



Enhancement of biomethane yield rate in anaerobic co-digestion of cattle dung and untreated vegetable waste through the amendment of water-hyacinth biochar

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ABSTRACT

This study investigates the effect of biochar (BC) amendment on the anaerobic co-digestion (AcoD) of cattle dung (CD) and untreated vegetable waste (VW) performed at different co-substrates mixing ratios (CMR) of 70:30, 60:40, 50:50, 40:60, and 30:70 under ambient and mesophilic conditions. The mesophilic BC-added digester with a CMR of 60:40 exhibited 13.19 % and 39.26 % higher cumulative methane yield (CMY) than the corresponding uncontrolled digesters (CMR 60:40) with and without BC-added over 45 days of experiments. Subsequently, considering CMR 60:40, another group of biochemical methane potential experiments was conducted at ambient conditions, incorporating varying amounts of BC, viz. 5, 10, 15, 20, and 25 gL⁻¹. The digester comprising 15 gL⁻¹ BC achieved the highest CMY, recommending it as the optimal amount of BC addition for the AcoD of CD and VW. This study discloses that BC addition is a significant approach to increasing CH₄ yield.

1. Introduction

Open dumping of biodegradable waste, such as cattle dung (CD) and vegetable waste (VW), significantly impacts the environment. It contaminates soil and water through leachate generation and contributes to air pollution by releasing hazardous gases (Ren et al., 2018). The primary greenhouse gases (GHG) released from CD and VW are carbon dioxide (CO₂), methane (CH₄), dinitrogen oxide and fluorinated compounds, contributing to weather change. US Environmental Protection Agency reported worldwide food waste shares around 8 % of anthropogenic GHGs (U.S. EPA, 2021). Likewise, the livestock share roughly 18 % of GHGs globally. Further, livestock is responsible for the generations of agricultural CH₄ (80 %) and anthropogenic CH₄ (35 %), which is equivalent to 2.2 billion tons of CO₂ (Sejian et al., 2016). In this regard, the United Nations (UN) takes various steps to reduce global warming. UN Climate Change Conference (COP21) held in Paris, France, aims to put efforts to limit warming to 1.5 °C above pre-industrial levels, and substantial emission reductions must be accomplished worldwide by 2030 (COP28, 2023).

Among the several approaches for lowering emissions, anaerobic digestion (AD) can also be recognised as an appropriate and adaptable

technology for treating biodegradable waste products and harnessing renewable energy. It also offers a cost-effective and sustainable solution for lowering emissions. The AD process produces biogas via microorganisms in an oxygen-free environment. However, microbial activities and growth are influenced by many factors, such as the substrates' degradability, temperature, alkalinity, and mineral concentrations (Basumatary et al., 2024). Moreover, biogas generation relies on feedstocks' total solid (TSC) and volatile solid (VSC) contents. Thus, preparing the slurry within the desired range is essential to achieve a higher biogas yield. Additionally, blending additive materials has vital benefits for improving the AD system.

Various investigators studied the impact of biochar (BC) incorporation on AD of organic waste. BC is a carbon-rich material produced by pyrolysing, gasifying, or hydrothermal carbonising biomass without oxygen (Ighalo et al., 2023). Earlier studies reported that blending BC with anaerobic substrates improves the performance of the AD process. Adding an optimum quantity of BC increases the CH₄ generation rate, enhances operational stability, accelerates the evolution of anaerobic bacteria, immobilises microorganisms, and improves biogas quality (Chen et al., 2023; Zhao et al., 2021). The key benefits realised in the AD system demonstrate that BC possesses robust physical and chemical

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Table 1
Experimental design for co-digestion.

| Co-substrate mixing ratio | CD: VW (m/m ^a) (i) | CD (g) (ii) | VW (g) (iii) | Water (g) (iv) | Total (g) (v) = Σ (ii) + (iii) + (iv) |
|---------------------------|--------------------------------|-------------|--------------|----------------|----------------------------------------------|
| CMR 70:30 | 70:30 | 245 | 105 | 350 | 700 |
| CMR 60:40 | 60:40 | 210 | 140 | 350 | 700 |
| CMR 50:50 | 50:50 | 175 | 175 | 350 | 700 |
| CMR 40:60 | 40:60 | 140 | 210 | 350 | 700 |
| CMR 30:70 | 30:70 | 105 | 245 | 350 | 700 |

^a m/m stands for mass by mass basis, g stands for gram.

properties that enhance the AD process. Recent studies have reported that BC is a highly porous material that provides colonisation space for microorganisms (Li et al., 2022). Additionally, several functional groups, alkaline elements, and interspecies electron exchange capacity of BC boost the biogas generation in the anaerobic co-digestion (AcoD) process (Kumar et al., 2021). The BC can mitigate the generation of ammonia and volatile fatty acids, preventing AcoD failure (Sun et al., 2019). The performance of AcoD with BC is influenced not only by the physico-chemical properties of BC but also by the types of substrates and their mixing ratio.

Even though numerous studies have examined the AcoD of CD and untreated VW, there is limited literature on the viability of adding water hyacinth biochar (WHBC) in the co-digestion, particularly with varying CD and VW mixing ratios. The BC derived from various feedstocks may significantly differ in its effectiveness in enhancing AD performance. As water hyacinth is available in the North-East India, this substrate is considered for the preparation of BC in the present study. Compared with other BC prepared from available biomass such as tree leaves (Sahota et al., 2018), citrus peel BC (Jiang et al., 2020) and chicken manure (Pan et al., 2019), WHBC was found to have a higher surface area and pore size. The low hydrogen/carbon (H/C) ratio in the WHBC could be a favourable property to enhance CH₄ production upon its addition to AcoD. Furthermore, a highly alkaline nature of WHBC indicates its hydrophobicity and is a desirable property of adsorption capacity to mitigate inhibition and attract microbes to the BC surface. Thus, use of WHBC in the AcoD will be beneficial. This study conducted laboratory-scale AcoD experiments using the different co-substrate mixing ratios (CMR) of CD and untreated VW with and without the blending of WHBC. The primary goals of the current investigations are (i) to identify the optimal CMR for AcoD of CD and VW, (ii) to study the effect of WHBC incorporation on AcoD of CD and VW, and (iii) to evaluate the most appropriate amount of WHBC to be added for maximising CH₄ production. Further, a prediction of the CH₄ generation potential was executed using a modified Gompertz model. The impact of operating parameters, including pH value and temperature, on CH₄ generation rate was also studied.

