogen ratios, dilute inhibitory compounds, enhance substrate utilization, and promote stable microbial activity and biogas production (Azarmanesh et al., 2023, Gu et al., 2020, Karki et al., 2022). The selection of suitable co-substrates is therefore an important factor in achieving stable digestion, particularly when heterogeneous municipal, agricultural, and wastewater-derived wastes are treated within a single digestion system.

The organic fraction of municipal solid waste (OFMSW) represents a major biodegradable component of municipal waste and contains substantial amounts of readily degradable organic matter. Increasing municipal waste generation associated with population growth, urban expansion, and changing consumption patterns has intensified the need for sustainable treatment and resource-recovery strategies (Liu et al., 2023, Wang et al., 2022, X. Zhang et al., 2023). Anaerobic digestion provides a suitable route for converting the biodegradable fraction of OFMSW into biogas, however, process performance may be improved by combining OFMSW with substrates having complementary physicochemical characteristics.

Waste activated sludge (WAS) is an inevitable by-product of biological wastewater treatment, and its production continues to increase as wastewater treatment infrastructure expands (Grobelak and Spinosa, 2023). Anaerobic digestion can reduce sludge volume and recover energy, however, the biodegradation of WAS is often constrained by the limited accessibility of intracellular and particulate organic matter (Abudi et al., 2016, Potdukhe et al., 2021).

Accordingly, thermal, chemical, ultrasonic, and combined pretreatment methods have been investigated to enhance sludge disintegration and increase organic matter solubilization before anaerobic digestion (Giwa et al., 2025). A particularly important application of combined thermal–alkaline pretreatment is to improve the availability of biodegradable organic compounds for microbial conversion thereby enhancing biogas production and quality (Azarmanesh et al., 2023, Giwa et al., 2025).

Rice straw is one of the most abundant lignocellulosic agricultural residue and can also be a very good feedstock to produce renewable bioenergy (Rathour et al., 2023).Because of its complex lignocellulosic structure and high lignin content, however, it is difficult for microbes to access and less biodegradable under anaerobic digestion (Deng et al., 2023). Open-field burning remains a common disposal practice that is still prevalent in many areas of agriculture, which can release pollutants into the air and increase greenhouse gas (de Souza et al., 2026, Sanoja-Lopez et al., 2024). Several new physical, chemical, biological and in combination available methods of pretreatment have been therefore tried to break the recalcitrant structure of rice straw and make it highly accessible for further conversion in anaerobic digestion system (Meenakshisundaram et al., 2022, Woźniak, Kuligowski et al., 2025).

Hydrogen peroxide (H_2O_2) treatment can promote delignification and improve the accessibility of structural carbohydrates to microbial degradation (Limeneh et al., 2025, Toghiani et al., 2024).

In our previous work, batch anaerobic co-digestion of OFMSW, TWAS, and rice straw (RS) was evaluated under different substrate mixing ratios. The outcomes of that study provided the basis for the semi-continuous investigation presented here (Abudi et al., 2020, Abudi et al., 2016, Abudi, Hu, Xiao, et al., 2016). These investigations demonstrated that thermo-alkaline pretreatment of TWAS and H_2O_2 pretreatment of RS could improve substrate biodegradability and biogas production. However, batch biochemical methane potential experiments primarily describe the ultimate biodegradability of substrates and provide limited information on reactor adaptation and performance under successive increases in organic loading. The long-term response of a semi-continuous co-digestion system combining OFMSW with two selectively pretreated co-substrates under mesophilic and thermophilic conditions therefore requires further investigation.

Accordingly, this study investigated the semi-continuous anaerobic co-digestion of OFMSW, TWAS, and RS under mesophilic and thermophilic conditions. The specific objectives were to evaluate the effects of substrate-specific pretreatment and stepwise increases in organic loading rate from 2 to 4 g VS L^{-1} d^{-1} on biogas production, COD removal, and reactor stability, to assess the associated pH and volatile fatty acid responses during loading transitions, and to compare the performance of pretreated and untreated reactor configurations. A preliminary energy assessment was also conducted to estimate the additional recoverable energy associated with pretreatment-enhanced biogas production. The main contribution of this work is the long-term evaluation of a semi-continuous three-substrate co-digestion system integrating thermo-alkaline pretreatment of TWAS with H_2O_2 pretreatment of RS under progressively increasing OLR conditions.

MATERIAL AND METHODS

Feedstocks and inoculum

Three feedstocks were employed in this study, namely the OFMSW, thickened waste activated sludge (TWAS), and rice straw (RS). TWAS was evaluated in both untreated and thermo-alkaline-pretreated forms, whereas RS was used in untreated and H_2O_2-pretreated forms. The OFMSW mixture was prepared using representative household food and green waste components, following the procedure previously described by (Abudi et al., 2016).

Anaerobic inoculum was collected from a municipal wastewater treatment plant in Wuhan, China, whereas TWAS was collected from the thickened sludge line of the municipal wastewater treatment plant in Wuhan, China. After collection, OFMSW, TWAS, and the inoculum were stored at 4 °C until use. Rice straw (RS) was obtained from farmland located in the Wuhan region, China., thoroughly dried, and mechanically milled to a particle size of less than 5 mm before the experiments. The processed material was then sealed in vacuum bags and stored under ambient conditions until use in the digestion experiments. Table 1 summarizes the physicochemical properties of the three feedstocks together with the inoculum used in the experiments.

As summarized in Table 1, OFMSW was characterized by the highest TCOD and sCOD values, with an sCOD/TCOD ratio of 52.2%, suggesting a relatively large soluble organic fraction. Rice straw, on the other hand, exhibited the highest TS and VS contents because of its concentrated solid composition. TWAS was distinguished by the highest total nitrogen content (5.6% of TS), whereas the inoculum contained comparatively low levels of solids and organic matter.

A specific substrate mixing proportion at the ratio of 3:0.5:0.5 (OFMSW:TWAS:RS) was chosen for this study following a thorough analysis of data gained from our previous batch experiments (Abudi et al., 2020, Abudi et al., 2016). This ratio mix specifically has been established and confirmed as the ideal combination after preliminary investigations undertaken at the same time where it was repeatedly expressed in that this specific final formulation gave significantly higher volumetric biogas yield than all

Table 1. Physicochemical characteristics of the feedstocks and inoculum used in this study

Parameters	Unit	OFMSW	TWAS	RS	Inoculum
pH	-	3.5 ± 0.04	6.4 ± 0.00	6.2 ± 0.02	6.5 ± 0.05
TS	%	23.3±0.34	14.2±0.16	92.6±0.31	2.3±0.39
VS	%	20.2±0.26	6.7±0.09	70.4±0.22	1.4±0.35
VS / TS	%	88.5	47.4	76.0	60.0
TCOD	mg/L	210667±3581	37040±1332	ND	19040±1351
sCOD	mg/L	110050±1610	12800±750	ND	5600±452
sCOD/TCOD	%	52.2	34.6	ND	29.4
Alkalinity	mg/L as $CaCO_3$	500±12	1500±21	ND	667±14
Total carbon	% of TS	45.6±0.7	35.4±1.3	40.9±1.1	ND
Total nitrogen	% of TS	2.1±0.2	5.6±0.15	0.7±0.04	ND

Note: ND = not determined, values are presented as mean ± standard deviation.

other experimental tested scenarios. In addition, based on the specific total carbon and total nitrogen contents of the components reported in Table 1, this specialized mix had a calculated TS C/N ratio of roughly 18.5:1. This value matches the scientific literature recommendations for AD processes: a C/N ratio range of 15–20 has been widely accepted as values that are optimal for stability and efficiency (Mao et al., 2019, Mata-Alvarez et al., 2014). The high content of nitrogen, both inherently and relatively, in TWAS, which was calculated to be as high as 5.6% (%wt on a total solid basis), plays a crucial role in balancing the extremely C-rich OFMSW and the lignocellulosic RS for effective C/N co-composition and co-digestion. It translated to this inherent elemental balancing act that indeed offered us some remarkable nutrient conditions that were also highly synergistic in nature and are absolutely vital nutrients to ensure strong, unabated microbial activity during the biological degradation phase.

