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Biochar as a catalyst for methane enhancement in anaerobic digestor containing cow dung, food waste, and rice straw: An experimental and statistical study

P. Sivakumar [a], R. Saravanane [a], S. Mohan [b], B. Sankar [c,*]

[a] Department of Civil Engineering, Puducherry Technological University, Puducherry, India
[b] Department of Civil Engineering, Indian Institute of Technology (IIT) Madras, Tamil Nadu, India
[c] Department of Civil Engineering, Mangalam College of Engineering, Kerala, India

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ABSTRACT

There is a growing interest in meeting the rising energy demand from a more sustainable source. Biomass energy has the potential to act as a sustainable and environmentally friendly alternative to fossil fuels and help achieve net-zero emissions in the near future. This study proposes an economically feasible method to enhance biogas efficiency by co-digesting cow dung (CD), food waste (FW), rice straw (RS), with the addition of Coconut husk Bio-Char (BC). The present research aims to study the variation in the biogas yield from biochar addition by monitoring the alteration in the influential parameters such as pH, temperature, total solids (TS), volatile solids (VS), volatile fatty acids (VFA), and carbon to nitrogen ratio (C/N). The biochar addition stabilized both pH and temperature due to its intrinsic properties by transforming intermediates like H_2S and CO_2. It also significantly increased the VFA accumulation and degradation attributed to the buffering ability of the biochar. The methane yield of blends with biochar was significantly higher than that of the blends without biochar. The mixture CD 30: FW 50:RS 20 containing biochar showed a peak methane yield of 165.08 mL. The statistical model developed using response surface methodology (RSM) predicted the methane yield with an accuracy of 99.07 % and a statistical significance level of 0.05. The accuracy of the RSM model was validated by comparing it with the existing Gompertz kinetic model. The performance evaluation error metrics, Coefficient of Correlation (R), and Root Mean Square Error (RMSE) results were observed to be 0.966, 0.925, and 62.89 mL/gVS, 87.24 mL/gVS for RSM model and Gompertz model, indicating the superior performance of the RSM model developed in this study.

1. Introduction

The transition from fossil fuels to sources of renewable energy for achieving carbon neutrality has generated significant discussion. Biomass has the potential to act as a sustainable and environmentally friendly alternative to fossil fuels in the production of biofuels. India, a culturally diverse nation, relies heavily on the agriculture sector, which contributes to 15 % of its GDP. In India, there is an increase in demand for sustainable energy sources due to a growing population, which ultimately leads to more waste generation. Many reports have stated that household wastes, animal waste, dairy wastes, and agricultural waste are accumulating uncontrollably. This calls for an urgent need to minimize such waste sustainably without causing ill effects to the environment. Annually, India produces around 126 million tonnes of rice straw, with an average yield ratio of 0.45. About 16 % of agricultural residues in India are burned in the fields, with rice straw making up 60 % of this amount. Due to the lack of affordable and profitable methods for utilizing rice straw, farmers often resort to burning it or leaving it in the fields until the next cultivation season. This practice contributes to air pollution (CO, SO_2, NOx) and the emission of greenhouse gases (700 mg CH_4/kg and 19 mg N_2O/kg) (Brennan and Owende, 2010; Olatunji et al., 2021).

Even though the raw materials needed for the production of biogas in large quantities are available in India, the efficiency and utility are rather minimal. Also, the complex structure of biomass, comprising of cellulose, hemicellulose, and lignin, poses a substantial challenge to their efficient use (Alalwan et al., 2019). The past studies revealed extensive research carried out to improve the efficiency of the biogas

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* Corresponding author.
E-mail address: sankarboomibalan130@gmail.com (B. Sankar).

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(Brémond et al., 2021; Divya et al., 2015; Kouzi et al., 2020; Pöschl et al., 2010). The efficiency of biogas is dependent on numerous factors such as substrate composition, pH, temperature, carbon to nitrogen ratio, organic loading rate (OLR), volatile solids (VS), volatile fatty acids (VFA), and hydraulic retention time (HRT).

The production of biogas involves anaerobic digestion (AD) of the feedstocks, which has several interconnected organic degradation stages, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The installation of reactors can be done in a single or two-stage process. Several studies have reported the advantages of two-stage AD over single-stage as it hinders the nutrient availability for the microbes through VFA build-up and harmful intermediates, which ultimately yield to lower productivity of methane. In the initial hydrolytic/acidogenic phase, VFAs and hydrogen were produced. Subsequently, acetogenic bacteria convert VFAs into CH_3COOH, which is then transformed into CH_4 and CO_2 by methanogenic bacteria during the acetogenesis/methanogenesis phase (Djimtoingar et al., 2022).

1.1. Literature review

1.1.1. Parameters influencing the efficiency

Dahiya et al. (2015) investigated the effects of the pH content of the acidogenic fermentation of the food waste on altering the productivity of volatile fatty acids (VFA). It was observed that the higher degree of alkalinity in the co-digestion stages buffers the acidification process and H_2 production from the food waste. Wang et al. (2014) studied the anaerobic digestion of the food waste along with activated sludges collected from a settling water treatment plant (aerobic) and brewery reactor bed (anaerobic). The study investigated the effects of co-digestion on the hydrolysis rate and volatile fatty acid (VFA) production rate. It was observed that the hydrolysis rate improved with the addition of anaerobic sludge compared to aerobic sludge at all the pH ranges. The VFA yield and ammonium nitrogen concentration were observed to be maximum at a pH value of 6. Dareioti et al. (2014) investigated the effects of pH of the substrates such as olive mill wastewater, cow manure, and cheese whey in a ratio of 55:5:40 on the production of VFA and hydrogen. The maximum VFA yield was observed at a pH value of 6.5, whereas the hydrogen production was maximum for pH value of 6.

Wongarmat et al. (2022) investigated the co-digestion of sugarcane waste along with biogas effluent. The acidogenic phase was separated in order to act as a pretreatment for the sugarcane cakes. It was observed that if the substrate–inoculum ratio (SIR) is greater than the optimum, then the yield of the system will be reduced because a higher proportion of inoculum will degrade organic matter at a higher rate, which will be subsequently converted to VFAs, making the digestor more acidic. Another study by Wainaina et al. (2019) highlighted that the proportion of VFA from acidogenic phase, such as acetic acid, propionic acid, and butyric acid at a constant pH may not be the same for different substrates. It depends on the proportion of cellulose, hemicellulose, proteins, and lipid constituents of the organic substrate involved in the degradation. The accumulation of VFA is governed by two major factors, the acidogenic reaction process and the inhibition of methanogens in the early stages.

It has been widely accepted that for efficient biogas production, maintaining the C/N ratio between 20 and 30 is ideal, as bacteria consume carbon 30 times more quickly than nitrogen (Muthudineshkumar and Anand, 2018). Kwietniewska and Tys (2014) stated that if the C/N ratio is too low, then the percentage of nitrogen (protein content) present in the substrate is high. Anaerobic degradation of such a substrate will increase the ammonia content of the system, and a simultaneous increase in VFA of the system as a result of the effect of ammonia on bacteria. Kushwaha et al. (2022) in their study proposed an easier and cost-effective method of controlling the C/N ratio in the optimum ranges with the addition of agricultural residue as a co-digestor. Their study involved animal manure, which typically has a high nitrogen content, so to achieve the optimal C/N ratio, agricultural waste is added and allowed to co-digest.

The optimum temperature for methanogenesis is 37 °C, which is not the same for hydrolysis, acetogenesis, and acidogenesis. If all the phases of anaerobic degradation are in the same reactor, temperature of the reactor cannot be controlled, and it varies according to the prevailing conditions inside the reactor (Nie et al., 2021). Vanegas and Bartlett (2013) studied the effect of temperature on the gas production and anaerobic digestion from seaweed-based biomass. The temperature profiles in the digestive reactor were altered from 20 to 45 ℃ for a period of 54 days. The highest methane yield of 184 mL methane /g of volatile solids was obtained for a temperature of 35 ℃. The study by Nie et al. (2021) revealed that the temperature profile greatly alters the cellular enzymes involved in the hydrolysis rate and plays a pivotal role in the hydrogen gas, methane gas, and VFA production. The synergy between the enzymes provided metabolic pathways for the accumulation of VFAs.

Ammonia is one of the most inhibiting parameters in the process of biogas production through anaerobic digestion through hydrogen-enriched pathways. Xiao et al. (2022) studied the effects of co-digestion of activated carbon along with pig manure on the organic loading rate and ammonia concentrations. It was reported that the methane yield is maximum when the partial pressure of hydrogen is 0.5 atm, and the thermophilic methanogens have high resistance towards high ammonia loads. Ammonia is a factor dependent on HRT, SRT, OLR. As the OLR increases, the residence time of solids inside the reactor is shorter and hence there is incomplete degradation of organic matter in the reactor, leading to lower ammonia production.

1.1.2. Usage of biochar

Ngo et al. (2024) investigated the effectiveness of biochar as a co-digestor in anaerobic digestion in the enhancement of methane production from the existing chicken manure. It was reported that the addition of biochar led to a porous structure where acclimation was possible for the microbes. An increase of 80 % in COD was observed together with a reduction in the ammonia concentration, which further reduced ammonia stresses in microbes. The biochar particles provided shielding for microbes from ammonia, due to that their activity rate did not diminish even at increased time periods. A study by Ambaye et al. (2021) mentioned that the biochar efficiency varies depending on the chemical composition of the source material and the stability of carbon content, acting as sequesters to inhibitors. The quality of biochar depends on pyrolysis temperature, feedstock, and heating rate. Chen et al. (2024) highlighted the importance of usage of biochar in anaerobic digestion as a circular economy approach. The study investigated the addition of biochar as a cost-effective method for the acceleration of thermophilic anaerobic digestion. The methane yield from the biochar addition improved up to 18 % due to the growth of electroactive bacteria such as Clostridia. The total volatile fatty acids and the cumulative methane yield were found to be maximum for the reactor containing biochar. The study by Basumatary et al. (2024) examined the variation in methane production by different substrate mixing ratios, temperature, and biochar proportions. The percentages of biochar were varied from 5 to 25 g/L. The results from the study indicated that the maximum methane yield was attained for a substrate mixing ratio of 40:60 (cow dung: cooked food waste) with a biochar addition of 15 g/L. Kulabako et al. (2025) studied the co-digestion properties of water hyacinth together with cow dung in the presence of biochar made from wood and fecal sludge. The utilization of water hyacinth was driven by the sustainability perspective by minimizing the environmental hazard in the tropical regions, and also a cost-effective alternative for waste management.