2. Materials and methods

2.1. Feedstock collection, preparation and characterisation

The feedstocks employed in AcoD were CD and VW. The VW was obtained from the dining hall of a hostel at IIT Guwahati, India. The

Table 2
Characterisation of co-substrates and their slurries.

| Parameters | Raw CD | Raw VW | Co-substrates mixing ratio | | | | |
|------------|--------------|--------------|----------------------------|--------------|--------------|--------------|--------------|
| | | | CMR 70:30 | CMR 60:40 | CMR 50:50 | CMR 40:60 | CMR 30:70 |
| MC (%) | 84.08 ± 0.26 | 91.65 ± 0.02 | 91.77 ± 0.09 | 92.14 ± 0.07 | 92.86 ± 0.04 | 93.31 ± 0.10 | 94.21 ± 0.15 |
| TS (%) | 15.92 ± 0.17 | 8.37 ± 0.02 | 8.23 ± 0.09 | 7.86 ± 0.06 | 7.14 ± 0.04 | 6.69 ± 0.09 | 5.79 ± 0.15 |
| VS (%TS) | 83.50 ± 0.10 | 94.25 ± 0.14 | 74.70 ± 0.17 | 75.93 ± 0.11 | 77.00 ± 0.03 | 81.06 ± 0.05 | 83.32 ± 0.06 |
| C/N ratio | 25.82 ± 1.10 | 18.75 ± 0.29 | 22.81 ± 0.13 | 21.92 ± 1.02 | 21.26 ± 0.54 | 20.52 ± 1.06 | 19.14 ± 0.25 |
| pH | 6.39 ± 0.09 | 4.31 ± 0.05 | 5.98 ± 0.09 | 5.76 ± 0.10 | 5.21 ± 0.08 | 5.03 ± 0.06 | 4.66 ± 0.05 |

main components of untreated VW were green mustard leaves, eggplants, potatoes, cabbages, long gourds, radish leaves, and other vegetables. Before being introduced into the digester for AcoD, the VW was ground using a mixer grinder (Bajaj Model: GX 8750 W) and stored at a temperature of 4 °C. The CD was gathered from a farm near the institute. Fresh WH was obtained from the IIT Guwahati campus and cleaned with water before being chopped into small pieces (about 3 mm). After natural drying for 5–6 days, it was put in an oven (Optics Technology, India) for 24 h at 105 ± 2 °C to eliminate wetness. Subsequently, BC was prepared through a pyrolyser (Das and Co., India) at 550 °C, setting the heating rate at 10 °C min⁻¹ for 1 h by supplying argon gas to make the reactor oxygen-free (Basumatary et al., 2024). Before being added to digesters, the WHBC was ground using a mixer grinder until it could pass through a mesh size sieve of 100 μm.

The MC, TSC and VSC of substrates were evaluated employing the standard methods reported by APHA (2017). The ultimate analysis of

Table 3
Physico-chemical properties of WHBC.

| Investigation | Parameter | Unit | Value |
|------------------------|------------------|--------------------|--------------|
| Proximate analysis | MC | % | 3.82 ± 0.43 |
| | AC | % | 19.59 ± 0.27 |
| | VM | % | 13.64 ± 0.14 |
| | FC | % | 62.96 ± 0.05 |
| CHNSO study | C | wt% | 41.35 ± 0.11 |
| | H | wt% | 1.67 ± 0.08 |
| | N | wt% | 3.34 ± 0.06 |
| | S | wt% | 0.52 ± 0.03 |
| | O | wt% | 53.11 ± 0.10 |
| | H/C | – | 0.04 ± 0.00 |
| | O/C | – | 1.28 ± 0.03 |
| AAE metals | K | wt% | 9.47 ± 0.02 |
| | Ca | wt% | 5.73 ± 0.09 |
| | Na | wt% | 1.41 ± 0.07 |
| | Mg | wt% | 1.11 ± 0.08 |
| Others elements | P | wt% | 3.12 ± 0.09 |
| | Cl | wt% | 5.23 ± 0.10 |
| Physisorption isotherm | S _{BET} | m ² /g | 14.10 ± 0.11 |
| | V _p | cm ³ /g | 0.05 ± 0.01 |
| | d _p | nm | 11.94 ± 0.05 |
| Others analysis | pH | – | 10.07 ± 0.06 |
| | EC | dS/m | 33.28 ± 0.85 |

Table 4
Functional group of FT-IR analysis for WHBC.

| Bond | Functional group | Frequency from this study (cm ⁻¹) | Frequency from the literature (cm ⁻¹) | Reference |
|------|---------------------|-----------------------------------------------|---------------------------------------------------|-------------------------|
| O–H | Alcohol, phenol | 3610 | 3650–3600 | NIU (2023) |
| C=C | Alkene, aromatic | 1409 | 1700–1400 | Shen et al. (2015) |
| C–O | Alcohol | 1037 | 1200–1000 | Domingues et al. (2017) |
| C–H | Aromatic | 874 | 900–690 | Shen et al. (2015) |
| C–H | Aliphatic, aromatic | 562 | – | Shen et al. (2015) |