Experimental setup and procedure

Semi-continuous anaerobic co-digestion experiments were performed under mesophilic and thermophilic conditions using four laboratory-scale reactors. The experimental program was designed to evaluate the effects of operating temperature, organic loading rate (OLR), hydraulic retention time (HRT), and substrate pretreatment (TWAS and RS) on reactor performance. Three HRTs of 16, 11, and 8 days were investigated. As the reactors were operated without sludge recycling, the sludge retention time (SRT) was considered equivalent to the HRT. Corresponding OLRs of 2, 3, and 4 g VS L^{-1} d^{-1} were applied at HRTs of 16, 11, and 8 days, respectively. During operation, each reactor was fed immediately after the corresponding sample was withdrawn. The operating conditions for each reactor are summarized in Table 2 and Figure 1-A.

Reactor configuration and operation

Glass reactors with a total volume of 1 L were used as semi-continuous anaerobic digesters (Figure 1B). Each reactor was sealed with a rubber stopper fitted with two openings. One opening was connected to a 10-cm tube for substrate feeding, whereas the second was fitted with a 5-cm tube connected to a 1-L Tedlar bag for biogas collection. An additional port was provided for effluent withdrawal and remained securely sealed with a screw-compression clamp during operation.

The working volume of each reactor was maintained at 600 mL, including 500 mL of anaerobic inoculum. Prior to start-up, nitrogen gas was used to purge the reactors and establish anaerobic conditions. In the start-up phase (days 1–9), pH was regulated if necessary by the addition of small amounts of 6 M NaOH to avoid too much acidification and thus, process failure. The amount of NaOH added in all reactors was 4.2 mL in Reactor A, 3.8 mL in Reactor B, 2.5 mL in Reactor C, and 2.2 mL in Reactor D. Such regulation was done to keep pH above 6.5 in the beginning phases of acidogenesis. From day 9 onwards, no more pH regulation was carried out, allowing the microorganism consortium to regulate its own pH homeostasis. The digesters were then placed in a temperature-controlled water-bath shaker operating at approximately 90 rpm. Mesophilic and thermophilic operating temperatures were maintained at 35 and 55 °C, respectively.

Each loading condition was maintained for three HRT cycles. The OLR was increased stepwise by progressively reducing the HRT, allowing reactor performance and microbial adaptation to be evaluated at each loading stage.

Table 2. Operating conditions of the experimental reactors

Reactor	Substrate mixture (OFMSW: TWAS:RS)	Pretreatment	Digestion condition
A	3.0:0.5:0.5 (thermo-alkaline-pretreated TWAS + H_2O_2-pretreated RS)	Yes	Mesophilic
B	3.0:0.5:0.5 (untreated TWAS + untreated RS)	No	Mesophilic
C	3.0:0.5:0.5 (thermo-alkaline-pretreated TWAS + H_2O_2-pretreated RS)	Yes	Thermophilic
D	3.0:0.5:0.5 (untreated TWAS + untreated RS)	No	Thermophilic

Note: TWAS was subjected to thermo-alkaline pretreatment, whereas RS was pretreated with H_2O_2. Mesophilic and thermophilic reactors were operated using the same substrate mixing ratio to examine the influence of operating temperature and substrate pretreatment on reactor performance.

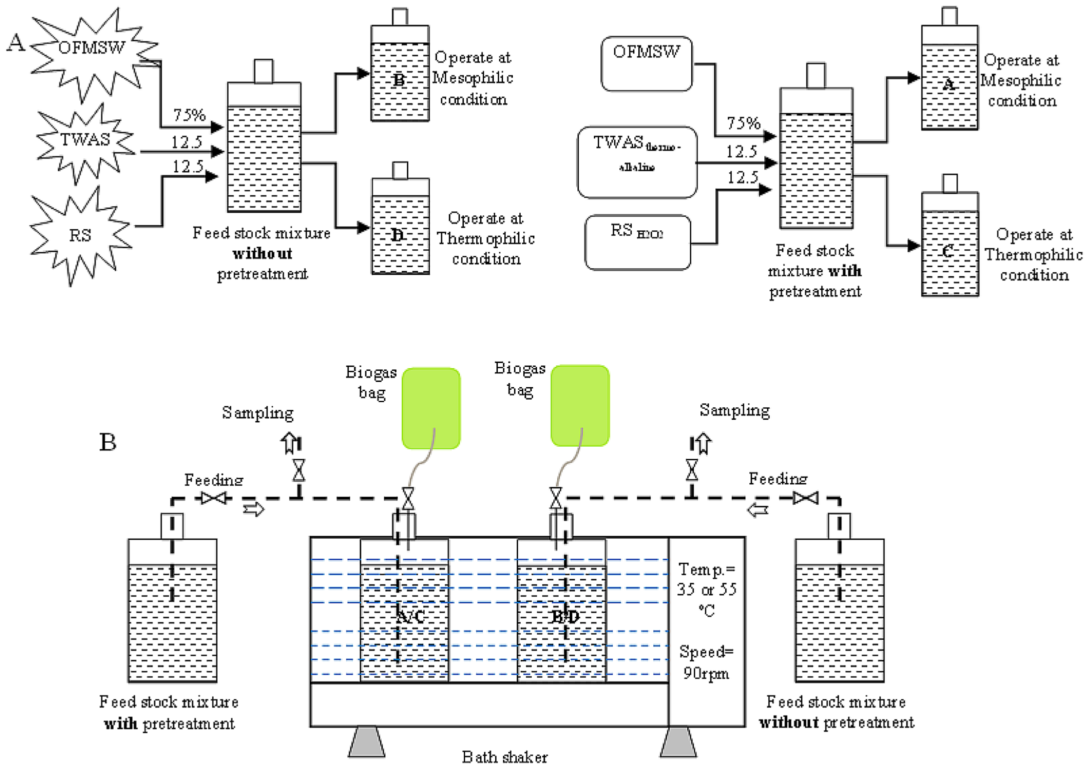

Figure 1. Experimental setup of the semi-continuous anaerobic co-digestion process: (A) substrate pretreatment and reactor allocation under mesophilic and thermophilic conditions, (B) laboratory-scale reactor configuration and operation

Analytical methods

Water displacement was used to monitor biogas production on a daily basis. Volumetric biogas production ($m^3\ m^{-3}\ d^{-1}$) was calculated by normalizing the measured biogas volume to the reactor working volume and operating time. Specific biogas yield was determined by dividing the measured biogas volume by the concentration of VS added to the reactors. Cumulative biogas production was calculated by summing the daily biogas volumes throughout the operating period. TS, VS, TCOD, soluble chemical oxygen demand (sCOD), and pH were measured according to Standard Methods for the Examination of Water and Wastewater (APHA, 2017). Volatile fatty acids (VFAs) and alkalinity were quantified using the FOS/TAC titration procedure described by (Voß et al., 2009).