1.1.3. Optimization of biogas yield

To maximize biogas production, parametric optimization of the anaerobic digestion process is essential. In the conventional approach, optimization is done by altering one parameter at a time while keeping the other parameters constant. This allows for the impact of each individual parameter to be understood. However, this method requires a large number of experiments and consumes a lot of time. Additionally, it is costly and does not allow for the determination of interactions between parameters (Thirugnanasambandham, 2017). Nowadays, various optimization techniques such as the RSM, Artificial Neural Network (ANN), and Genetic Algorithm (GA) are widely used to optimize process parameters (Biancofiore et al., 2017; Mondal et al., 2023; Pérez-Ortiz et al., 2003). Past researchers have used such optimization techniques to effectively predict the biogas production from different input variables. Deepanraj et al. (2017) studied the optimization of process parameters involved in the digestion of food waste. The study utilized Taguchi-gray analysis technique by assigning input and output variables. The input parameters chosen were total solid concentration, pH, temperature, C/N ratio, and pretreatment, whereas the output was methane yield. The study provided mathematical regression models to predict the methane yield with an accuracy of about 93.1 %. Wang et al. (2013) studied the optimization of anaerobic digestion of cow manure using RSM to predict the hydrogen production. The input variables were taken as the varying pH, and temperature, whereas the solid retention time, organic loading rate, and stirring were kept constant. The model developed from the central composite design of RSM was a polynomial quadratic with coefficient of determination value of 0.63. Iweka et al. (2021) analysed the optimization techniques like RSM with the incorporation of a programming language like Python to effectively optimize the co-digestion of corn chaff and cow manure. The substrate ing ratios and hydraulic retention time were selected as the input parameters, where the methane yield was designated as the output parameter. The study concluded that the optimum substrate mixing ratio as 1:1.55 (corn chaff: cow manure), and 37 37-day retention time gave the maximum yield of methane with 68 % total composition.

1.2. Aim and scope

The novelty of the present study lies in its systematic investigation of substrate mixing ratios and their impact on both physico-chemical properties and biogas yield, which addresses a major research gap. Additionally, the incorporation of biochar (BC) into domestic and agricultural waste mixtures remains an underexplored area, and this study uniquely evaluates its influence on digestion performance. The present research aims to study the variation in the biogas yield by monitoring the alteration in the influential parameters such as pH, total solids (TS), volatile solids (VS), volatile fatty acids (VFA), and carbon to nitrogen ratio (C/N). To assess the performance of an anaerobic co-digestion system, it is essential to create an accurate biogas yield forecasting model. Hence, the development of a statistical model to accurately predict methane yield based on key input parameters (pH, TS, VS, VFA, and C/N ratio) further distinguishes this research. The present study has significant relevance in the field of sustainable energy production and waste management, which are in alignment with the Sustainable Development Goals.

2. Materials

The raw materials for the present research were procured from the nearby sources. Food waste and vegetable waste were collected from the Puducherry Technological University hostel (12.01453° N, 79.84456° E). The major types of food waste are of South Indian style, namely boiled rice, idly, sambar, variety rice, etc. Fresh Cow dung is collected in a nearby local byre. While collecting the cow dung in the byre, details about the cattle's health are also collected. The paddy straws were collected from nearby agricultural fields near Puducherry. The freshly collected straws were cleaned and chopped into fine pieces of size ranging from 1 mm to 1 cm using a chaff cutter. The coconut husk was obtained from a farm in Dindigul district of Tamil Nadu, India. The husk was grinded into fine pieces and dried in a hot air oven at 100 ℃ for a period of 24 h. Pyrolysis was carried out on the dried husk after the removal of moisture at an incremental rate of 10 ℃ per minute up to 500 ℃ in an air-tight chamber (Lee et al., 2013). The vapor residence time for the intermediate rate pyrolysis was maintained at 30 min. The biochar product yield was obtained in a weight percentage of 30 % per input feed. The biochar is then finely ground and sieved to 100 μm before adding it to the digestor chamber. Fig. 1 shows the collected raw materials.

2.1. Characterization of substrates

The substrates from the present study were characterized based on the moisture content, TS, total volatile solids (TVS), and total organic carbon (TOC). The moisture content and the total solid concentrations are determined by the thermogravimetric method, where the samples were initially oven dried at 105 ℃ for about 24 h and the final result was obtained by measuring the weight subtracting from the wet fractions. To determine the TVS, the oven-dried samples were kept in a muffle furnace and heated upto 500 ℃ for a period of 2 h. The weight difference between the oven-fried and furnace-heated samples is taken as the percentage of TVS. TOC was measured using the loss from ignition method. To determine pH, the substrate samples were diluted in water with suitable mixing ratios, and the pH value was measured using a pH meter. Finally, C/N ratio of all the samples after removing the moisture content was measured using CHNS semi-macro elemental analyzer. The results provided in Table 1, such as moisture content, VS, and C/N ratio, influence microbial activity and biogas production. From the table, it can be seen that CD and RS have moderate to high total solids, indicating their suitability for co-digestion with drier RS. The volatile solids and organic carbon content suggest a good potential for biogas production, especially from RS (Karki et al., 2022). However, rice straw's high C/N ratio (77.1) may lead to nitrogen deficiency, requiring balancing with nitrogen-rich substrates like CW (Yadvika et al., 2004). The acidic pH of FW (4.5) could inhibit microbial activity and require buffering during anaerobic digestion (Zhang et al., 2014).

2.2. Characterization of biochar

The physical properties of the biochar play an important role in the digestion characteristics of the substrate. The major functionally important physical parameters are specific surface area, volume, size of pores, and pH. The specific surface area is measured with a BET analyzer using the gas adsorption method. Vacuum gas removal was performed on biochar before testing. To determine the pore volume and diameter, both BET analyzer and the mercury porosimeter are utilized to determine the accurate pore volume. The average of the results was taken as the final result. In order to determine pH, the biochar sample was diluted in water with a mixing ratio of 1:10, and the pH value was measured using a pH meter. Initially, proximate analysis was carried out on the grounded biochar to determine the moisture content, ash content, carbon content, and volatile substances. The moisture content was determined using the specifications provided by (ASTM, 2014). The ash content was determined using the recommendations of (ASTM D1102–84 2013). The test for detecting volatile matter was carried out according to (ASTM, 2011). The carbon content was finally estimated by subtracting the mass percentage of moisture content, ash content, and volatile matter from the total weight of the biochar. The elemental compositions identified were carbon, nitrogen, oxygen, hydrogen, and sulfur. The results of the physical and chemical properties obtained from the testing are given in Table 2. The high fixed carbon (68.66 %) and carbon content (74.65 %) indicate that the BC can enhance microbial activity and buffer organic loading in anaerobic digestion (Pan et al., 2019). Its porous structure (BET surface area 52.13 m^2/g, 2.96 nm pores) provides a habitat for microbes, improving digestion efficiency (Aramrueang et al., 2022). The alkaline pH (9.29) helps stabilize pH

Fig. 1. Raw materials collected and processed.

Table 1
Initial characterization of substrates.

Parameters	Cow dung	Food and vegetable waste	Rice straw
Moisture content (%)	81.2 ± 0.5	90.8 ± 1.3	6.6 ± 1.2
Total solids (%)	18.8 ± 0.9	9.2 ± 0.7	93.4 ± 0.3
Total volatile solids (%)	14.5 ± 1.8 (84 % of TS)	8.1 ± 1.2 (88 % of TS)	80.5 ± 0.8 (86 % of TS)
Total organic carbon (%)	43.2 ± 0.6	58.1 ± 1.6	47.2 ± 1.4
pH	8.89	4.5	8.1 ± 0.2
C/N ratio	18.2 ± 0.4	31.4 ± 1.2	77.1 ± 0.8

Table 2
Physical properties, composition, and elemental analysis of biochar.

Parameters	Tested value
Physical properties	
BET surface area	52.13 m^2/g
Pore volume	0.069 cm^2/g
Average pore diameter	2.96 nm
pH	9.29 ± 0.10
Composition (%)	
Moisture content	3.32 ± 0.06
Ash content	11.24 ± 0.23
Volatile substance	16.78 ± 0.21
Fixed carbon content	68.66 ± 0.11
Elemental analysis (%)	
Carbon	74.65 ± 0.21
Nitrogen	4.45 ± 0.08
Oxygen	1.43 ± 0.09
Hydrogen	0.19 ± 0.12
Sulfur	19.28 ± 0.15

levels in an acidic digestion environment. Moderate nitrogen levels in BC support microbial growth. The ash content in the BC can help regulate the pH levels by transforming the intermediates, such as H_2S and CO_2.