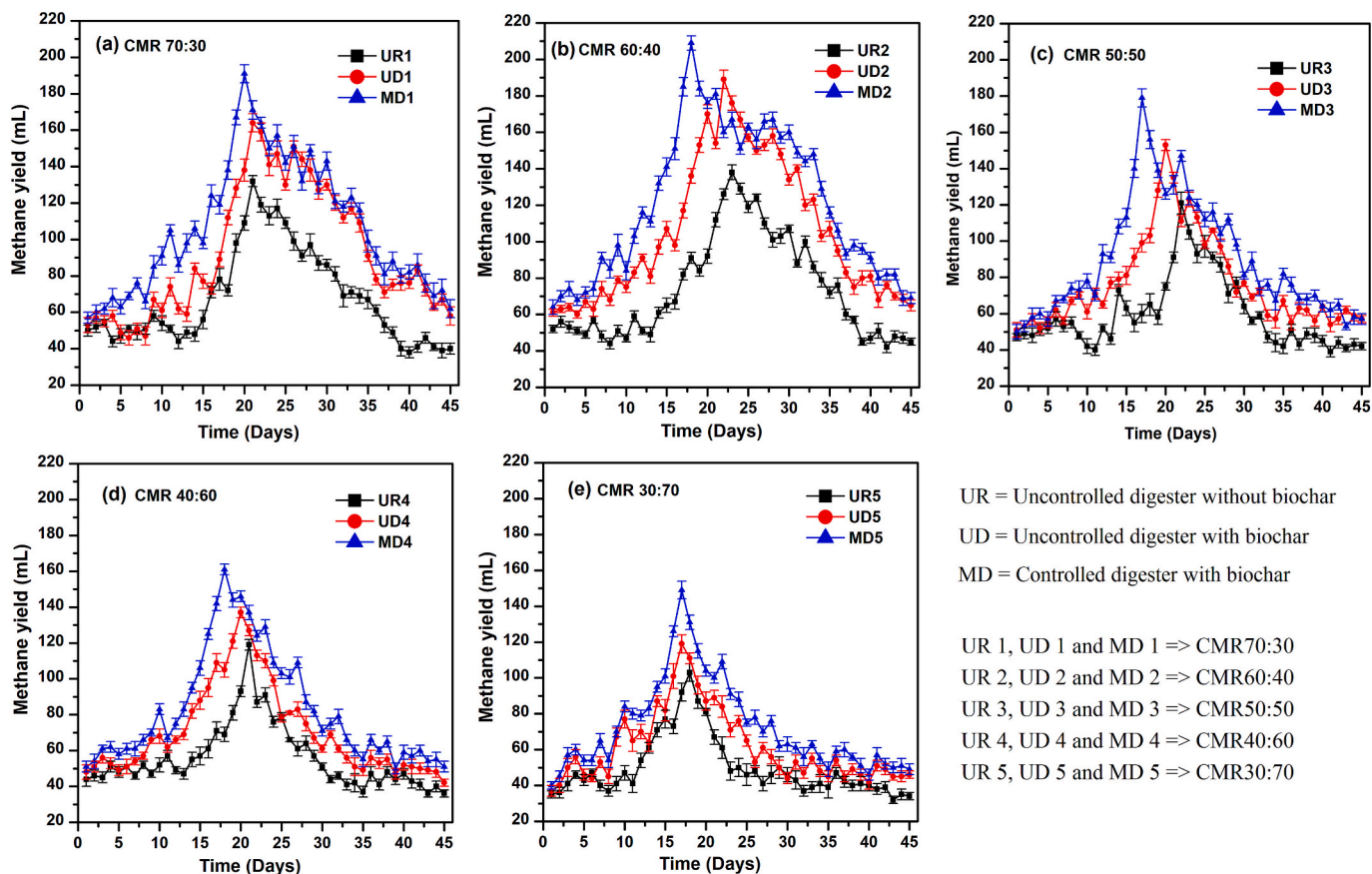


Fig. 1. Daily CH₄ yield from the digesters with CMRs of (a) 70:30, (b) 60:40, (c) 50:50, (d) 40:60, and (e) 30:70.

the substrates and BC was performed according to ASTM: D5373-16 using a CHNSO analyser (Euro EA, Italy). The proximate analysis of BC was performed using ASTM methods viz. E871-82, D1102-84, and E872-82 to evaluate MC, ash content (AC), and volatile matter (Basumatary et al., 2024). The fixed carbon (FC) was assessed by calculating the weight difference. The morphological and microstructure of BC were inspected by a scanning electron microscope (SEM) (Zeiss, Germany). The BET surface area (S_{BET}), pore size (d_p), and pore volume (V_p) of BC were analysed with a Brunauer–Emmet–Teller (BET) analyser (Quantachrome Instruments, USA). The BC was degassed for 5 h at 200 °C prior to analysis. To assess the presence of mineral matters such as alkali and alkaline earth (AAE) elements in biochar, energy-dispersive X-ray spectroscopy (EDX) (Zeiss, Germany) was employed. The X-ray diffractometer (XRD) (Rigaku Technologies, Japan) was used to identify the crystalline compounds of BC. The scanning range (2 θ) was set between 10° to 70° with a scanning rate of 15°/min. Further, the surface functional groups of BC samples were assessed by a Fourier transform infrared (FT-IR) spectrometer (PerkinElmer, Singapore, Model: Spectrum two) in the 4000–400 cm⁻¹ wave range. The alkalinity level of WHBC was measured by referring to methods illustrated by Liu et al. (2022) using a pH analyser.

2.2. Biochemical methane potential (BMP) experimental set up

Initially, three sets of BMP tests were conducted at different CMRs of CD: VW, such as 70:30, 60:40, 50:50, 40:60, and 30:70 on a wet mass basis parallelly in triplicate, as depicted in Table 1. The objective of selecting the different CMRs was to determine the optimal CMR for achieving the highest methane yield. The BMP test was performed according to the methodology outlined by Basumatary et al. (2024). The schematic diagram and photograph of BMP tests (Fig. S1) are

incorporated in the supplementary materials. The feedstock and distilled water were mixed in a 1:1 ratio. No inoculum was added in the BMP tests, considering CD as the active feedstock. Entire BMP tests were conducted under uncontrolled and controlled temperatures for 45 days. In the set I experiment, no BC was used. However, 10 g of BC, prepared from WH at the pyrolysis temperature of 550 °C, was added arbitrarily to each of the digesters in sets II and III. Additionally, sets I and II experiments were performed at uncontrolled (ambient) temperature, while the temperature of set III was maintained at 37 ± 2 °C using a hot water bath (Equitron Medica, India). Hereafter, considering the best CMR detected in sets I, II and III, another set of the experiment (set IV) was carried out by blending varying quantities of BC (in gL⁻¹): 5, 10, 15, 20, and 25 to recognise the appropriate amount of BC required to be mixed for highest methane production.

2.3. Modified Gompertz model analysis

The CH₄ generation potential from the different CMRs of CD and VW was determined by the modified Gompertz model (MGM) equation. Several researchers have acknowledged the MGM equation as a precise prediction analysis for CH₄ generation potential (Syaichurrozi, 2018; Tian et al., 2020). The MGM equation demonstrates the highest accuracy in predicting cumulative CH₄ yield potential, CH₄ yield rate, and the time required to achieve the highest CH₄ yield compared to other kinetic models. The model parameters were evaluated by curve-fitting the BMP test data (Zhang and Wang, 2021) of Y_C and t using Matlab R2022b, as depicted in Eq. (1).

$$Y_C = Y_p \exp \left[- \exp \left\{ \frac{Y_R \cdot e}{Y_p} (\lambda - t) + 1 \right\} \right] \quad (1)$$