For sCOD analysis, samples were centrifuged at 4000 rpm for 15 min, and the resulting supernatant was analyzed according to standard procedures (APHA, 2017, Lipps et al., 2023).

Carbon and nitrogen concentrations in the substrates were measured with a Vario EL elemental analyzer (Elementar Analysensysteme GmbH, Germany). The structural carbohydrate composition of rice straw, including cellulose, hemicellulose, and lignin, was analyzed according to the Van Soest fiber analysis method (Van Soest et al., 1991).

Statistical analysis

All experiments were conducted in duplicate, and the results are presented as mean ± standard deviation (SD). Statistical analyses were performed using IBM SPSS Statistics 20.0 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was used to evaluate differences among treatments, with statistical significance established at $p < 0.05$.

RESULTS AND DISCUSSION

The influence of substrate pretreatment, operating temperature, and organic loading rate on the performance of the semi-continuous anaerobic co-digestion system treating OFMSW, TWAS, and RS was investigated. Reactor performance, process stability, and biogas production are presented and discussed in the following sections.

Thermophilic versus mesophilic digestion performance

Thermophilic digestion consistently outperformed mesophilic operation throughout the experimental period, particularly when thermo-alkaline-pretreated TWAS and H_2O_2-pretreated rice straw were used as co-substrates (Table 3). Under mesophilic conditions, volumetric biogas production rates ranged from 0.76 to 1.45 m^3 m^{-3} d^{-1} for pretreated substrates and from 0.81 to 1.44 m^3 m^{-3} d^{-1} for untreated substrates. By contrast, thermophilic operation produced noticeably higher volumetric biogas production rates. Values ranged from 1.17 to 2.13 m^3 m^{-3} d^{-1} for pretreated substrates and from 0.89 to 1.80 m^3 m^{-3} d^{-1} for untreated substrates.

Under the initial operating condition (OLR = 2 g VS L^{-1} d^{-1}, HRT = 16 days), Reactor C recorded the highest specific biogas yield, reaching 15.66 m^3 kg^{-1} VS. Throughout the experimental period, Reactor C maintained the highest overall biogas production among the four reactors (Table 3 and Figure 2). The improved performance is likely associated with the combined influence of thermophilic operation and substrate-specific pretreatment. Together, these factors enhanced the breakdown of complex organic matter, increased substrate availability, and supported microbial conversion into biogas.

In addition, the shorter HRT (from 16 to 8 days) in connection with the increased OLR led to a reduction in the time for the contact between substrate and microorganisms and enzymatic hydrolysis. This issue is especially relevant for slowly growing microorganisms like methanogens and cellulolytic bacteria, which need additional time to be able to effectively work with the substrate (Peces et al., 2021). A shorter HRT may be one of the reasons for the washout of slowly growing microorganism groups, which reduces the efficiency of substrate conversion. A similar pattern was observed for COD removal. Under thermophilic conditions, COD removal ranged from 57% to 73% for pretreated substrates and from 53% to 68% for untreated substrates. The corresponding values under mesophilic conditions were slightly lower, ranging from 54% to 69% and from 51% to 65%, respectively. These findings suggest that thermophilic operation maintained efficient organic matter conversion across the investigated loading conditions. Cumulative biogas production followed the same pattern, with Reactor C maintaining the highest overall performance (Figure 2). By the end of the experiment, Reactor C produced 31,886 mL of cumulative biogas, exceeding the corresponding values for Reactors A, B, and D by 18.6%, 25.0%, and 23.2%, respectively. This overall improvement appears to result from the combined effects of thermophilic operation and substrate-specific pretreatment. Thermo-alkaline pretreatment promoted TWAS disintegration and solubilization, while H_2O_2 pretreatment improved the accessibility of the lignocellulosic fraction of RS for anaerobic conversion.

The improved performance observed under thermophilic conditions is consistent with recent studies demonstrating that increasing digestion temperature enhances hydrolysis, microbial metabolic activity, and substrate conversion during anaerobic co-digestion could improve hydrolysis, microbial metabolic activity and conversion of substrate. In comparison to conventional mesophilic digestion, Hu and Shen reported that thermophilic co-digestion brought significantly higher methane yield (Hu and Shen, 2024).

Similarly, Lee et al. observed enhanced substrate degradation kinetics and biogas productivity under thermophilic operation through increased microbial activity (Lee et al., 2024). Murillo-Roos et al. further demonstrated that thermophilic digestion promoted microbial growth, reduced the required retention time, and enhanced methane-rich biogas production (Murillo-Roos et al., 2022). The performance improvement observed in the present study therefore cannot be attributed solely to the thermophilic operating regime but rather to the combined effects of elevated temperature and substrate-specific pretreatment. The enhanced performance observed in Reactor C is likely related to the complementary roles of the two pretreatment strategies. Thermo-alkaline pretreatment increased the accessibility of TWAS, whereas H_2O_2 pretreatment improved the biodegradability of rice straw, facilitating more efficient anaerobic conversion.

Effect of organic loading rate on daily biogas production

The effect of progressively increasing the organic loading rate (OLR) on average daily VS-normalized biogas production was determined under both mesophilic and thermophilic operating conditions. The reactors were operated at

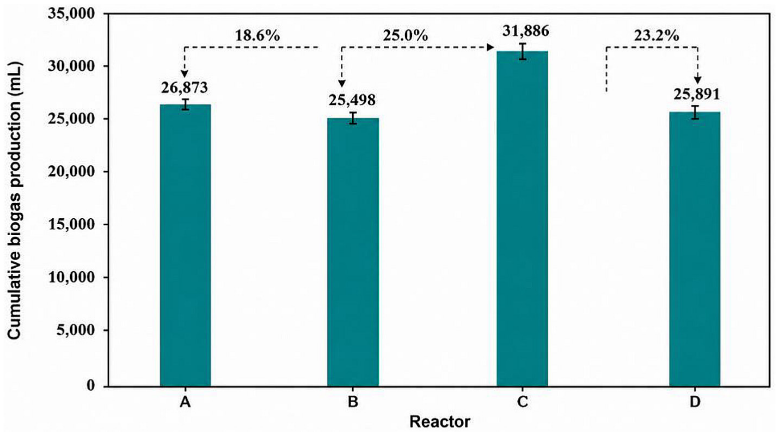

Figure 2. Final cumulative biogas production of the four semi-continuous anaerobic co-digestion reactors over the experimental period. Values represent mean ± standard deviation (n = 2). Percentages indicate the increase in cumulative biogas production achieved by Reactor C relative to Reactors A, B, and D, respectively

Table 3. Operating conditions and performance of the semi-continuous anaerobic co-digestion reactors

Reactor	Substrate condition	Temp.	OLR	HRT	HRT cycles	Operating duration	Influent COD	Volumetric biogas production	Specific biogas yield	COD removal
Unit		°C	g VS L^{-1} d^{-1}	d	-	d	g/L	m³ m^{-3} d^{-1}	m³ kg^{-1} VS	%
A	Pretreated (TWAS: thermo-alkaline; RS: H_2O_2)	35	2	16	3	48	1.15	0.76±0.11	13.19±0.21	54
			3	11	3	33	1.70	1.12±0.13	7.43±0.13	62
			4	8	3	24	2.25	1.45±0.12	6.55±0.16	69
B	Untreated (TWAS and RS)	35	2	16	3	48	1.25	0.81±0.06	12.79±0.31	51
			3	11	3	33	1.90	0.94±0.13	6.83±0.20	58
			4	8	3	24	2.50	1.44±0.15	6.12±0.21	65
C	Pretreated (TWAS: thermo-alkaline; RS: H_2O_2)	55	2	16	3	48	1.15	1.17±0.08	15.66±0.12	57
			3	11	3	33	1.70	1.71±0.10	6.69±0.28	65
			4	8	3	24	2.25	2.13±0.12	6.64±0.21	73
D	Untreated (TWAS and RS)	55	2	16	3	48	1.25	0.89±0.15	12.73±0.23	53
			3	11	3	33	1.90	1.65±0.07	7.86±0.24	61
			4	8	3	24	2.50	1.80±0.11	5.31±0.15	68

OLRs of 2, 3, and 4 g VS L^{-1} d^{-1}, corresponding to HRTs of 16, 11, and 8 days, respectively. Each loading stage was maintained for three consecutive HRT cycles before the OLR was increased to allow adequate microbial adaptation and performance evaluation.