Scanning electron microscopy (SEM) was utilized to determine the surface morphology of the biochar particles using back-scattered electrons. The initial preparation of the sample involved cleaning the debris, mounting the sample in a vent chamber with carbon tape, followed by gold coating for uniform electron emissions to get high-resolution images. The sample was analyzed using Zeiss model equipment. An X-ray diffractometer (XRD) was used to determine the crystalline structure of biomass. The testing range of 2 theta was between 10° and 90°. The XRD instrument was operated at an electron intensity of 35 mA and a voltage of 40 kV. To identify the functional groups present in the sample, PerkinElmer spectrum two model-based Fourier transform infrared spectra (FTIR) was used. The SEM, XRD, and FTIR analyses are shown in Fig. 2. The SEM image shows a highly porous structure, which enhances microbial colonization and surface area for adsorption in anaerobic digestion. The biochar from the present study had a larger surface area, less rough, and clearer in appearance compared to the residue biochar (Sheng et al., 2022), tomato plant biochar (Wiedner and Glaser, 2013) and digestate biochar (Duong et al., 2025). The XRD spectra reveal crystalline phases like $CaCO_3$, $CaSO_4$, K_2SO_4, and $Cu(OH)_2$, indicating the presence of mineral components that can buffer pH and support microbial processes. FTIR peaks at 3442, 1569, and 1124 cm^{-1} confirm functional groups like –OH, $C{=}C$, and C–O, essential for surface reactivity and nutrient retention (Zheng et al., 2021).

3. Experimental methods

3.1. Seeding and feed substrate

Seed sludge used in this study was digestate collected from an already existing batch reactor, which was operated under mesophilic conditions for 30 days with cow dung as a substrate. The digestate was filtered with a wired mesh of 0.5 mm diameter to remove suspended

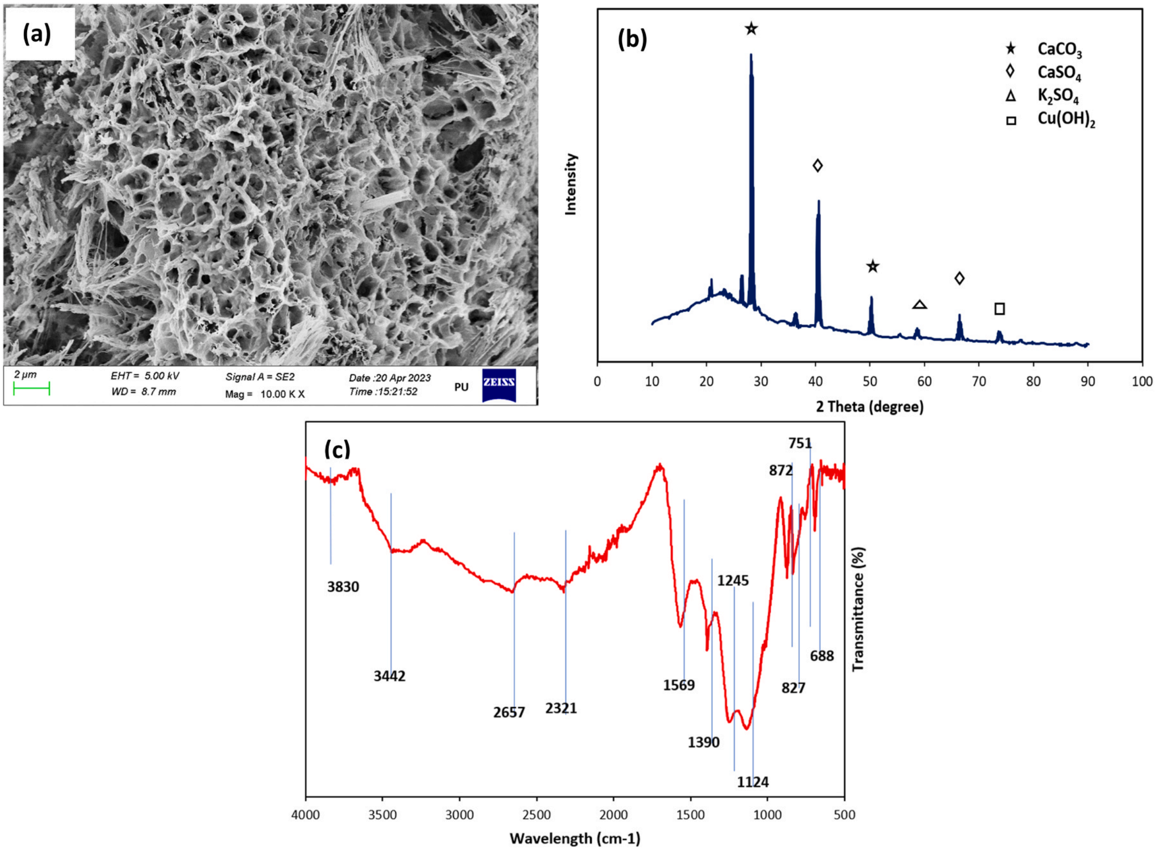

Fig. 2. (a) SEM image; (b) XRD spectra; and (c) FTIR spectra of biochar.

fibrous and coarse materials. The filtered digestate was heated for 1 h at 700 ℃ to remove methanogens.

3.2. Reactor design

The Batch reactor used to carry out experiments was a 10-litre container with a 2-litre headspace. A Total of 8 reactors were established with different combinations of organic feed. The reactors with different feed proportions are detailed in Table 3. The contents were mashed and homogenized manually with a movable disc weighing about 2 kg rotating at a speed of 10 RPM for 1 min. Water is added to the homogenized waste to maintain the total solid concentration at 8 %. Gas shut-off check valve, along with the gas tube is connected to the mouth of the reactors. In the initial stage of the experiment, nitrogen gas was inserted into the airtight digester in order to create an anaerobic environment. A provision was made for the collection of samples for intermittent analysis of the physical and chemical parameters. The temperature of the environment was maintained between 35 and 40 ℃ to maintain a controlled environment for the bacterial digestion.

Table 3
Experimental design of mixture ratios.

Reactor no.	Designation	Mixture ratios (set 1)	Mixture ratios (set 2)	
R1	Cow dung: Food waste	100:0	100:0	BC−15 g/L
R2	Cow dung: Food waste	0:100	0:100	BC−15 g/L
R3	Cow dung: Food waste	70:30	70:30	BC−15 g/L
R4	Cow dung: Food waste	30:70	30:70	BC−15 g/L
R5	Cow dung: Food waste	50:50	50:50	BC−15 g/L
R6	Cow dung: Food waste: Rice straw	50:30:20	50:30:20	BC−15 g/L
R7	Cow dung: Food waste: Rice straw	30:50:20	30:50:20	BC−15 g/L
R8	Cow dung: Food waste: Rice straw	40:40:20	40:40:20	BC−15 g/L

3.3. Experimental setup

Two sets of experimental designs were planned, one without biochar and the other with biochar. The mixture ratios with cow dung: food wastes 100:0 and 0:100 were taken as the reference blends to compare the influence of rice straw and biochar addition. A total of 8 mixture ratios were selected for the design of experiments, detailed in Table 3. The optimum quantity of coconut husk biochar was chosen from the

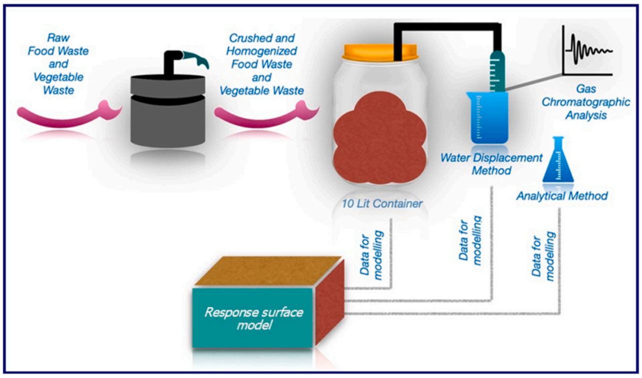

Fig. 3. Schematic diagram of R2 setup and experimental method.

literature as 15 g/L (Basumatary et al., 2024), was added in the second set of the experiments. The schematic diagram of the methodology can be seen in Fig. 3.

The volume of gas produced was measured using the principle of water displacement. The schematic representation of the test setup employed is shown in Fig. 4. The digestor was connected to an airtight beaker containing diluted NaOH with a concentration of 1.5 N. Another pipe immersed in the NaOH solution is connected to an external collection where the solution will be displaced. This setup ensures the estimation of the total volume of methane alone, as the CO_2 in the gas reacts with NaOH to form carbonates. The volume of the NaOH solution displaced will be a direct indicator of the total methane produced for a particular volume of the feed (Barua et al., 2018). The silicon pipe used was made sure of any potential leaks. The measurement was recorded for over 50 days of hydraulic retention time. The temperature was frequently monitored to prevent any uncontrolled digestion.

3.4. Statistical analysis

3.4.1. Response surface methodology

Response Surface Methodology (RSM) consists of mathematical technique that can be used to effectively correlate ‘n’ number of independent variables with one or more output parameters as a response. Also, it can be used to design and develop statistical models that can estimate the interactions between input factors, and analyse the effects of influencing parameters on the output (Ma et al., 2022). For this purpose, a commercially available design expert-based Stat Ease software was used to develop the statistical model (Design-Expert, 2012). The RSM model relies on fitting the experimental data to the linear, quadratic, and polynomial functions by reducing the least square errors. This method is more reliable when more number of independent variables and datapoints are involved (Szpisják-Gulyás et al., 2023). The effects and the quantitative function relationship between the substrates on the volume of methane production were analysed. Major influencing parameters were considered as the control factors such as pH, VS, VFA, mixing ratio, and C/N ratio. The mathematical expression given in Eqa. 1 best describes the structure of the RSM model.