where Y_C , Y_p , Y_R , λ and e designate the accumulative specific methane

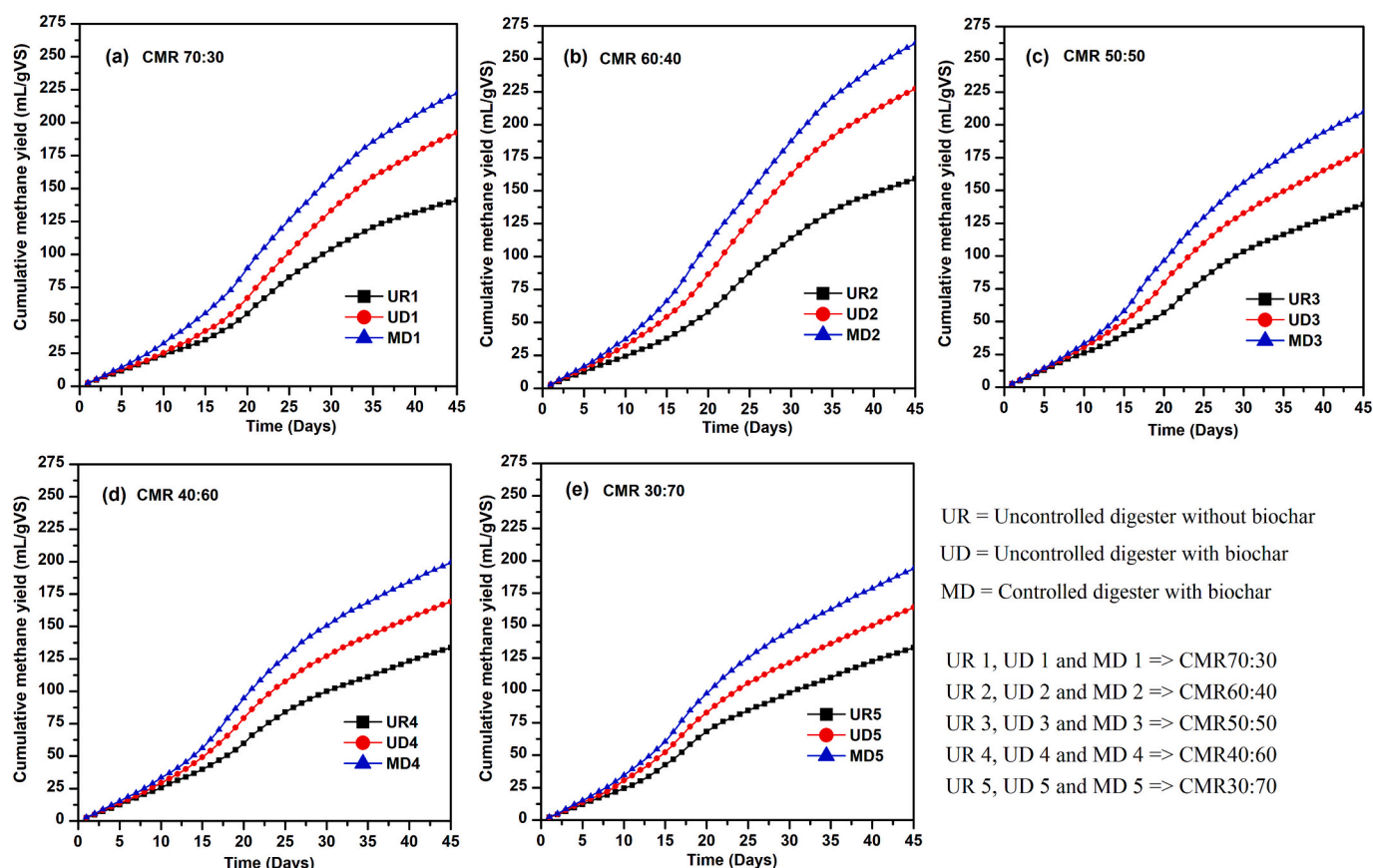


Fig. 2. Cumulative CH₄ yield from the digesters with CMRs of (a) 70:30, (b) 60:40, (c) 50:50, (d) 40:60, and (e) 30:70.

Table 5

Impact of biochar addition on improving AD performance.

| Reference | Substrates | Temperature and HRT of the AD process | Type of biochar | Pyrolysis temperature | Dosage of BC | Performance |
|----------------------|-------------------|---------------------------------------|--------------------|-----------------------|-----------------|------------------------------------------|
| Suthar et al. (2022) | CD | 35 °C, 35 days | Water hyacinth | 350 °C | 0.5–1.5 % (v/v) | CH ₄ increased by 9.33–19.8 % |
| Sun et al. (2022) | FW | 37 °C, 30 days | Corn straw | 600 °C | 10 g/L | CMY increased by 22.90 % |
| Wang et al. (2024) | KW | 37 °C, 90 days | Straw digestate | 500 °C | 15 g/L | CH ₄ increased by 20.8 % |
| Li et al. (2022) | CS and SS (1:1) | 36 °C, 30 days | Corn straw | 500 °C | 10 g/L | CMY increased by 7.96 % |
| Kaur et al. (2020) | SS and FW (1:7) | 35 °C, 30 days | Wheat straw pellet | 550 °C | 10 g/L | CMY increased by 24.59 % |
| This study | CD and VW (60:40) | 37 °C, 45 days | Water hyacinth | 550 °C | 10 g/L | CMY increased by 39.26 % |

Where CD: Cattle dung, FW: Food waste, KW: Kitchen waste, CS: Corn straw, SS: Sewage sludge, VW: Vegetable waste, and CMY: Cumulative CH₄ yield.

yield (SMY) at the given time (t) in days (d), highest CH₄ yield potential, highest CH₄ generation rate, lag phase time, and $e = 2.7183$, a constant, respectively.

3. Results and discussions

3.1. Characterisation of substrates and their slurries

Table 2 illustrates the MC, TSC, VSC, and pH values of raw CD, raw VW and their slurries prepared at different CMRs viz. 70:30, 60:40, 50:50, 40:60, and 30:70. Evaluating these parameters is essential to identify the appropriateness of a feedstock for anaerobic digestion, as they extensively impact the performance of the AcoD process and, subsequently, CH₄ production. In this investigation, the MC of CD and VW were 84.08 ± 0.26 % and 91.65 ± 0.02 %, respectively, whereas for the slurries with different CMR, the value of MC ranged from 91.70 % to 94.21 %. A significant amount of MC in the slurry supports

microorganisms during the hydrolysis and acidogenesis stages. Insufficient moisture levels can hinder the AD process by promoting acid accumulation (Odejobi et al., 2021; Osei-Owusu et al., 2023). Conversely, higher MC in the feedstock enhances microbial activity in anaerobic digestion (Lay et al., 1997). Previous scientific studies have highlighted the substantial impact of TSC on the AcoD of waste biomass (Indren et al., 2020; Johnravindar et al., 2022). High TSC can inhibit mass transfer between anaerobic bacteria and feedstock, reducing CH₄ generation (Johnravindar et al., 2022). Abbassi-Guendouz et al. (2012) witnessed a higher CH₄ yield rate for TSC of 10 %, which declined as the TSC increased from 10 % to 30 %. In this study, TSC for various CMRs of CD and VW ranged from 5.90 to 8.23 %, while TSC for raw CD and raw VW was 15.90 % and 8.37 %. The TSC of cattle dung was achieved to be consistent with the outcome found by Wang et al. (2022), whereas the TSC for vegetable waste was consistent with the result described by Bouallagui et al. (2009). The VS (% TS) value signifies that the substrates comprising degradable materials can be transformed into biogas. The VS