Figure 3 shows that Reactor C exhibited the highest average daily VS-normalized biogas production at OLRs of 2 and 3 g VS L^{-1} d^{-1}, reaching approximately 326- and 302-mL g^{-1} VS added d^{-1}, respectively. At an OLR of 2 g VS L^{-1} d^{-1}, its production exceeded those of Reactors A, B, and D by approximately 19.9%, 23.0%, and 11.3%, respectively. At an OLR of 4 g VS L^{-1} d^{-1}, the VS-normalized biogas production of Reactor C (272 mL g^{-1} VS added d^{-1}) differed only marginally from that of Reactor A (271 mL g^{-1} VS added d^{-1}). Nevertheless, Reactor C remained the best-performing reactor when all loading stages were considered. The superior performance of Reactor C is likely attributable to the synergistic influence of thermo-alkaline pretreatment of RS, and thermophilic digestion. Thermo-alkaline pretreatment promoted sludge disintegration and increased the release of soluble organic matter, whereas H_2O_2 pretreatment made the lignocellulosic fraction of rice straw more accessible for anaerobic degradation. When these pretreatment strategies were combined with thermophilic digestion, substrate utilization became more efficient, providing favorable conditions for microbial conversion and sustained biogas production. The reactor configurations exhibited distinct responses to the progressive increase in OLR. As the OLR increased from 2 to 4 g VS L^{-1} d^{-1}, the average daily VS-normalized biogas production of Reactor C gradually decreased from 326 to 302 and finally to 272 mL g^{-1} VS added d^{-1}. A comparable response was recorded for Reactor D, where VS-normalized biogas production declined from 293 to 270 and then to 240 mL g^{-1} VS added d^{-1}. In contrast, Reactor A exhibited a marked decrease at an OLR of 3 g VS L^{-1} d^{-1} and partially recovered at 4 g VS L^{-1} d^{-1}, whereas Reactor B showed a comparable response. Although higher OLRs increased total daily biogas production, the corresponding biogas yield per unit of added VS did not increase proportionally.

The reduction in the VS-normalized biogas yield with the rise in the OLR from 326 to 272 mL g^{-1} VS added d^{-1} in Reactor C may be associated with a number of related reasons. Firstly, the increase in OLR at high values (3–4 g VS L^{-1} d^{-1}) might have led to the fact that the hydrolysis step of decomposition of organic material, specifically its lignocellulose fraction in case of rice straw and particulate organic fraction of TWAS, could have been limiting. Despite the positive effect of H_2O_2 and thermo-alkaline pre-treatment in terms of improving the availability of substrates, the hydrolytic efficiency of the microbial population was not enough to handle the additional load of substrates. This assumption is supported by the findings of (Peces et al., 2021, Pramanik et al., 2018).

Similar responses have been reported in previous studies, where thermophilic anaerobic co-digestion combined with appropriate substrate pretreatment enhanced hydrolysis and substrate utilization under elevated organic loading conditions (Giwa et al., 2025, Hu and Shen, 2024, Lee et al., 2024). Consistent with these observations, the present findings indicate that integrating thermo-alkaline pretreatment of TWAS with H_2O_2 pretreatment of RS under thermophilic conditions enhances the performance of semi-continuous anaerobic co-digestion by improving overall biogas production.

Daily and cumulative biogas production profiles

Daily and cumulative biogas production profiles illustrate the temporal response of the reactors to stepwise increases in organic loading rate (OLR). The profiles shown in Figure 4 illustrate reactor behavior during semi-continuous operation as the OLR increased progressively from 2 to 4 g VS L^{-1} d^{-1}. Each increase in OLR was followed by a brief fluctuation in daily biogas production before the reactors stabilized under the new operating conditions. Such short-term responses are consistent with microbial acclimation to changes in substrate loading and substrate availability during semi-continuous digestion. Although daily production temporarily fluctuated, cumulative biogas generation continued to increase steadily in every reactor throughout the experiment.

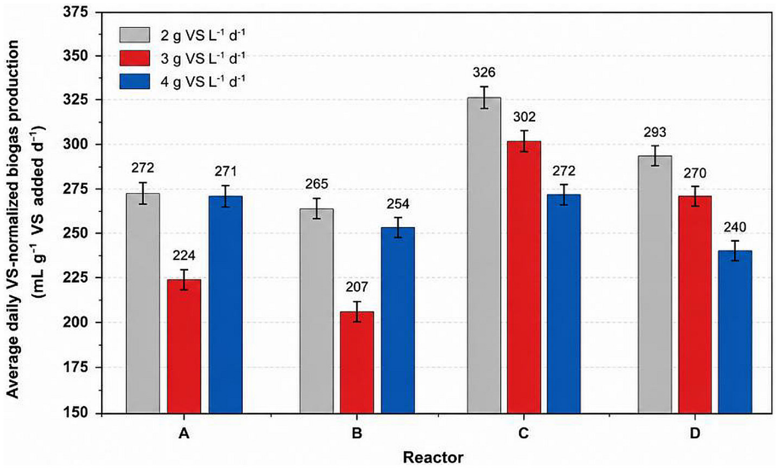

Figure 3. Comparison of average daily VS-normalized biogas production among the four semi-continuous anaerobic co-digestion reactors operated at three organic loading rates (2, 3, and 4 g VS L^{-1} d^{-1}). Error bars represent standard deviation (n = 2)

Reactor C maintained the highest daily biogas production among all four reactors over the entire experimental period. During the first operating stage at an OLR of 2 g VS L^{-1} d^{-1}, the maximum daily biogas production reached 1174 mL d^{-1} on day 10. The corresponding maximum daily production values in Reactors A, B, and D were 54.5%, 44.2%, and 31.3% lower, respectively. The same response pattern was evident after increasing the OLR to 3 and 4 g VS L^{-1} d^{-1}, with Reactor C reaching maximum daily biogas production values of 1710 and 2216 mL d^{-1}, respectively. Overall, the thermophilic pretreated reactor retained its production advantage even as the organic loading rate increased progressively.