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i<j}^{n} \beta_{ij} X_i X_j \ldots + e(X_i, X_j, \ldots X_n) \qquad \ldots \quad (1)$$

Where, Y is the experimental output parameter value (methane yield); β_0, β_i, β_{ij} are the regression coefficients; X_i, X_j,. X_n are the investigated factors and ‘n’ is the number of factors. The different statistical metrices given in Eqa. 2 and 3, to determine the reliability of models, are coefficient of correlation (R) and the root mean square error (RMSE). The BMP_{act} and BMP_{pre} are the actual and predicted methane potential of the bio feed.

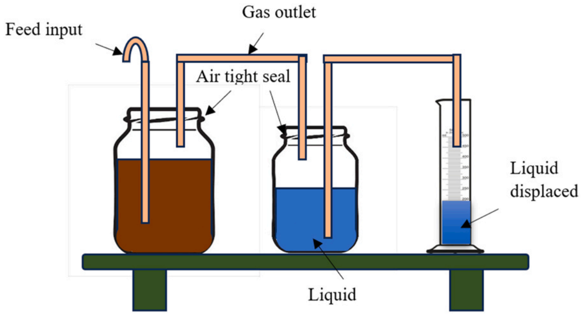

Fig. 4. Schematic representation of the test setup for liquid displacement method.

$$R = \frac{\sum_{i=1}^{n} (BMP_{act,i} - \overline{BMP_{act}})(BMP_{pre,i} - \overline{BMP_{pre}})}{\sqrt{\sum_{i=1}^{n} (BMP_{act,i} - \overline{BMP_{act}})^2} \sqrt{\sum_{i=n}^{n} (BMP_{pre,i} - \overline{BMP_{pre}})^2}} \quad \ldots \qquad (2)$$

$$RMSE = \sqrt{\frac{1}{N} \sum_{i=1}^{n} (BMP_{act,i} - BMP_{pre,i})^2} \ldots \qquad (3)$$

3.4.2. Validation with kinetic model

The results of the experimental program are compared with the mathematical model derived from the Gompertz equation (Zhang and Wang, 2021). Several studies have reported the reliability of the modified form of the Gompertz model. The cumulative methane potential at time “t” of the blends can be obtained by the Eqa. 4.

$$MP(t) = MP_{\max} \quad .\exp\left(- \exp\left[\frac{\mu_m . e}{MP_{\max}} \quad (\lambda - t) + 1 \right] \right) \quad \ldots \qquad (4)$$

Where, $MP(t)$ and $MP_{\max}$ represents the methane potential of the blends at any given time t (days) and maximum methane potential, respectively. The value μ_m represents the production rate of methane. λ represents the initial microbial lag phase time inside the digester. The value of e is Euler’s number, given by the exponential of 1 (2.718). The accuracy of the model is calculated using R and RMSE and compared with the performance of the RSM model.

4. Results and discussion

4.1. Characterization of mixture samples for anaerobic digestion

The samples containing various mixture ratios of the substrates were tested for TS (%), MC (%), VS (%), pH value, and C/N ratio. The results of the characterization can be correlated with the methane production and can be used to determine the influential parameters and their ranges. Furthermore, examining the physicochemical parameters helps stabilize the digestion process (Raposo et al., 2012). The sampling parameters studied for various substrate mixture ratios include CD 100: FW0, CD 0: FW 100, CD 70: FW 30, CD 30: FW 70, CD 50: FW 50, CD 50: FW 30: RS 20, CD 30: FW 50: RS 20, and CD 40: FW 40: RS 20. The results of the test program are presented in Table 4. From the table, it can be seen that the moisture content holds a significant portion of the samples. It is pertinent that the moisture levels are in such high ranges, as it accelerates the hydrolysis and acidogenesis phases of the anaerobic digestion. Any significant lowering of the moisture content could result in an imbalance that could lead to rapid acid production by acid-producing bacteria, which slows down the activation of methanogenic bacteria (Avinash and Mishra, 2023). In the present study, the higher MC levels ensured the functioning of bacteria and enzyme production. The TS (%) concentrations in the blends from the present study were between 10 and 20. The TS levels are crucial for microbial cellular metabolism and reproduction as it govern the mass transfer between microbes and the surrounding nutrients. Many past studies have demonstrated the microbial inactivity and methane depletion due to the higher concentration levels of TS up to 30 % (Motte et al., 2013; Wang et al., 2020). Hence, in the present research, the TS levels were

Table 4
Initial tested parameters of the sample.

Mixture ratios	MC (%)	TS (%)	VS (%)	pH	C/N ratio
CD 100: FW0	84.26	15.74	77.31	5.74	26.71
CD 70: FW 30	87.45	12.55	76.79	5.71	18.79
CD 50: FW 50	86.3	13.7	77.69	5.55	16.62
CD 30: FW 70	86.15	13.85	81.95	5.42	16.08
CD 0: FW 100	81.12	18.88	82.34	5.28	14.61
CD 50: FW 30:RS 20	87.55	12.45	77.93	5.84	17.79
CD 40: FW 40:RS 20	86.8	13.2	79.66	5.65	16.25
CD 30: FW 50:RS 20	86.65	13.35	79.43	5.66	16.47

maintained between 10 and 20. The VS % in the blends was found to be between 75 % and 95 % of the TS. The VS content ensures the availability of organic matter for the digestion process. It can be seen that the VS content was higher in FW. pH values were observed to be lower for blends containing higher percentages of FW. The addition of RS increased the pH value from 5.71 to 5.84 for CD 70: FW 30 mixture, 5.42–5.66 for CD 30: FW 70 mixture, and 5.55–5.65 for CD 50: FW 50 mixture. The results of the C/N analysis indicate the nutritional value of the substrate for the microbes to thrive (Salangsang et al., 2022). The C/N ratio of the CD 100 % mixture was found to be maximum because of the low nitrogen content. The total carbon content as a percentage of TS was observed to be more or less the same as 40 ± 4.5 % for all the blends, whereas the nitrogen content varied from 1.4 % to 2.96 %. The C/N ratio from the present study was lower than the ideal ranges reported in the past studies. To offset such shortcomings, biochar was added to the blends, and its effect on the methane yield was determined.

4.2. pH value

4.2.1. Without the addition of BC

Maintaining pH in the digester is crucial for the performance of the bacteria in efficiently digesting the substrate. It has been reported that the optimal pH range for the maximum methane production is between 6 and 7.5 (Cioabla et al., 2012; Gonde et al., 2023). In the present study, the pH value of all the samples was monitored every day throughout the 50-day period of digestion. Fig. 5(a) and 5 (b) show the variation in pH of mixture samples containing CD: FD and CD: FW: RS without the addition of biochar. It can be seen from Fig. 5(a) that the pH value of the mixture containing 100 % CD showed the highest value as a result of the buffering capacity of the CD, which has been reported in earlier studies (Acosta et al., 2021). Whereas, the pH values of the mixture containing 100 % FW were the lowest due to the spices and cooking oil typically present in the food wastes. Even though the initial pH values of 100 % FW were lower, a gradual rise in the values can be observed over time. The co-digestion of CD and FW slightly increased the pH levels due to the presence of CD and the buffering effect. A decline in pH trend was observed for all the samples up to a 7-day period, indicating the activation of microbes in producing acids. The further increase in the values corresponds to the consumption of acids by the methane-producing bacteria. The pH values for all the blends containing CD and FW varied consistently within the acidic range. The addition of RS significantly balanced the pH range throughout the digestion process. The pH values of the blends CD 50: FW 30:RS 20 and CD 30: FW 50:RS 20 were observed to be in the optimal ranges.

4.2.2. With the addition of BC

The addition of biochar in the blends helped stabilize the pH values due to the intrinsic characteristics of biochar, producing a highly alkaline solution (Zhao et al., 2021). Fig. 6(a) and 6 (b) show the variation in the pH of samples containing CD: FD and CD: FW: RS with the addition of biochar. A 16.6 % increase in the pH was observed for the blend containing 100 % CD due to the addition of 15 % BC. Such an increase in the alkalinity of the blends can be attributed to the presence of essential ions like Na, K, and Mg. Studies have reported that the ash content in the biochar helps regulate the pH levels by transforming the intermediates such as H_2S and CO_2, which further increases the methane yield (Nie et al., 2024). The co-digestion of CD and FW, along with the addition of BC, improved the pH levels significantly. A 15.5 % increase in the pH value was observed on 15th day for the CD 30: FW 70 mixture containing BC when compared to the same mixture without the addition of BC. The addition of BC in the blends containing RS further balanced the pH levels necessary for the anaerobic digestion, as seen in Fig. 6(b).

4.3. Volatile solids

4.3.1. Without the addition of BC

The volatile solids (VS) present in the digester before, during, and after the digestion can represent the amount of organic matter consumed for the production of biogas. In the present study, VS was measured daily throughout the 50-day digestion period by collecting a small amount of sample from the digester. The results obtained from the test program are shown in Figs. 7 and 8. The initial VS concentration of the samples from set 1 (without BC) was between 77 % and 83 %. As expected, the VS concentration declined over time, irrespective of the sample mixture ratios, due to the conversion of organic matter into gas. The sample containing 100 % CD revealed the least VS concentration, which degraded in a similar trend compared to other blends. But after 24 days of digestion, the degradation trend was more flat, indicating the inefficiency in the conversion of organic matter into gas. In the case of 100 % FW mixture (max VS concentration), the degradation was smoother. The co-digestion results showed the positive effects of substrate mixing from the more consistent and steeper degradation. The constantly declining curves of blends CD 30: FW 70 and CD 50: FW 50 show the potential of substrate mixing in increasing the efficiency of digestion, seen in Fig. 7(a). The reduction in the VS%, from start till end of the digestion, for CD 30: FW 70 mixture was 26.11 %, which was 6 % more compared to the mixture containing 100 % FW. The mixing of RS in the substrate improved the pH levels, which improved the thriving environment for the microbes, which ultimately led to more VS consumption and gas conversion (Oosterkamp, 2020). Fig. 7(b) shows the VS degradation trends of the blends containing RS. The mixture containing CD 30: FW 50:RS 20 showed an imbalance in pH in the earlier stages due to the rapid acid buildup caused by the acidogens. After 15 days of the digestion period, the decline in VS was observed to be rapid and consistent with a 30 % reduction when calculated from the beginning and end of the anaerobic digestion.