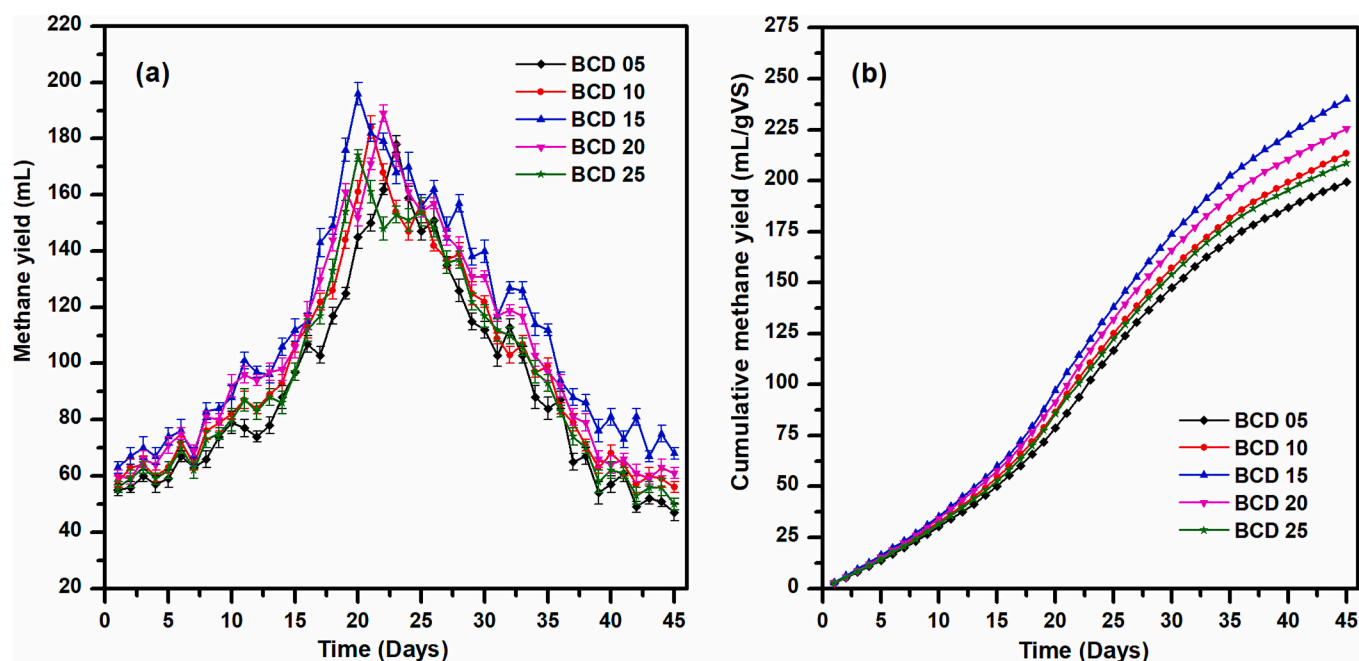


Fig. 3. (a) DMY and (b) CMY from the digesters with the addition of different quantities of BC.

(% TS) value for different CMRs was detected to be 74.70–83.32 %. Further, the pH value of the slurry reduced from 5.98 to 4.66 due to the addition of a higher amount of VW, which was below the desired range. Therefore, improvement of pH value is essential for the growth of microorganisms. This study used biochar prepared from water hyacinth to enhance the pH value. The outcome of BC addition to the AcoD process is elaborated in the following section. The C/N ratio is crucial in determining the level of nutrients for anaerobic bacteria. For the feedstocks with a high C/N ratio, the methanogens consume nitrogen rapidly, which results in lower biogas yield. On the other hand, the feedstocks with an excessively low C/N ratio increase the risk of ammonia inhibition, which is toxic to methanogens and leads to inefficient utilization of carbon sources (Kothari et al., 2014; Mao et al., 2015). Hence, maintaining C/N ratios within the optimal range is essential for achieving higher methane yields. In this study, the C/N ratio for CD and VW were 25.82 ± 1.10 and 18.75 ± 1.07 , whereas, for slurry, their values were found in the range of 19.00–23.00 for different mixing ratios. The C/N ratio of the current study was obtained nearly in the optimum range of 20–30, as its value was high for cattle dung. Therefore, the AcoD of co-substrates with low and high C/N ratios can improve anaerobic digestion.

3.2. Results of characterisation of biochar

The physicochemical properties and structures of BC effect the AcoD process (Ovi et al., 2022). Table 3 illustrates the characterisation of BC. The pH level of WHBC was in the alkaline range (10.07 ± 0.06) due to the existence of AC and volatilisation of acidic functional groups (Kumar et al., 2021). The AAE materials (K, Ca, Mg and Na) enhance the alkalinity of co-digestion (Basumatary et al., 2024). Further, some elements like C, N and P assist the growth and metabolism of microbes (Liu et al., 2023). The H/C and O/C values were obtained to be 0.04 ± 0.00 and 1.28 ± 0.03 , respectively. These low values of H/C and O/C signify the high hydrophobicity and adsorption capacity of organic pollutants like ammonium (Johnravindar et al., 2022; Xie et al., 2022). The surface textural properties of BC were also analysed. The S_{BET} , V_p and d_p were detected as $14.10 \pm 0.11 \text{ m}^2\text{g}^{-1}$, $0.05 \pm 0.01 \text{ cm}^3\text{g}^{-1}$, and $11.94 \pm 0.05 \text{ nm}$, respectively. The porosity nature of biochar offers a surface area for the adsorption of carbon dioxide in the AD process (Aramrueang et al.,

2022). Further, the SEM image of WHBC (Fig. S2 (a)) signifies biochar's porous structure that provides areas for the colonisation of anaerobic bacteria. The wavelength of FTIR spectra (Fig. S2 (b)) detected is presented in Table 4. These functional groups provide direct interspecies electron transfer (DIET) and raise the progress of microbes in the AcoD system (Shen et al., 2020). The WHBC displays the functional groups, including C—H vibrational groups (aromatic and aliphatic) at 874 and 562 cm^{-1} , stretching vibrations of saturated and unsaturated alcohols C—O at 1037 cm^{-1} and aromatic C=C stretching at 1409 cm^{-1} . Additionally, a low peak at 3610 cm^{-1} in FTIR spectra represents O—H stretching. Besides, XRD spectra (Fig. S2 (c)) detected at various 2-theta (2θ) values confirmed the presence of CaCO_3 (29.36° , calcite), CaSO_4 (40.98° , 66.76°), KOH (30.86° , 50.37°) and K_2SO_4 (58.76°) which provide buffering effect in AcoD process (Jiang et al., 2020).

3.3. Methane production

The daily and cumulative methane yield at various CMRs, such as 70:30, 60:40, 50:50, 40:60, and 30:70, without and with BC addition under control and uncontrol temperatures are presented in Figs. 1 and 2. The CH_4 production rate was low at the initial phase of the AcoD process. This could be because the oxygen in the reactors restricts the growth of microbes and CH_4 yield at the initial phase of the AD operation (Deepanraj et al., 2017). Subsequently, a high rate of CH_4 yield was observed, likely due to the speedy growth of microbes. Afterwards peak period, CH_4 generation declined. The high peak methane generation was attained between the end of the 2nd and 4th weeks of the AcoD period.