One distinguishing feature of Reactor C was its ability to recover rapidly after each increase in OLR. Peak daily biogas production generally occurred approximately 2–7 days after the loading rate was increased. This relatively short lag period likely represents the time required for microbial acclimation and the establishment of metabolic equilibrium following the increase in substrate loading. Greater substrate availability probably supported a faster microbial response following each increase in organic loading. Taking together, the observed production patterns demonstrate that Reactor C remained operationally stable under progressively increasing loading conditions Cumulative biogas production followed an

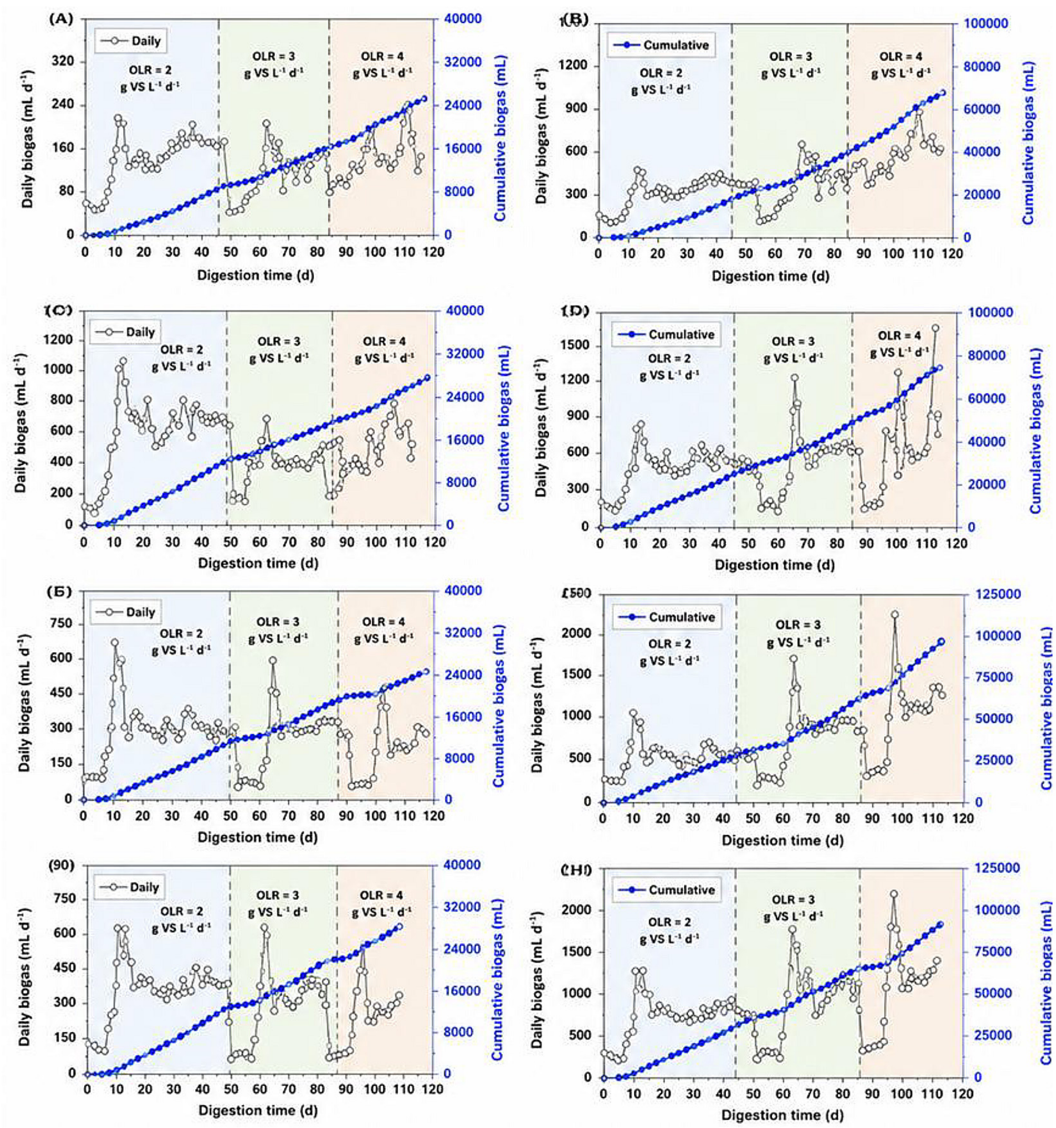

Figure 4. Temporal profiles of daily (left axis) and cumulative (right axis) biogas production for the four semi-continuous anaerobic co-digestion reactors operated at organic loading rates of 2, 3, and 4 g VS L^{-1} d^{-1}. Vertical dashed lines indicate the transition between consecutive loading stages

uninterrupted upward trend throughout the study (Figure 4). Reactor C displayed the steepest cumulative production trend, particularly during the later operating stages. Despite temporary fluctuations in daily biogas production after each OLR transition, cumulative biogas production continued to increase without prolonged stagnation, indicating continued biogas generation throughout the successive loading stages.

The temporal production patterns observed in Reactor C are consistent with previous studies reporting stable reactor performance under thermophilic digestion at elevated organic loading rates (Giwa et al., 2025, Hu and Shen, 2024). In the present study, the absence of prolonged declines in cumulative biogas production following OLR transitions further indicates that the reactor maintained stable operation throughout the experimental period.

Effect of organic loading rate on average daily biogas production

Figure 5 presents the influence of organic loading rate (OLR) on average daily biogas production during semi-continuous anaerobic co-digestion. In contrast to the VS-normalized response described in Section 3.2, this section examines the absolute daily biogas production of each reactor.

A progressive increase in average daily biogas production was observed in all reactors as the OLR increased from 2 to 4 g VS L^{-1} d^{-1}. Reactor C consistently exhibited the highest average daily biogas production at all investigated OLRs. Its production increased from approximately 705 mL d^{-1} at an OLR of 2 g VS L^{-1} d^{-1} to 1090 mL d^{-1} at 3 g VS L^{-1} d^{-1} and 1370 mL d^{-1} at 4 g VS L^{-1} d^{-1}. This corresponds to an increase of approximately 54.6% from 2 to 3 g VS L^{-1} d^{-1} and a further increase of 25.7% following the increase to 4 g VS L^{-1} d^{-1}. The other reactors also showed increasing daily biogas production as the OLR increased, although the extent of the response varied among the different reactor configurations.

The higher absolute biogas production recorded in Reactor C most likely resulted from the combined effects of thermophilic digestion and substrate-specific pretreatment. Together, these operating conditions promoted more efficient substrate utilization and maintained stable biogas generation as the OLR increased A comparison with the VS-normalized results presented in Figure 3 highlights the difference between total daily biogas production and biogas yield expressed per unit of added VS. Daily biogas production increased steadily with increasing OLR in all reactors (Figure 5). In contrast, the VS-normalized biogas yield remained relatively stable at lower OLRs but declined under the highest loading condition in Reactor C (Figure 3). Increasing the OLR therefore increased daily biogas output, although the conversion efficiency expressed per unit of added VS became lower. These findings demonstrate why both absolute biogas production and substrate-normalized performance should be evaluated when assessing semi-continuous anaerobic co-digestion systems.

The growth in the amount of produced biogas on a daily basis (Figure 5) and the decrease in the normalized biogas yield (Figure 3) represent an important operational compromise in semi-continuous anaerobic digestion process. Although the use of higher OLRs implies the introduction of larger amounts of biodegradable material, resulting in increased production of biogas, it leads to a decrease in the efficiency of its conversion due to a combination of factors: hydrolysis limitation, accumulation of VFA and decreased HRT as discussed in Section 3.2. This fact proves the importance of considering both parameters while optimizing the OLR in semi-continuous co-digestion process. The thermophilic pretreated reactor (Reactor C) proved to be the most tolerant to such drop in the efficiency.