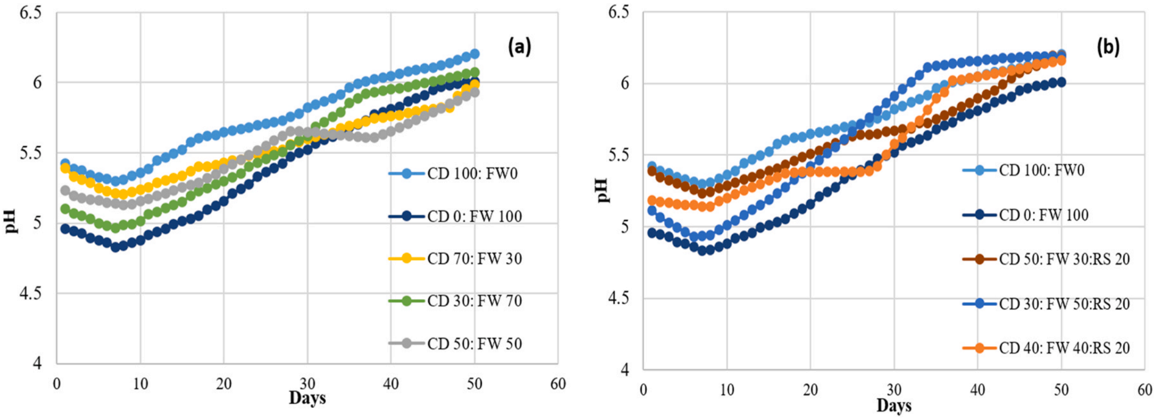

Fig. 5. pH variations in mono and co-digestion blends containing (a) CD and FW (without BC); (b) CD, FW, and RS (without BC).

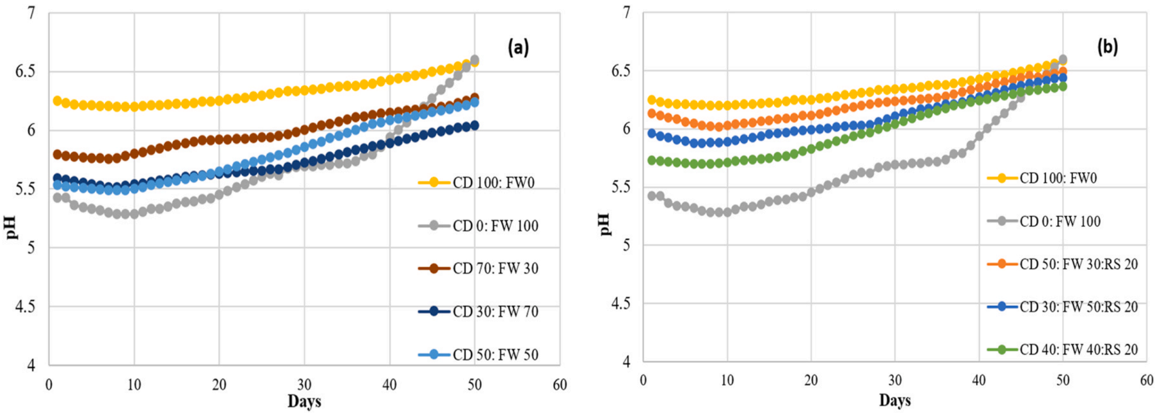

Fig. 6. pH variations in mono and co-digestion blends containing (a) CD and FW (with BC); (b) CD, FW, and RS (with BC).

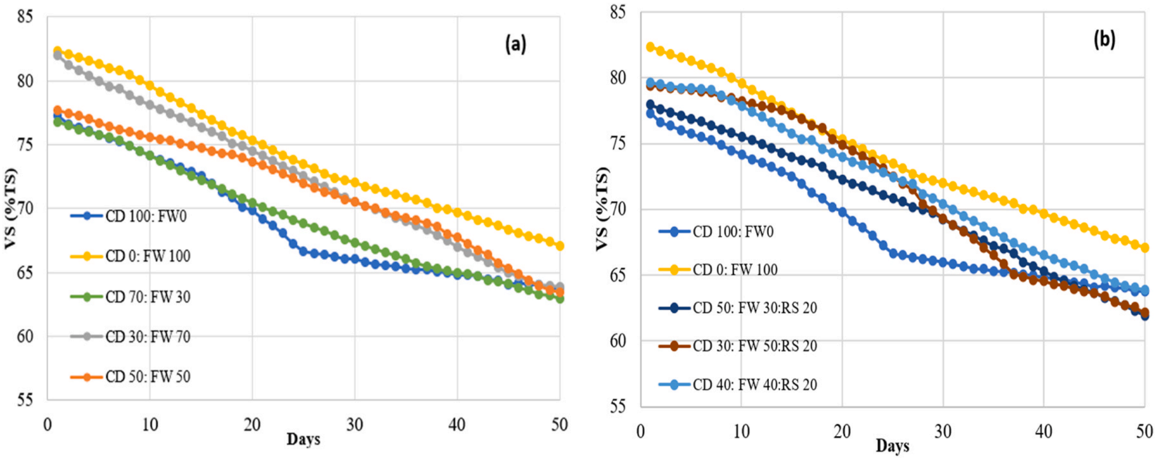

Fig. 7. VS concentrations in mono and co-digestion blends containing (a) CD and FW (without BC); (b) CD, FW, and RS (without BC).

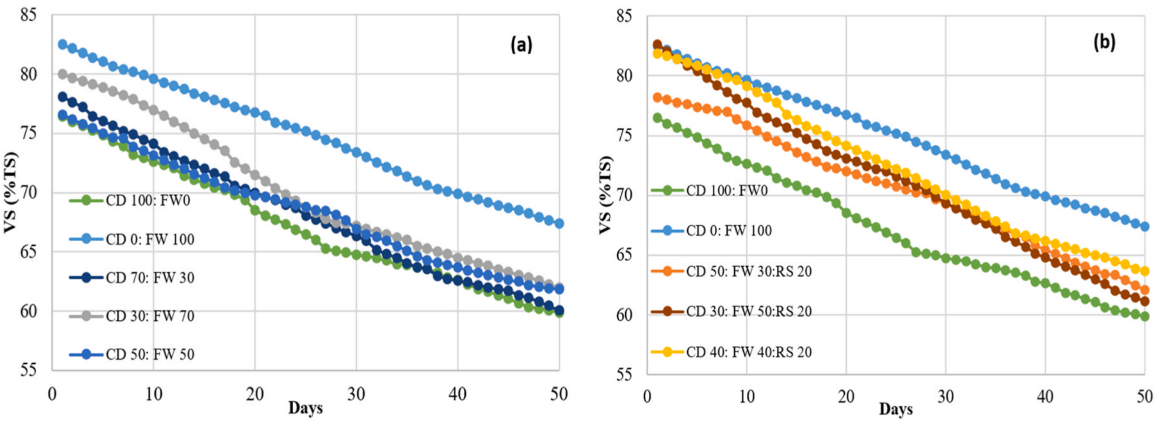

Fig. 8. VS concentrations in mono and co-digestion blends containing (a) CD and FW (with BC); CD, FW, and RS (with BC).

4.3.2. With the addition of BC

The addition of biochar helped improve the degradation of volatile matters more consistently. For set 2 blends, the VS content varied from 78 % to 85 %. The degradation curves of CD, FW, co-digestion of CD and FW, and mixture containing CD, FW, and RS with the addition of 15 % biochar are shown in Fig. 8(a) and (b). The reduction rate of VS also signifies the methane-producing potential of the blends containing biochar. A 27 % and 20.4 % degradation of VS was observed for blends containing 100 % CD and 100 % FW, when measured from the beginning to the end of digestion. In the co-digestion mixture CD 30: FW 70, a 28.8 % reduction in VS was observed, which was 2.5 % higher compared to the same mixture without the addition of BC. This indicates the effectiveness of BC in assisting the microbes in converting organic matter to gas. Also, the inclusion of RS along with biochar causes positive synergistic interactions, which lead to more consistent and steeper degradation of organic matter (Ma et al., 2020). The mixture CD 30: FW 50:RS 20 containing BC showed 33.5 % reduction at the end of the digestion period, which was 3.5 % more compared to the same mixture without BC addition. The VS variations significantly correspond to the methane-producing potential of all the blends from the present study.

4.4. Volatile fatty acids

4.4.1. Without the addition of BC

The role of VFA in the methane production has been reported in several past studies (Jiang et al., 2018; Wang et al., 2009). The VFAs are intermediate products formed during the acidogenesis stage of anaerobic digestion. The VFAs are mainly composed of acetate, butyrate, and propionate, which are formed by the breakdown of organic matter in the substrate by the acidogens. These VFAs are then consumed by the methanogens in the successive stages to form methane. The concentration of VFAs was tested in three distinctive periods 10-day, 20 day-and 50-day to record the maximum, intermediate, and final VFA concentration levels, such that the variation trend can be observed. The accumulation and degradation of VFAs measured in all the sample blends are shown in Figs. 9 and 10. From Fig. 9, it can be seen that the mixture containing CD 100 % showed the least VFA accumulation and a slower degradation trend, mostly attributed to the nutrient deficiency. Whereas, the 100 % FW mixture indicated more volumetric VFA accumulation. The co-digestion improved the acidic compounds formation with the mixture containing CD 30: FW 70, showing an improvement of 15 % over the CD 100 % mixture in a 10-day retention time. The inclusion of RS balanced the pH levels and assisted with the nutrient requirement for the microbes which led to the higher volumetric accumulation and faster degradation of VFAs, seen from Fig. 9. The mixture containing CD 30: FW 50:RS 20 showed the maximum value of 24000 mg/L for a 10-day retention period, which was 20 % and 15 % more compared to the CD 100 % and CD 30: FW 70 blends, respectively.