Biochar can enhance biogas generation by improving the microbial environment in the digester. The BC-added digesters conducted at ambient and mesophilic temperatures achieved a higher daily methane yield (DMY) rate compared to the corresponding ambient digesters without BC. The BC-added digesters with CMRs of 70:30, 60:40, 50:50, 40:60, and 30:70 was found to be 26.53, 30.04, 22.62, 20.95, 18.91 % higher volume of cumulative methane yield (CMY) than respective digesters without BC respectively, conducted at ambient temperature, for the HRT of 45 days. Likewise, the BC-added mesophilic digesters generated 36.42, 39.26, 33.47, 32.90 and 31.47 % higher amounts of CMY than the uncontrolled digesters. This study confirms that the AD system can be improved by adding BC. This investigation also concludes

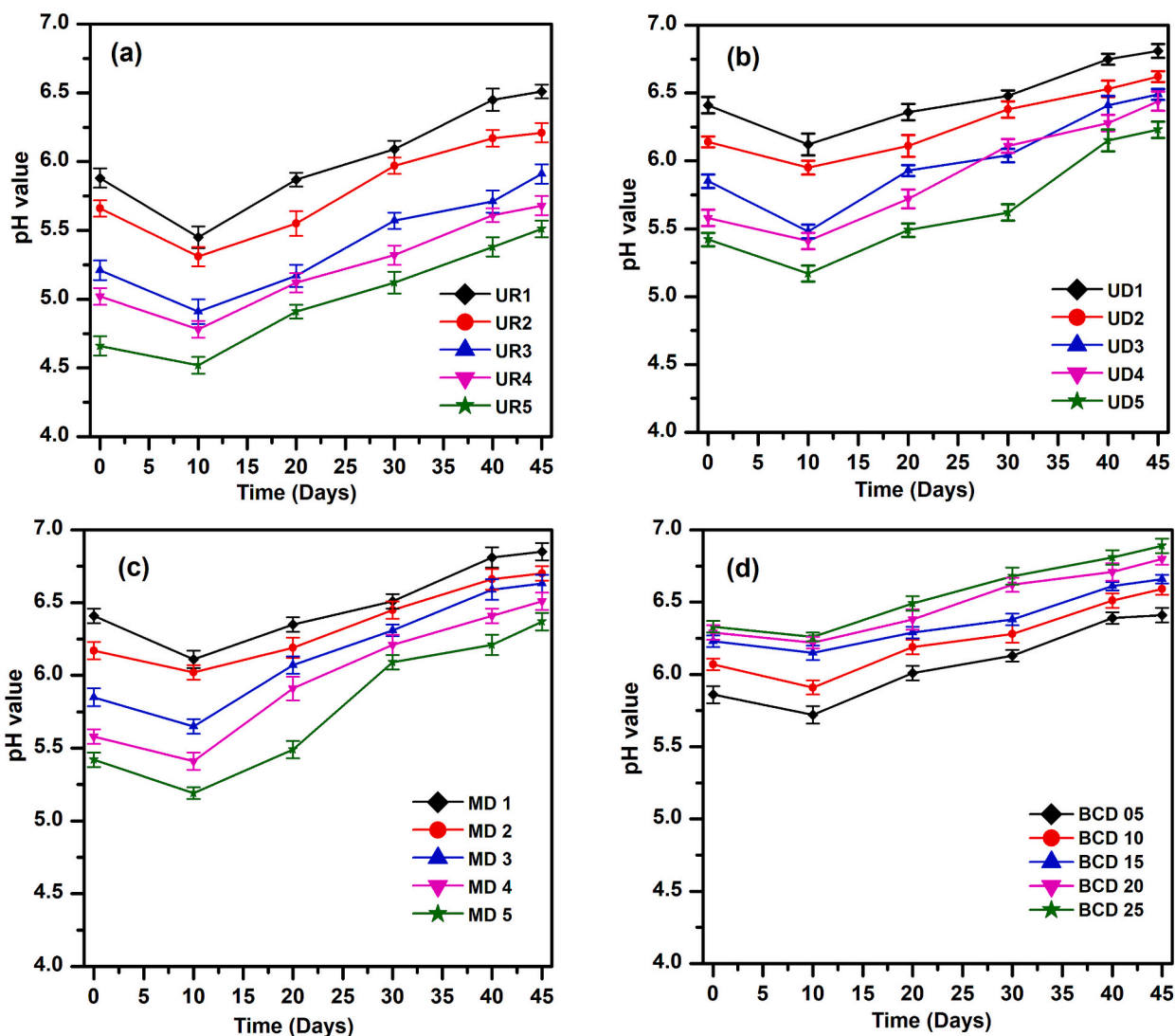


Fig. 4. Variations of pH value for the digesters at (a) ambient temperature without BC, (b) ambient temperature with BC, (c) mesophilic temperature with BC, and (d) ambient temperature with varying amounts of BC for SMR 60:40.

that maintaining the reactor temperature at mesophilic conditions can increase CH_4 generation. A typical comparison between the results of present work and the reported research works is presented in Table 5. This comparative study reveals that the rate of CH_4 generation potential depends on the type of substrates, CMR, types of BC, and pyrolysis temperature of BC. Therefore, analysis of various parameters impacting the methane production rate is essential. Previous studies revealed that BC provides the colonisation areas for anaerobic bacteria and serves as the adsorbent for substances, such as ammonia and limonene, which may hinder co-digestion (Wambugu et al., 2019). The alkaline nature of BC improves methane concentration via a reaction of carbon dioxide and H_2S with alkaline materials available in ash, consequently enhancing the biogas quality (Wang et al., 2017). Various elements and properties of BC, which influence the improvement of the CH_4 production rate, are demonstrated in Table 3. The importance of these properties for AcoD has already been discussed in Section 3.2. Additionally, BC is a thermal insulator that primarily benefits mesophilic environments, where steady temperatures are necessary for efficient methanogenesis. In this study, the highest CH_4 yield was achieved at CMR 60:40 for all groups of BMP experiments. The SMY obtained for CMR of 60:40 at HRT of 45 days were 158.98, 227.24 and 261.75 $\text{mLg}^{-1}\text{Vs}^{-1}$ for groups I, II and III, respectively. At CMR of 60:40, the C/N ratio and MC were 21.9 ± 1.02 and 92.14 ± 0.07 %, respectively, indicating the optimal values for

achieving the highest CH_4 yield in the AcoD of CD and VW. These values were found to align with the optimum range reported in various studies. The current investigation demonstrated that the CMR of 60:40 can be recommended as the most likely CMR for the co-digestion of CD and untreated VW.

In addition, it is desirable to incorporate a suitable dose of BC into the digester to achieve a higher methane yield. In this regard, another group of BMP experiments (set IV) was conducted at ambient conditions by adding varying quantities of water hyacinth BC, such as 5, 10, 15, 20, and 25 gL^{-1} . These digesters were designated as BCD 05, BCD 10, BCD 15, BCD 20, and BCD 25, respectively. The daily and cumulative CH_4 yield for set IV are presented in Fig. 3. The SMY for digesters BCD 05, BCD 10, BCD 15, BCD 20, and BCD 25 were found to be 199.28, 213.40, 240.07, 225.51, and 208.62 mLCH_4/gVS for HRT of 45 days. Moreover, BCD 15 exhibited 16.99 %, 11.11 %, 6.06 %, and 13.10 % higher CMY than BCD 05, BCD 10, BCD 20, and BCD 25, respectively. This study reveals that blending 15 gL^{-1} water hyacinth-based BC in the CMR of 60:40 can be realised as the most significant CMR for the AcoD of CD and VW. Optimisation of CMR for AcoD provides numerous advantages, including boosting the methane yield rate, enhancing process stability, mitigating inhibition, utilising various substrates, nutrient recycling, improving system performance, and supporting environmental sustainability.