Earlier studies also reported that higher organic loading rates, when combined with suitable pretreatment strategies, can enhance methane production, especially under thermophilic operating conditions. Bi et al. reported increased methane production as the OLR increased from 2.5 to 5.5 g VS L^{-1} d^{-1} (Bi et al., 2025). Similarly, Zhang et al. found that thermophilic co-digestion enhanced methane production by improving acidogenic, acetogenic, and methanogenic conversion (X. Zhang et al., 2024). The available evidence agrees well with the present results and indicates that the thermophilic pretreated system maintained higher daily biogas production as the organic loading rate increased.

This difference can be explained by the greater amount of biodegradable organic matter introduced at higher OLRs. As a result, total daily biogas production increased, whereas the conversion efficiency per unit of added volatile solids became lower.

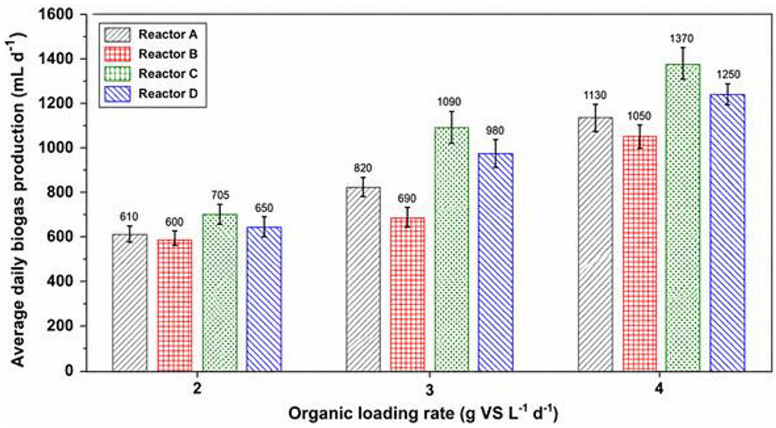

Figure 5. Mean daily biogas production obtained from the four semi-continuous anaerobic co-digestion reactors operated at different organic loading rates (OLRs). Error bars represent ± standard deviation (n = 2)

pH and VFA dynamics in relation to reactor performance

Figure 6 presents the temporal profiles of pH and volatile fatty acid (VFA) concentrations measured throughout the semi-continuous anaerobic co-digestion process. A temporary decline in pH was observed in all reactors, although the timing differed among them. The decrease occurred during days 2–9 in Reactor A, days 1–9 in Reactor B, and days 7–9 in Reactors C and D. The early decline in pH occurred simultaneously with increasing VFA concentrations, indicating active hydrolysis and acidogenesis. During this period, acid production temporarily exceeded its conversion by acetogenic and methanogenic microorganisms. To prevent excessive acidification during the initial acclimation period, pH was adjusted by adding 6 M NaOH. After this initial adjustment, pH gradually returned to near-neutral values and remained stable for the remainder of the experiment. The short-term pH changes were also measured after step changes in OLR, especially between successive operating stages. These pH variations corresponded closely with increases in VFA concentrations which represented temporary shifts in the relative production and consumption of acids among the microbial communities in response to the greater amount of substrate. The accumulation of VFAs following each OLR shift (Figure 6) shows a temporary mismatch between the acidogenesis and methanogenesis activities. Due to the greater increase in acidogenic activity relative to acetogenic and methanogenic activities at the higher OLR levels, there was an increase in the concentration of VFAs and low conversion rate. Even though these did not affect inhibition of the process, these were among the reasons for the low yield of biogas. Following microbial acclimation, VFA concentrations approached a relatively stable state, and pH was mostly above 7.0 during the operation period. As the OLR increased, temporary accumulation of VFAs was followed by degradation towards relatively stable concentrations, which signified adaptation of the anaerobic microbial consortia to rising substrate loading. Temporal responses were also seen in the daily biogas production profiles (Figure 4) as day-to-day fluctuations in production had parallels in VFA concentration periods. Thermophilic reactors showed a good recovery rate in general after OLR changes, especially for Reactor C. The coupled pH–VFA response also provides valuable insight into reactor stability. Short-term VFA accumulation following each increase in OLR indicates that acid production briefly exceeded its subsequent microbial conversion. As reactor operation stabilized, pH recovered and VFA concentrations gradually returned to stable levels, indicating that microbial acid consumption had regained equilibrium with acid production.

It is important to highlight that the above-mentioned pH manipulations took place only at the beginning of the experiment (from days 1 to 9) in order to avoid serious acidification and the failure of the system – an ordinary procedure carried out in lab experiments on anaerobic digestion (Abudi et al., 2016, Voß et al., 2009). No further pH regulation was conducted for the rest of the experiment. Thus, the pH and VFA changes that occurred during subsequent OLR variations (from days 10 to 105) are due to the inherent capacity of the system and reflect real stability conditions.

The relationship between pH and VFAs has been widely recognized as a key indicator of anaerobic digestion stability. Wu et al. described the close relationship between pH and VFA dynamics (Wu et al., 2024), while Yellezuome et al. reported that changes in OLR markedly affected microbial activity and community structure during anaerobic co-digestion (Yellezuome et al., 2024). Similarly, De Castro et al. linked improved buffering capacity with stable pH conditions and sustained methane generation (de Castro et al., 2025). Overall, the present results support previous observations by showing that temporary fluctuations in pH and VFA were characteristic of normal microbial acclimation to increasing organic loading rather than evidence of sustained process instability.

Comparative performance with previous anaerobic co-digestion studies

Only a limited number of published studies have reported semi-continuous anaerobic co-digestion of OFMSW using two selectively pretreated co-substrates. Accordingly, the proposed system was assessed by comparing its performance with previously published studies involving organic wastes, sewage sludge, and lignocellulosic residues. Direct comparisons should be interpreted cautiously because reactor performance depends on differences in feedstock characteristics, pretreatment strategies, reactor configuration, digestion temperature, hydraulic retention time, and organic loading rate. Nevertheless, the available literature provides a useful basis for evaluating the relative performance and distinctive features of the proposed co-digestion approach. Representative studies covering different pretreatment strategies and digestive conditions are summarized in Table 4. The selected studies were chosen because they investigated anaerobic co-digestion systems employing substrate pretreatment, semi-continuous or continuous operation, or operating conditions comparable to those examined in the present study.

Most previous studies have focused on the anaerobic co-digestion of two substrates or on evaluating individual pretreatment methods prior to anaerobic digestion. Thermal and alkaline pretreatments are commonly applied to improve sludge disintegration and organic matter solubilization, whereas physical, chemical, and combined pretreatment methods have mainly been investigated to enhance the biodegradability of lignocellulosic residues. As summarized in Table 4, most reported systems were batch-operated or involved a limited number of co-substrates, and these approaches generally improved methane or biogas production compared with untreated substrates.