4.4.2. With the addition of BC

The addition of BC in all the blends from the present study significantly improved the VFA accumulation in the early stages of digestion and simultaneously reduced the concentration towards the end of the digestion. Such enhancement in the performance of the mixture substrate can be attributed to the buffering ability of the BC. The alkaline nature of the BC helps maintain the nutrient balance, pH balance, and the absorption of hydrogen ions and the inhibiting elements such as K, Na, and Si, which have been reported in earlier studies (Aramrueang et al., 2022; Viaene et al., 2024). The variation in the VFA content in the blends from the present study is shown in Fig. 10. It is clearly evident from the figure that the addition of BC improved the VFA accumulation irrespective of the blends, and also, the degradation was higher. It can be seen that the mixture containing CD 100 % showed the least VFA accumulation and a slower degradation trend, mostly attributed to the nutrient deficiency. The co-digestion improved the acidic compounds formation with the mixture containing CD 30: FW 70 showing an improvement of 13.2 % over the CD 100 % mixture at a 10-day retention time period. The mixture containing the inclusion of RS (CD 30: FW 50: RS 20) showed the maximum value of 29000 mg/L for a 10-day retention period, which was 26.2 % and 11.5 % more compared to the CD 100 % and CD 30: FW 70 blends, respectively.

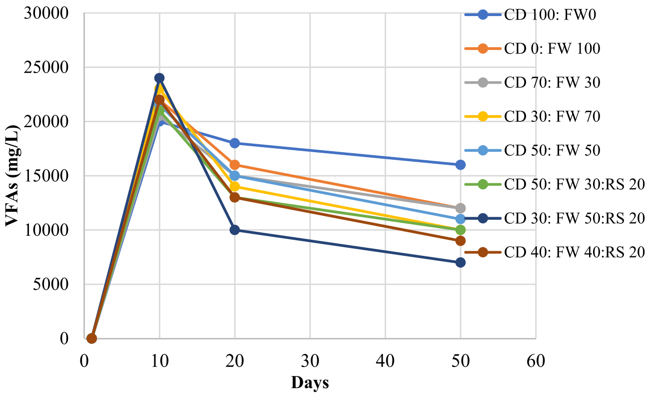

Fig. 9. VFAs in mono and co-digestion blends without BC.

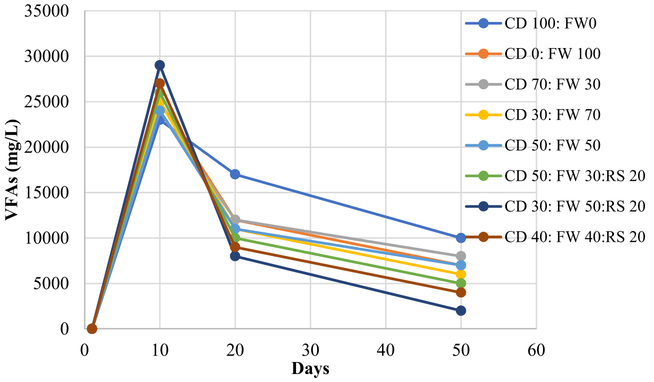

Fig. 10. VFAs in mono and co-digestion blends with BC.

4.5. C/N ratio

Maintaining the C/N ratio in a digester is an important criterion as it directly governs the energy supply and the cell building in microbes. The optimal ranges reported in past studies are 20–30 (Shahbaz et al., 2020; Zheng, Cai, et al., 2021). The high C/N ratio indicated a slowing down of the digestion process, whereas a low C/N ratio can lead to the accumulation of toxic ammonia that inhibits the methanogen from gas conversion. From the present study, the substrate characterization revealed an increase in the nitrogen contents for the FW, which is the reason for the lowering of the C/N ratio. Blends with co-digestion of CD and FW tend to alter the C/N ratio depending on the composition of FW. Hence, monitoring the C/N ratio can help find a relation between the substrate characteristics at any given time and the corresponding methane potential. The variations in the C/N ratio of all the blends over the digestion period can be mapped to the methane production and used to understand the microbial mechanisms. The C/N ratio trends of all the blends with and without BC are shown in Figs. 11 and 12. It can be observed that the C/N ratio of all the blends reduced over time due to the breakdown of carbon. The mixture containing 100 % CD showed the maximum value, which indicated the unavailability of nitrogen for the microbes to break down more organic components (Xiao, Zan, et al., 2022). From Figs. 11 and 12, it can be seen that the addition of BC led to a more stable and controlled reduction in the C/N ratio across all blends, suggesting improved nutrient retention and balanced microbial activity. In blends without BC, the C/N ratio dropped rapidly in the early stages, indicating faster nitrogen loss or inefficient degradation. The stability

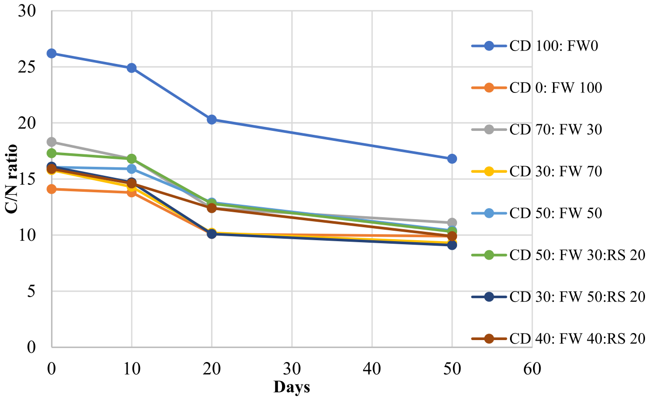

Fig. 11. C/N ratio variation in blends without BC.

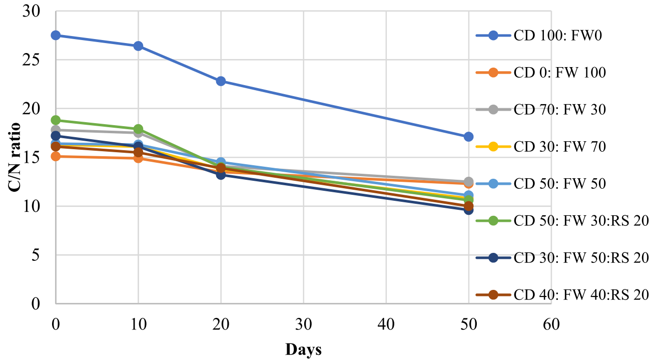

Fig. 12. C/N ratio variation in blends with BC.

can be attributed to BC's buffering capacity and improved microbial activity.

4.6. Methane yield

4.6.1. Without the addition of BC

The methane yield was measured daily throughout the digestion period from the liquid displacement method. The daily methane yield in mL for the CD, FW, CD: FW, and CD: FW: RS blends with and without the addition of BC are shown in Figs. 13 to 16. It was observed that the production of methane began early on in the first day itself and slowly progressed up to an 8-day period due to the initiation of microbial activity. Afterwards, a significant rise in the methane yield was observed up to 20th day. From Fig. 13, it can be seen that the methane yield of co-digestion of CD and FW was more compared to mono-digestion. The 10-day methane yields of CD 100 % and FW 100 % blends were 65.1 mL and 71.58 mL, respectively. The methane yield of the co-digestion blend containing CD 30: FW 70 was observed to be 87.47 mL, which was 34.3 % and 22.19 % more compared to the CD and FW mono-digestion, indicating the effectiveness of co-digestion for maximum methane yield. The peak of methane yield for the 100 % CD mixture was observed to be 111.34 mL in the 18th day, whereas for FW 100 % it was 125.76 mL on the 18th day, and for the CD 30: FW 70 mixture it was 136.26 mL on the 16th day. The observations reveal that co-digestion not only accelerates the methane yield but also helps to achieve maximum yield. The effectiveness of co-digestion has also been reported in past studies. The inclusion of RS in the substrate increased the daily methane yield. The RS content played a pivotal role in the system balance by regulating the pH and nutrient content within the digester (Ngan et al., 2019). The peak yield of methane from blends CD 50: FW 30:RS 20, CD 30: FW 50:RS 20, and CD 40: FW 40:RS 20 were observed to be 135.8 mL, 165.08 mL, and 146.42 mL, respectively. The peak value of CD 30: FW 50:RS 20 mixture was 48.26 %, 31.26 %, and 28.82 % more compared to 100 % CD, 100 % FW, and CD 30: FW 70 mixture. The corresponding cumulative methane yield represented in mL/gVS is shown in Fig. 17.

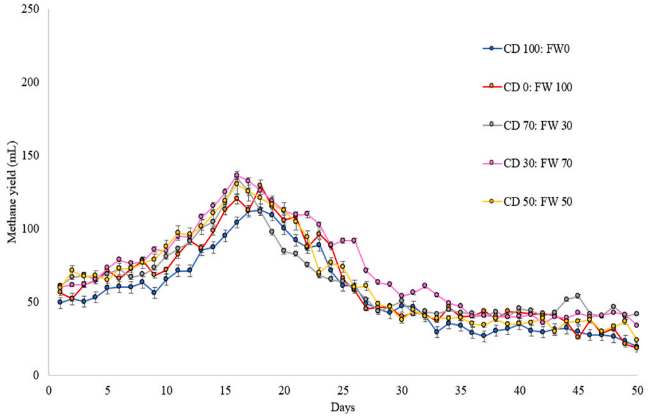

Fig. 13. Daily methane yield in mono and co-digestion blends containing CD and FW (without BC).