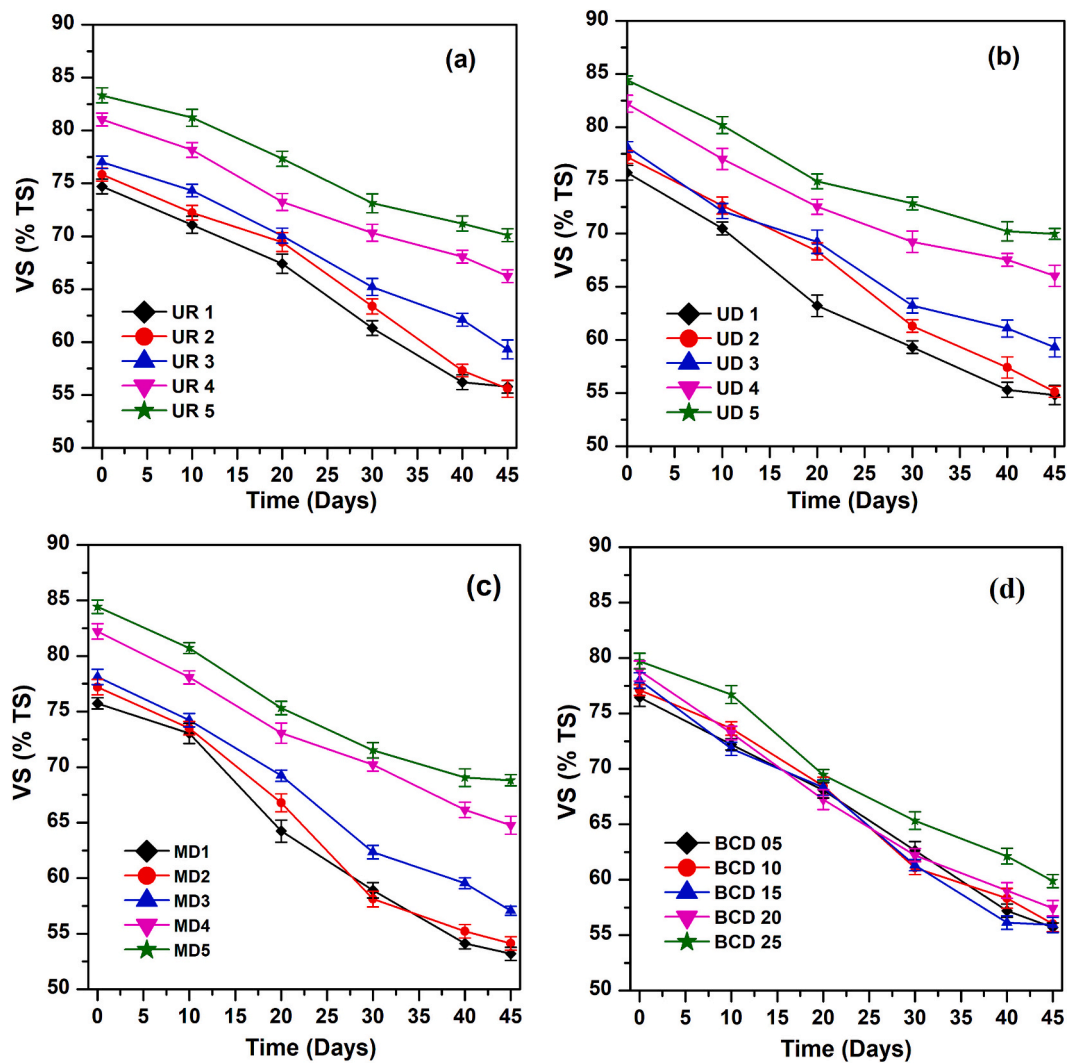


Fig. 5. Reduction of VS (% TS) concentration under (a) ambient temperature without BC, (b) ambient temperature with BC, (c) mesophilic temperatures with BC, and (d) ambient temperature with different quantities of BC for CMR 60:40.

Table 6
Modified Gompertz model parameters for AcoD of CD and VW.

| CMR | Y_F (mL/gVS) | | | Y_R (mL/gVS-day) | | | λ (day) | | | Y_C (mL/gVS) | | | R^2 | |
|-------|---------------------|--------|--------------------|---------------------|-------|--------------------|---------------------|---------|--------------------|----------------|---------------------|--------|-------|--------------------|
| | Digester without BC | | BC added digesters | Digester without BC | | BC added digesters | Digester without BC | | BC added digesters | | Digester without BC | | | BC added digesters |
| | Set I | Set II | | Set III | Set I | | Set II | Set III | Set I | Set II | Set III | Set I | | |
| 70:30 | 154.67 | 226.28 | 250.00 | 4.39 | 6.03 | 6.93 | 6.47 | 6.14 | 6.86 | 141.31 | 192.33 | 222.26 | 0.99 | |
| 60:40 | 174.82 | 240.47 | 275.23 | 4.89 | 7.17 | 8.23 | 6.11 | 5.36 | 6.77 | 158.98 | 227.24 | 261.75 | 0.99 | |
| 50:50 | 150.42 | 206.77 | 224.99 | 4.11 | 5.58 | 6.79 | 5.05 | 5.59 | 5.93 | 139.39 | 180.12 | 209.52 | 0.99 | |
| 40:60 | 144.84 | 188.84 | 220.28 | 4.02 | 5.41 | 6.54 | 4.57 | 5.36 | 5.72 | 133.65 | 169.07 | 199.20 | 0.99 | |
| 30:70 | 145.91 | 177.15 | 209.66 | 4.11 | 5.16 | 6.27 | 4.29 | 4.49 | 4.89 | 132.88 | 163.86 | 193.90 | 0.99 | |

Set I and Set II are at ambient temperature, Set III is at mesophilic temperature, and CMR and BC stand for co-substrate mixing ratio and biochar, respectively.

3.4. pH value and temperature

Fig. 4 depicts the pH value recorded at ten-day intervals for all the groups of the BMP test. The decrease in pH value was observed during the first week; however, it was detected to be increased in the second week afterwards. This phenomenon occurs because acidogenic and acetogenic bacteria produce acids and CO₂ during the initial phase of co-digestion. Afterwards, the pH value rises due to the consumption of acids by methane-generating bacteria (Kwiatkiewska and Tys, 2014). The pH

range for set I was obtained to be 4.6 to 6.5, while the incorporation of WHBC into sets II and III increased the pH levels to a range of 5.4 to 6.8. In set IV, the pH value was observed to rise as the quantity of WHBC increased. Adding 25 g/L WHBC resulted in a higher pH value of 6.3–6.9, whereas 5 g/L WHBC addition led to a lower pH range of 5.8–6.4. Several AAE metals, which were detected in the EDX analysis of the BC, improve the alkalinity level of the reactors (Aramrueang et al., 2022). Additionally, it is necessary to optimise the quantity of BC required to maintain the suitable pH range for different substrates.