In the present study, three different waste streams (OFMSW, TWAS, and RS) were integrated within a semi-continuous anaerobic co-digestion process. Unlike previous studies, the pretreatment strategy was selected according to the characteristics of each individual substrate. Thermo-alkaline pretreatment was applied to TWAS

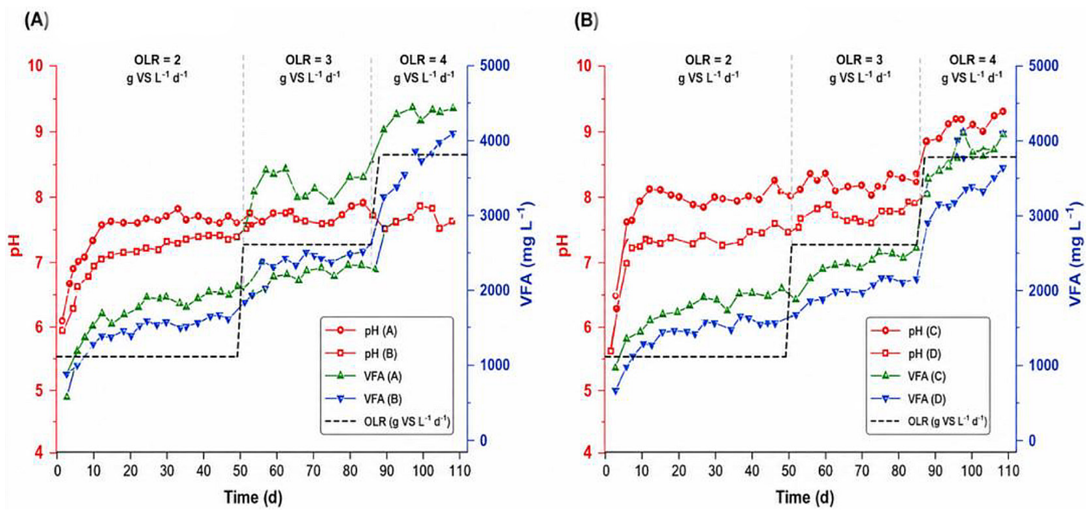

Figure 6. Temporal changes in pH and volatile fatty acid (VFA) concentrations for (A) mesophilic and (B) thermophilic semi-continuous anaerobic co-digestion reactors operated under OLRs of 2, 3, and 4 g VS L^{-1} d^{-1}. Vertical dashed lines represent the transitions between successive loading stages

Table 4. Comparison of the operating conditions and process performance of the present study with previously reported anaerobic co-digestion systems

Feedstocks	Pretreatment strategy	Operation mode	Temp. (°C)	Main performance outcome	Reference
Food waste (FW) and cattle manure (CM)	Thermal pretreatment resulted in a 62–81% increase in methane yield.	Batch	37	Thermal pretreatment increased methane yield by 62–81%	(Arelli et al., 2018)
Food waste (FW) and waste activated sludge (WAS)	Alkali, alkali–thermal, thermal, and ultrasonic pretreatments	Batch	35	Ultrasonic pretreatment produced the highest methane yield (326.3 mL CH_4 g^{-1} VSS removed) during FW–WAS co-digestion, corresponding to a 56.2% improvement over the untreated control.	(Naran et al., 2016)
Chicken litter (CL), food waste (FW), and wheat straw (WS)	Alkali and sequential alkali–acid pretreatments of CL and WS	Batch	37	Pretreatment applied individually to either CL or WS increased biogas production by up to 50%. When both substrates were pretreated, total biogas production increased by 80% and 88% following alkali and sequential alkali-acid pretreatments, respectively.	(Zahan and Othman, 2019)
Waste activated sludge (WAS) and wheat straw (WS)	Hydrothermal pretreatment at 125, 150, and 175 °C	Batch	37	The highest cumulative specific methane yield (225.7 mL CH_4 g^{-1} VS) was obtained during co-digestion of WAS and WS pretreated at 175 °C.	(Tian et al., 2020)
Food waste (FW) and waste activated sludge (WAS)	Anaerobic biological co-pretreatment for 24 h	semi-continuous mode	35	Co-digestion using biologically co-pretreated FW and WAS resulted in a 24.6% increase in methane yield and a 10.1% improvement in solids reduction compared with the untreated control.	(J. Zhang et al., 2017)
Rice straw (RS) and swine manure (SM)	Microwave, alkaline, and combined microwave–alkaline pretreatments of rice straw	Batch	35	Rice straw pretreatment improved biogas production during high-solids co-digestion with swine manure, with the combined microwave–alkaline pretreatment providing the greatest enhancement.	(Qian et al., 2019)
Waste activated sludge (WAS) and source-sorted biowaste (OFMSW)	None	Continuous	37 and 55	Operation under thermophilic conditions increased the specific biogas yield from 0.34 to 0.49 m^3 kg^{-1} TVS, corresponding to an improvement of approximately 45–50% relative to mesophilic digestion.	(Cavinato et al., 2013)
Food waste (FW) and sewage sludge (SS)	Thermal pretreatment of sewage sludge at different temperatures	Batch	37	Among the investigated temperatures, thermal pretreatment at 140 °C produced the highest cumulative biogas production (5685 mL), representing an approximately 29.6% improvement relative to the untreated system.	(Aboulfotoh et al., 2022)
Wheat straw (WS) and sunflower meal (SM)	Thermal pretreatment of the selected co-digestion mixture at 120–180 °C	Batch	Digestion temperature not reported	Co-digestion using a substrate mixture of 67% WS and 33% SM increased biogas production by 61%. Applying thermal pretreatment at 180 °C further improved biogas yield by 94.3% relative to the untreated control.	(Rajput and Hassan, 2021)

OFMSW co-digested with TWAS and rice straw (RS)	TWAS: thermo-alkaline; RS: H_2O_2 pretreatment	Semi continuous	35 and 55	Selective substrate pretreatment combined with thermophilic co-digestion improved final cumulative biogas production by 18.6–25.0% relative to the other reactor configurations while maintaining stable performance across OLRs of 2–4 g VS L^{-1} d^{-1}.	Present study

to enhance sludge disintegration and organic matter solubilization, whereas H_2O_2 pretreatment was used to improve the accessibility of the lignocellulosic fraction in RS. This substrate-specific pretreatment approach differs from conventional studies in which a single pretreatment method was applied to the entire substrate mixture.

The comparison also indicates that thermophilic operation combined with substrate-specific pretreatment provided the best overall performance under the operating conditions investigated. Reactor C produced 18.6%, 25.0%, and 23.2% higher cumulative biogas production than Reactors A, B, and D, respectively. Moreover, stable biogas production was maintained throughout the semi-continuous operation as the OLR was progressively increased from 2 to 4 g VS L^{-1} d^{-1}. Unlike conventional batch studies, the present operating mode enabled reactor performance to be evaluated under successive loading stages and extended operating periods.

Overall, the main strength of this approach lies in combining substrate-specific pretreatment, multiple co-substrates, thermophilic digestion, and semi-continuous operation within a single anaerobic digestion system. Collectively, these comparisons demonstrate that integrating substrate-specific pretreatment with thermophilic semi-continuous co-digestion provides sustained performance under progressively increasing organic loading while maintaining stable reactor operation.

Preliminary energy and cost assessment

A preliminary energy and cost assessment was conducted to evaluate whether the additional biogas production obtained through substrate pretreatment could potentially contribute to offsetting the energy requirements associated with the pretreatment processes. Because the present investigation was performed using laboratory-scale semi-continuous reactors, the assessment was based primarily on the experimentally observed increase in cumulative biogas production between the pretreated and untreated reactor configurations. Accordingly, the analysis should be interpreted as an indicative energy assessment rather than a complete techno-economic evaluation of a full-scale anaerobic digestion facility.

The effect of substrate pretreatment was evaluated separately under mesophilic and thermophilic operating conditions. Under mesophilic conditions, Reactor A, which was fed with thermo-alkaline-pretreated TWAS and H_2O_2-pretreated rice straw, achieved a cumulative biogas production of 26,873 mL compared with 25,498 mL for the corresponding untreated Reactor B. Thus, substrate pretreatment resulted in an additional cumulative biogas production of 1.375 mL, corresponding to an increase of approximately 5.4%.