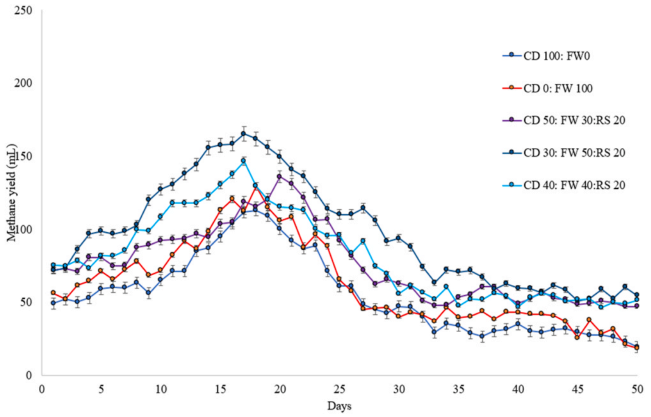

Fig. 14. Daily methane yield in mono and co-digestion blends containing CD, FW, and RS (without BC).

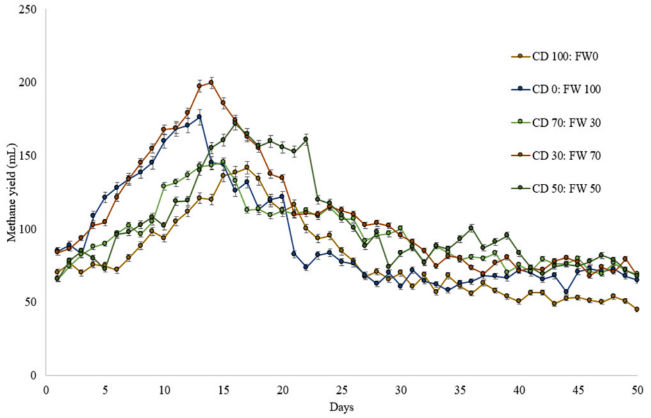

Fig. 15. Daily methane yield in mono and co-digestion blends containing CD and FW (with BC).

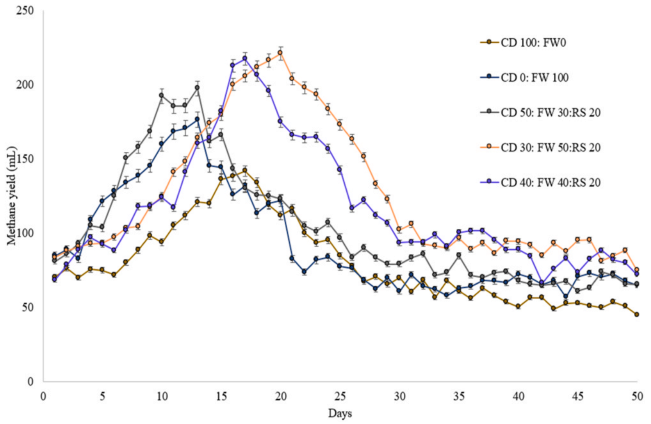

Fig. 16. Daily methane yield in mono and co-digestion blends containing CD, FW, and RS (with BC).

4.6.2. With the addition of BC

The addition of BC plays a pivotal role in the anaerobic digestion of substrates. The methane yield of blends with BC were significantly

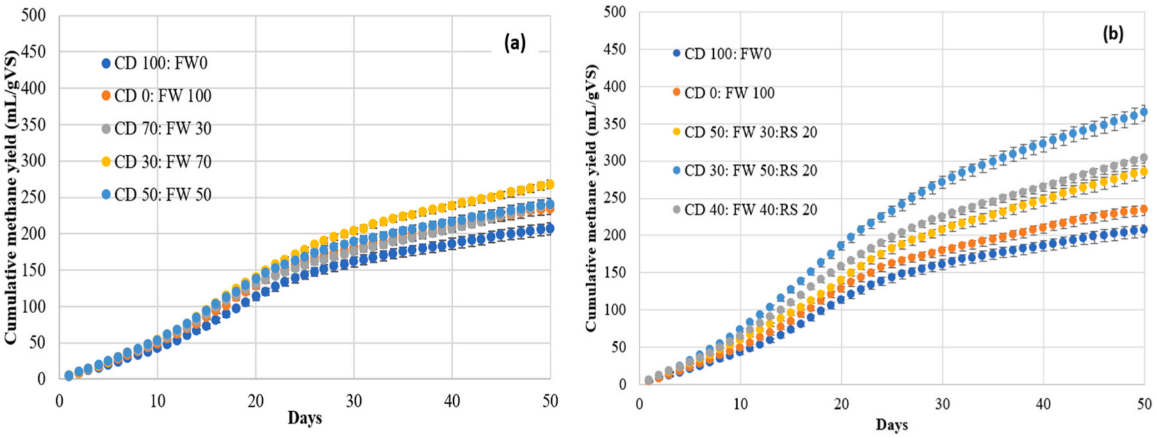

Fig. 17. Cumulative methane yield in mono and co-digestion blends containing (a) CD and FW (without BC); (b) CD, FW, and RS (without BC).

higher than that of the blends without BC. The intrinsic physicochemical properties of BC assist in the degradation process by providing a colonization area for the microbes. The porous and high surface area of the BC provides nucleation sites for the electron transfer, and also the functional groups of BC help microbes stick together and multiply (Wambugu et al., 2019). Further, the BC helps in adsorbing the impeding intermediate compounds such as NH_3 for the effective digestion process. By adsorbing such inhibitory compounds, the BC increases the microbial activity and maintains the chemical balance essential for maximum methane yield. Furthermore, the addition of BC creates an alkaline environment that helps maintain the nutrient balance, pH balance, and the absorption of hydrogen ions and the inhibiting elements such as K, Na, and Si, thereby improving the methane yield (Wang et al., 2017). The daily methane yield of the blends from the present study with the addition of BC is shown in Figs. 15 and 16. From the figure, it can be seen that the methane potential of all the blends improved significantly with the addition of BC. The 10-day methane yields of CD 100 % and FW 100 % blends were 93.78 mL and 159.72 mL, respectively. It can be seen that the FW performance improved by 123 % by the addition of BC. The methane yield of the co-digestion mixture containing CD 30: FW 70 was observed to be 167.6 mL, which was 78.7 % and 4,9 % more compared to the CD and FW mono digestion, indicating the effectiveness of co-digestion for maximum methane yield. The peak of methane yield for the 100 % CD mixture was observed to be 141.74 mL on the 17th day, whereas for FW 100 % it was 176.23 mL on the 13th day, and for the CD 30: FW 70 mixture it was 199.62 mL on the 14th day. The corresponding cumulative methane yield of mono and co-digestion represented in mL/gVS is shown in Fig. 18 (a). The inclusion of RS in the substrate along with BC increased the daily methane yield. The peak yield of methane from blends CD 50: FW 30:RS 20, CD 30: FW 50:RS 20, and CD 40: FW 40:RS 20 were observed to be 197.44 mL, 221.08 mL, and 217.35 mL, respectively. The peak value of CD 30: FW 50:RS 20 mixture was 55.9 %, 25.44 %, and 10.75 % more compared to 100 % CD, 100 % FW, and CD 30: FW 70 mixture. The corresponding cumulative methane yield represented in mL/gVS is shown in Fig. 18 (b). Hence, the present study highlights the improvement in the biogas efficiency from the addition of BC.

4.7. Statistical analysis using RSM technique

The crucial parameters involved in methane production are represented as categorical input factors. The central composite design (CCD) introduced by Box and Wilson was employed to optimize the factors involved in the effective methane production (Box and Wilson, 1951). The independent variables, such as pH, VS, VFA, C/N, and mixture ratios from the present study, are the categorical factors, and methane yield was chosen as the output variable. By assigning the axial central points to the variables, the model estimates the independent, linear interaction and quadratic relation between the factors (Szpisják-Gulyás et al., 2023). The Stat Ease design expert software tool was used to develop the design of experiments based on the categorical factors and output variable.