Table 7

Modified Gompertz model parameters for CMR 60:40 with varying quantities of BC addition.

| Digesters | Y_p (mL/gVS) | Y_R (mL/gVS-day) | λ (day) | Y_C (mL/gVS) | R^2 |
|-----------|----------------|--------------------|-----------------|----------------|-------|
| BCD 05 | 239.52 | 6.45 | 6.97 | 199.28 | 0.99 |
| BCD 10 | 245.05 | 6.81 | 6.72 | 213.40 | 0.99 |
| BCD 15 | 268.95 | 5.58 | 6.65 | 240.07 | 0.99 |
| BCD 20 | 239.26 | 6.22 | 6.75 | 225.51 | 0.99 |
| BCD 25 | 229.22 | 6.13 | 6.85 | 208.62 | 0.99 |

Temperature is the most crucial parameter for the evolution of microbes (Basumatary et al., 2021). In the present study, the temperature variations were recorded in intervals of 4 h for the uncontrolled digesters. However, the temperature of controlled digesters was not monitored since they were consistently maintained at a mesophilic temperature (37 ± 2 °C). The daily temperature variations recorded every 4 h for uncontrolled digesters ranged from 19.7 to 26.4 °C throughout the experimental period. The uncontrolled digesters' average temperature was below the mesophilic temperature range, resulting in a lower methane generation rate in these digesters. Thus, this study demonstrates that CH₄ production can be improved by maintaining the reactor's temperature in the mesophilic condition.

3.5. VS (% TS) degradation

Reduction in VS value helps to estimate the performance of the AD process. Fig. 5 illustrates the volatile solid reduction (VSR) analysis conducted at ten-day intervals for all BMP test sets. The VSR was evaluated using the Van Kleeck equation (Switzenbaum et al., 2003). The VSR in sets I, II and III were obtained to be 53.00–60.10 %, 57.00–63.70 %, and 59.30–65.15 %, respectively. The CMR 60:40 resulted in the highest VSR for all groups of experiments. Further, the mesophilic digesters were found to have a higher VSR value. The VSR value for CMR 60:40 of sets III was 65.14 %, higher than CMR 60:40 of sets I (60.10 %) and II (63.71 %). Similarly, the volatile solid reduction of the reactors BCD 05, BCD 10, BCD 15, BCD 20, and BCD 25 in set IV on the HRT of 45 days were calculated as 61.26, 62.20, 64.16, 63.77 and 62.05 %, respectively, exceeding the VSR for CMR 60:40 of set I. A decrease in VS favours the AcoD process, indicating increased biogas yield (Barua and Kalamdhad, 2017). The CMR with the highest VSR exhibited a higher CH₄ generation rate and vice versa.

3.6. Modified Gompertz model equation

Results of the MGM study are incorporated in Tables 6 and 7. The CMR of 60:40 resulted in a higher specific CH₄ production potential than other CMRs within their corresponding experimental groups, whereas the CMR of 30:70 exhibited a lower BMP value. Higher Y_p and Y_R values were obtained for the biochar-added mesophilic digester with a CMR of 60:40 compared to respective digesters with and without incorporation of BC performed at ambient temperature. Similarly, in the case of set IV, which was conducted by adding varying quantities of BC, 15 g/L biochar-added digester resulted in a higher CH₄ generation potential. The specific CMY potential for the digester with 15 g/L BC-added was 268.95 mL/gVS, whereas the 15 g/L BC-added digester exhibited a lesser potential (229.22 mL/gVS). Furthermore, BC 15 showed a higher value of Y_R (7.58 mL/gVS-day) compared to other reactors of set IV. This study confirms that biochar can enhance methane generation rate in the co-digestion of cattle dung and untreated vegetable waste, where 15 gL⁻¹ BC added may be considered the optimum amount of biochar required to be mixed. The curve-fitting plot of the MGM is provided in the supplementary materials (Fig. S3). The R^2 value was above 0.98, demonstrating the predicted and experimental results fit well.

4. Conclusions

Incorporating water hyacinth biochar into the AcoD of untreated vegetable waste with cattle dung has significantly enhanced the methane generation rate. This enhancement can be attributed to the porous nature of BC, which provides a colonial space for microbes. The macronutrients, alkali and alkaline earth metals of biochar enhance the slurry's alkalinity in the AD. Further, the functional groups (O—H, C—H, C=C, and C—O) increase the electron exchange among the electron donor and acceptor microorganisms. The most favourable CMR of CD and VW was identified at 60:40, as assessed by the BMP tests. Additionally, biochar-added mesophilic digesters with a 60:40 co-substrates ratio resulted in 39.26 % and 13.19 % higher CH₄ yield than those without and with biochar at ambient temperature for the BMP test period of 45 days. The addition of 15 gL⁻¹ BC to AcoD of CD and VW exposed the significant quantity of BC necessary for effective blending. This study determines that blending a suitable amount of BC into AcoD can boost CH₄ production. Further studies are needed to investigate the anaerobic co-digestion of different substrates with various co-substrate mixing ratios, using different types of biochar, to determine the ideal quantity of biochar to be added for maximising methane production.

Abbreviations

| | |
|-------|--------------------------------------------|
| AC | ash content |
| AcoD | anaerobic co-digestion |
| AD | anaerobic digestion |
| ASTM | American Society for Testing and Materials |
| BC | biochar |
| BET | Brunauer–Emmet–Teller |
| BMP | biochemical methane potential |
| CD | cattle dung |
| CMY | cumulative methane yield |
| DMY | daily methane yield |
| EDS | energy dispersive X-ray spectroscopy |
| FC | fixed carbon |
| SEM | scanning electron microscope |
| FT-IR | Fourier transform infrared |
| FW | food waste |
| HRT | hydraulic retention time |
| MC | moisture content |
| MGM | modified Gompertz model |
| CMR | co-substrates mixing ratio |
| SMY | specific methane yield |
| SDG | sustainable development goal |
| TSC | total solid content |
| UN | United Nations |
| VM | volatile matter |
| VSC | volatile solid content |
| VSR | volatile solid reduction |
| VW | vegetable waste |
| WH | water hyacinth |
| WHBC | water hyacinth biochar |
| XRD | X-ray diffractometer |

CRedit authorship contribution statement

Shayaram Basumatary: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Harrison Hihu Muigai:** Investigation, Formal analysis, Data curation. **Pranab Goswami:** Writing – review & editing, Visualization, Supervision, Conceptualization. **Pankaj Kalita:** Writing – review & editing, Validation, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that have or could be perceived to have influenced the work reported in this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biteb.2024.102013>.

Data availability

Data will be made available on request.

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