A substantially greater enhancement was observed under thermophilic conditions. Reactor C produced 31,886 mL of cumulative biogas, whereas the corresponding untreated Reactor D produced 25,891 mL. The additional biogas production attributable to the combined pretreatment strategy was therefore 5.995 mL, representing an increase of approximately 23.2%. These results indicate that the energetic benefit associated with substrate pretreatment was substantially greater under thermophilic digestion than under mesophilic operation.

To provide an indicative estimate of the energy associated with the additional biogas production, a methane content of 65% was assumed. Because methane composition was not directly measured in the present experiments, this value was adopted only as a representative assumption for preliminary energy estimation and should not be interpreted as an experimentally determined methane concentration. An approximate lower heating value of 10 kWh m^{-3} CH_4 and an electrical conversion efficiency of 40% for a combined heat and power (CHP) system were further assumed.

Based on these assumptions, the additional biogas generated by pretreatment under mesophilic conditions was equivalent to approximately 894 mL CH_4 and a recoverable electrical-energy equivalent of approximately 3.58 Wh. Under thermophilic conditions, the corresponding additional methane equivalent was approximately 3.897 mL CH_4, providing an estimated recoverable electrical-energy equivalent of approximately 15.59 Wh. The estimated incremental energy benefit under thermophilic conditions was therefore approximately 4.36 times that obtained under mesophilic conditions (Table 4).

The preliminary assessment indicates that substrate-specific pretreatment provided a substantially greater incremental energy benefit under thermophilic than mesophilic conditions. The approximately 4.36-fold difference in recoverable electrical-energy equivalent reflects the considerably larger increase in cumulative biogas production observed in Reactor C relative to Reactor D. This result is consistent with the higher biogas productivity and improved tolerance to increasing OLR previously observed for the thermophilic pretreated reactor. The combination of two pretreatment methods and thermophilic operation is believed to be responsible for the energy benefit experienced in Reactor C. TWAS disintegration and solubilization was enhanced with the thermo-alkaline pretreatment while the pretreatment with H_2O_2 increased the accessibility to the rice straw lignocellulosic fraction. Under thermophilic conditions, more substrates were converted into biogas thanks to an increase of biodegradable organic matter, and due to the hydrolysis and microbial conversion of the substrate which took place more quickly. Thus, the extra amount of energy that can be recovered from the improved biogas generation was significantly higher with thermophilic biogas generation. The initial estimation of cost of substrate pretreatment accounted for the use of NaOH, H_2O_2 and the cost of thermal energy.

However, extrapolation of laboratory-scale chemical and energy consumption to a mass basis requires a clearly defined scale-up procedure that accounts for substrate composition, pretreatment dosage, solids concentration, and process heat recovery. Therefore, a definitive cost saving per ton of treated substrate was not calculated in the present assessment. The previously estimated pretreatment cost components should be regarded as preliminary scale-up assumptions until validated through pilot- or demonstration-scale operation.

Nevertheless, the results provide a useful indication of the relative energy benefit of the proposed pretreatment strategy. The relatively limited increase in biogas production under mesophilic conditions suggests that the additional energy recovery may provide only a modest contribution toward pretreatment energy requirements. In contrast, the 23.2% increase in cumulative biogas production under thermophilic conditions resulted in a markedly greater incremental energy potential. It should also be recognized that the present calculation considers only the electrical-energy equivalent of the additional methane potentially recovered through a CHP system.

If the digester is operating under anoxically, there is potential for recovering heat from the CHP process for use within the digester or to partially

Table 5. Preliminary energy assessment of the additional biogas production associated with substrate pretreatment

Item	Unit	Mesophilic condition (A vs B)	Thermophilic condition (C vs D)
Cumulative biogas production, untreated reactor	mL	25,498	25,891
Cumulative biogas production, pretreated reactor	mL	26,873	31,886
Incremental biogas production due to pretreatment	mL	1,375	5,995
Increase in cumulative biogas production	%	5.4	23.2
Assumed methane fraction*	%	65	65
Estimated incremental methane volume	mL CH_4	894	3,897
Assumed methane energy content**	kWh m^{-3} CH_4	10.0	10.0
Assumed CHP electrical efficiency***	%	40	40
Estimated recoverable electrical-energy equivalent	Wh	3.58	15.59
Relative incremental energy benefit	–	1.00	4.36

Note: * Methane fraction was assumed to be 65% for preliminary energy estimation because methane composition was not directly measured in this study, ** An approximate methane energy content of 10 kWh m^{-3} CH_4 was adopted for indicative calculation, *** CHP = combined heat and power, an electrical conversion efficiency of 40% was assumed.

meet the digesters' heat needs during thermo-alkaline pretreatment. Further overall energy balance improvement in the proposed process could be achieved by utilizing such inside heat integration. In the present laboratory investigation, assessment of heat recovery, parasitic energy consumption, chemical recycling, digestate management, depreciation of equipment and other capital and operating costs were not evaluated. On a statistical basis, the results of the initial screening showed that a minor amount of energy may be recovered from TWAS and rice straw biomass through increased biogas production, as using selective pretreatment was advantageous in this case when implemented in combination with thermophilic semi-continuous co-digestion. The incremental recoverable energy for the thermophilic pretreated configuration was about 4.36 times higher than for the mesophilic pretreated configuration. Further pilot-scale experimental investigation and detailed techno-economic (TE) analysis of the proposed process could be carried out and the findings would be supported by these findings.

CONCLUSIONS

This study investigated semi-continuous anaerobic co-digestion of the OFMSW, TWAS, and RS under both mesophilic and thermophilic operating conditions Stepwise increases in OLR from 2 to 4 g VS L^{-1} d^{-1} were applied to evaluate reactor performance during successive loading stages. Based on the experimental findings, the following conclusions can be drawn:

- Selective pretreatment of the co-substrates improved biogas production under both temperature regimes. The enhancement was relatively limited under mesophilic conditions but was more pronounced under thermophilic operation.
- Thermophilic digestion generally achieved higher volumetric biogas production and COD removal than mesophilic digestion. Reactor C, which combined thermophilic operation with thermo-alkaline-pretreated TWAS and H_2O_2-pretreated RS, consistently delivered the highest overall biogas production among the reactor configurations evaluated.
- Increasing the OLR from 2 to 4 g VS L^{-1} d^{-1} enhanced volumetric biogas production without causing sustained process inhibition. Although temporary fluctuations in pH and VFA occurred after each OLR transition, both parameters gradually returned to stable levels, indicating successful microbial acclimation to the higher substrate loading.
- Combining substrate-specific pretreatment with thermophilic digestion consistently improved overall reactor performance. By the end of the operating period, cumulative biogas production in Reactor C exceeded that of Reactors A, B, and D by 18.6%, 25.0%, and 23.2%, respectively.
- Changes in pH and VFA profiles reflected normal reactor acclimation to successive increases in OLR. Temporary VFA accumulation during loading transitions was followed by pH recovery and stabilization of VFA concentrations, confirming stable semi-continuous reactor operation.
- The preliminary energy assessment showed that the additional biogas generated through substrate pretreatment yielded a greater incremental energy benefit under thermophilic operating conditions than under mesophilic digestion conditions. The estimated recoverable electrical-energy equivalent was approximately 4.36 times higher under thermophilic than mesophilic operation.
- Overall, combining thermo-alkaline pretreatment of TWAS and H_2O_2 pretreatment of RS with thermophilic semi-continuous co-digestion effectively enhanced the conversion of heterogeneous organic wastes into biogas. Future pilot-scale investigations should evaluate methane composition, pretreatment energy requirements, heat integration, and the overall techno-economic feasibility of the proposed process.

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