The RSM model was developed to optimize the methane yield of substrates of a wide range by assessing only the influence of pH, VS, VFA, and C/N ratio on the methane yield. The quadratic model developed from the RSM analysis is given in Eqa. 5. The model predicted the

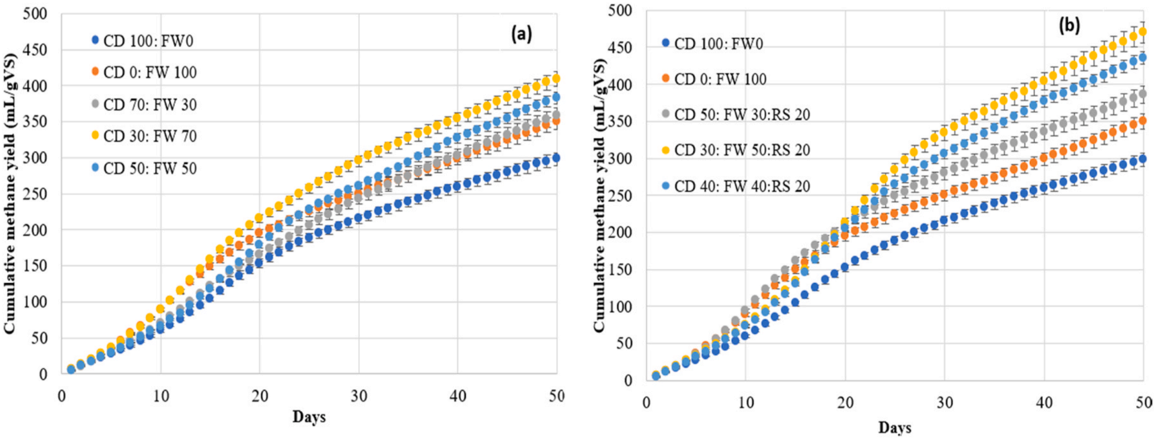

Fig. 18. Cumulative methane yield in mono and co-digestion blends containing (a) CD and FW (with BC); (b) CD, FW, and RS (with BC).

response of methane yield with a higher degree of accuracy. The coded coefficients and ANOVA results are given in Supplementary Table S1. Based on the analysis the standardized influential parameter plot, residual plot, fit analysis plot and error frequency plot were developed and can be found in Fig. S1 in the supplementary material. From the figure, it can be seen that almost 90 % of the standardized residuals lies between -20 and $+ 20$ mL/gVS, indicating that there are very limited outliers or extreme residual values.

$$\text{Methane (mL/gVS)} = -4404 + 8.67\ \text{Days} + 1270\ \text{pH} + 35.9\ \text{VS} - 0.01850\ \text{VFA} - 58.2\ \text{C/N} - 0.0833\ \text{Days*Days} - 59.94\ \text{pH*pH} - 0.029\ \text{VS*VS} + 0.000000\ \text{VFA*VFA} + 0.1270\ \text{C/N*C/N} - 1.515\ \text{Days*pH} + 0.1333\ \text{Days*VS} - 0.000475\ \text{Days*VFA} + 0.4011\ \text{Days*C/N} - 7.71\ \text{pH*VS} + 0.000545\ \text{pH*VFA} + 1.11\ \text{pH*C/N} + 0.000167\ \text{VS*VFA} + 0.575\ \text{VS*C/N} - 0.000050\ \text{VFA*C/N} \ldots. \quad (5)$$

4.8. *Gompertz kinetic model*

The modified Gompertz equation can be used to effectively estimate the biochemical methane potential at any given time “t”. Many studies have reported the accuracy and reliability of the kinetic model in predicting the methane potential. The kinetic parameters, such as lag phase time, methane production rate, and maximum methane potential, obtained from the present study for all the blends with and without the addition of biochar are presented in Table 5.

Table 5
Kinetic parameter for the anaerobic digestion of substrates with and without BC.

Mixture ratios	Lag phase, λ (days)	Methane production rate, μ_m (mL/gVS)	Maximum methane potential, MP_{max} (mL/gVS)
CD 100: FW0 (without biochar)	1.75	4.78	206.61
CD 0: FW 100 (without biochar)	1.24	5.41	233.83
CD 70: FW 30 (without biochar)	1.6	5.50	239.74
CD 30: FW 70 (without biochar)	0.92	6.04	267.82
CD 50: FW 50 (without biochar)	1.3	5.74	240.90
CD 50: FW 30:RS 20 (without biochar)	1.55	6.31	284.96
CD 30: FW 50:RS 20 (without biochar)	0.73	8.03	364.34
CD 40: FW 40:RS 20 (without biochar)	1.41	6.35	302.78
CD 100: FW0 (with biochar)	1.65	6.59	298.04
CD 0: FW 100 (with biochar)	1.03	8.23	349.61
CD 70: FW 30 (with biochar)	2.82	7.46	358.06
CD 30: FW 70 (with biochar)	1.89	9.15	409.18
CD 50: FW 50 (with biochar)	2.28	7.82	382.79
CD 50: FW 30:RS 20 (with biochar)	2.38	8.92	385.59
CD 30: FW 50:RS 20 (with biochar)	1.24	9.49	469.72
CD 40: FW 40:RS 20 (with biochar)	2.17	8.87	435.48

The performance of the RSM model with pH, VS, VFA, and C/N ratio as the input parameters is compared with the Gompertz kinetic model. The performance of both models was evaluated by the error metrics R, and RMSE. The experimental and predicted methane yield was compared for both the RSM and Gompertz kinetic models. The 800 data points plotted in the graph representing the deviations from the line of symmetry are shown in Supplementary Fig. S2. From the figure, two distinctive observations are seen. 1) The Gompertz kinetic model has a higher degree of accuracy in the initial stages of the digestion, and the data points beyond 100 mL/gVS show significant deviations from the line of symmetry. 2) The RSM model performs well in all the stages except for some data points with low methane yield, which can be seen in the lower left corner of the graph (see Supplementary Fig. S2). The linear trendline equation is shown along with the coefficient of determination, which indicates the accuracy of the RSM model. The average residual error for RSM and the Gompertz kinetic model was found to be 0.179 and 0.239, respectively. The ranges of the errors for both models were within the ranges of -1 to $+ 4$ % (see Supplementary Fig. S3). The methane potential prediction accuracy of the RSM model from the present study is significantly higher than the modified Gompertz model from another study containing water hyacinth (Quintana-Najera et al., 2022). Also, the performance evaluation error metrics R and RMSE results were observed to be 0.966, 0.925, and 62.89 mL/gVS, 87.24 mL/gVS for RSM and Gompertz model, respectively, shown in Fig. 22.

5. Conclusions

Based on the experimental program carried out on the digestate containing various proportions of CD, FW, and RW with and without the addition of BC, the following conclusions were drawn.

- Co-digesting various substrates enhances biogas production due to the positive interactions that occur within the digestion process, the increased microbial diversity from the different substrates, and the improved carbon-nitrogen nutrient balance facilitated by the co-digestion.
- Biochar addition as a co-digestor has great potential in improving the VFA concentrations and methane yield due to its porous microstructure, enabling the acclimation of microbes.
- The TS concentrations in the blends were between 10 % and 20 %, which were crucial for the microbial cellular metabolism and reproduction, as it govern the mass transfer between microbes and surrounding nutrients. The VS % levels were found to be between 75 % and 95 % of the TS. The VS levels increased for the blends containing FW.
- The pH values for all the blends containing CD and FW varied consistently within the acidic ranges. The addition of RS significantly

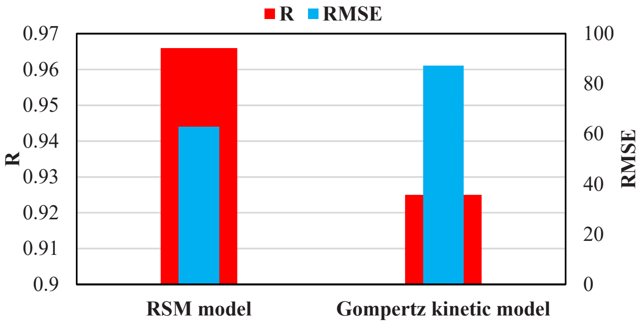

Fig. 22. Performance evaluation error metrics R and RMSE.

balanced the pH range throughout the digestion process. The ash content in the biochar help regulate the pH levels by transforming the intermediates such as H_2S and CO_2.

- The mixing of RS in the substrate improved the pH levels, which improved the thriving environment for the microbes, which ultimately led to more VS consumption and gas conversion. The mixture of CD 30: FW 50: RS 20 exhibited pH imbalances in the initial stages due to the rapid acid accumulation caused by acidogens. However, after 15 days of digestion, a significant and steady decline in volatile solids (VS) was observed, with a 30 % reduction when comparing the start and end of the anaerobic digestion process.
- The co-digestion improved the acidic compounds formation with the mixture containing CD 30: FW 70, showing an improvement of 15 % over the CD 100 % mixture in a 10-day retention time.
- The VFA accumulation in the mixture containing CD 30: FW 50:RS 20 showed the maximum value for a 10-day retention period, which was 20 % and 15 % more compared to the CD 100 % and CD 30: FW 70 blends.
- The methane yield of the co-digestion mixture containing CD 30: FW 70 was observed to be 87.47 mL, which was 34.3 % and 22.19 % more compared to the CD and FW mono-digestion, indicating the effectiveness of co-digestion for maximum methane yield.
- The methane yield of blends with BC was significantly higher than that of the blends without BC. The intrinsic physicochemical properties of BC assist in the degradation process by providing a colonization area for the microbes. The porous and high surface area of the BC provides nucleation sites for the electron transfer, and also the functional groups pf BC help microbes stick together and multiply.
- The quadratic model developed from the RSM analysis predicted the response methane yield in mL/gVS with a higher degree of accuracy and a significance level of 0.05. The coefficient of determination of the model was observed to be 99.07 %.
- The comparison of the RSM model and Gompertz kinetic model revealed two key inferences: (1) The Gompertz kinetic model exhibits higher accuracy during the early stages of digestion, but the data points beyond 100 mL/gVS show notable deviations from the symmetry line. (2) The RSM model performs well throughout all stages, except for a few data points with low methane yield.

The study shows improved methane yield and VFA accumulation through the novel integration of BC in co-digestion systems using CD, FW, and RS. It demonstrates in a unique way how BC's porous structure contributes to electron transfer and microbial enrichment. The application of both RSM and Gompertz models provides a comprehensive predictive understanding of the dynamics of methane generation. The optimized blend with BC achieved significant improvement in methane yield over mono-digestion, underscoring the synergy in substrate co-digestion.

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CRediT authorship contribution statement

R Saravanane: Writing – review & editing, Validation, Supervision, Project administration, Formal analysis, Conceptualization. **B Sankar:** Writing – review & editing, Software, Resources. **S Mohan:** Writing – review & editing, Validation, Project administration, Formal analysis. **P Sivakumar:** Writing – original draft, Methodology, Investigation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.clwas.2025.100388.

Data availability

Data will be made available on request.

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