**Effect of supplementing commercially available and locally prepared carbon materials in anaerobic digestion – Focusing on enhanced performance and potential mechanisms**

Tinku Casper D’ Silva1,2,3\*, Noémi N. Horváth-Gönczi4,5, Ottó Ribár5, Sameer Ahmad Khan1, Ram Chandra1,†, Virendra Kumar Vijay1, Zoltán Bagi5, Kornél L. Kovács5, Bhushan P. Gandhi3, Kirk T. Semple3

1Biogas Production, Enrichment, and Bottling Laboratory, Centre for Rural Development and Technology, Indian Institute of Technology Delhi, New Delhi – 110 016, India

2Kerala State Council for Science, Technology and Environment – National Transportation Planning and Research Centre (KSCSTE-NATPAC), Govt. of Kerala, Thiruvananthapuram, Kerala – 695 011, India

3Lancaster Environment Centre, Library Avenue, Lancaster University, Lancaster LA1 4YQ, UK

4Vertus Energy AG, 1010 Wien, Austria

5Department of Biotechnology and Microbiology, University of Szeged, 6726 Szeged, Hungary

*\*Corresponding authors:*  [casperdsilva23@gmail.com](mailto:casperdsilva23@gmail.com) (Tinku Casper D’ Silva)

[kovacs.kornel@bio.u-szeged.hu](mailto:kovacs.kornel@bio.u-szeged.hu) (Kornél L. Kovács),

**Abstract**

Anaerobic digestion (AD) is a potential approach to treat organic wastes for energy and resource recovery. Adding various carbon materials in AD is being studied owing to its positive effects on the overall governing mechanisms. This study investigated the effect of adding seven different carbon materials in AD reactors in two sets of experiments. In the first set, five commercially available carbon materials were added to the batch assay, treating α-cellulose under anaerobic and oxygen-stressed conditions. The activated carbon amended reactor steadily provided methane yield even under oxygen-stressed conditions. In the second set, locally prepared hydrochar (HC) and pyrochar (PC) derived from biogas slurry were added to the anaerobic co-digestion of organic wastes. The reactor amended with HC, and its process water (PW) provided 478 mL/g. VSinput of biomethane yield while the PC-amended reactor achieved 411 mL/g. VSinput. This enhancement was attributed to functional groups in HC, volatile acids in PW, and PC’s pH buffering properties. Further, the grey relational analysis revealed that the HC and PW amendment is the best route for improved AD process efficiency. In short, from the two sets of experiments, these findings suggest that activated carbon, PC, and HC (with PW) have potential for improving AD efficiency. However, further research is needed to evaluate their long-term effectiveness and scalability.

**Keywords:** Anaerobic digestion, Carbon materials, Enhanced biomethane yield, Pyrochar, Hydrochar

1. **Introduction**

Anaerobic digestion (AD) has been globally recognized for its dual benefits in renewable energy production and waste minimization contributing to the reduction of excess atmospheric carbon (Bastola et al., 2024; Subbarao et al., 2024). In the AD process, the waste biomass undergoes anaerobic degradation in which a set of microbial consortia utilizes the volatile solids (VS) to produce biogas primarily composed of combustible gas methane (~50 – 55 %v/v) and non-combustible carbon dioxide (45 – 50 %v/v) (Isha et al., 2021). The biochemical process in AD consists of at least 24 reactions that co-occur or in series (Batstone et al., 2002), making it a highly complex process to control. Numerous physico-chemical parameters are also involved in the process to be controlled, such as the substrate and operational pH, operational temperature, feeding rate, hydraulic retention time, the concentration of volatile fatty acids (VFAs), ammonia and alkalinity, and biological parameters like direct interspecies electron transfer (DIET) mechanism that decide the fate of process stability and methane productivity (D’ Silva et al., 2021; Batstone et al., 2002). Even a slight disturbance in the process stability can disrupt the overall system efficiency. Thus, the environmental/economic benefits of the AD system entirely rely on process control, biogas productivity, composition, and overall VS conversion efficiency (D’ Silva et al., 2021; Chandra et al., 2012).

In order to enhance these benefits, two prevailing concerns of AD, (a) almost equal methane to carbon dioxide volumetric ratio in the biogas produced and (b) sub-optimal VS conversion efficiency, need to be addressed. Recently, adding external additives in AD has become an interesting research topic for improving the methane content in biogas and VS utilization efficiency. Adding carbon-based materials such as biochar, activated carbon, carbon nanotubes and other carbon-based materials into the AD reactor was studied to enhance methane productivity or stabilize the overall bioprocess (Isha et al., 2023). These additives, in general, can play a crucial role in enhancing the AD process by promoting microbial growth and aggregation, accelerating the DIET mechanism, enhancing enzyme activity, and increasing buffering capacity. This leads to faster formation and utilization of volatile fatty acids, expedited H2 and CH4 production, and a shorter lag phase, ultimately resulting in higher-quality fermentation. Several researchers have extensively reviewed the general mechanisms and impact of different *“commercially available”* carbon materials (Khan et al., 2021; Nguyen et al., 2021; Abbas et al., 2021). It could be generally inferred that the reaction mechanism of the various additives differed greatly depending on independent characteristics. But the targeted output of methane enhancement was reported to be positively influenced in most cases. Therefore, this needs more investigation to validate how the carbon materials behaved under similar conditions to bridge the research gaps.

Further, a question that still arises is that what if these carbon materials are prepared locally? Will it have a positive impact on the methane content and biogas production? *“Locally prepared”* carbon materials derived from the thermal conversion of biomass are being investigated as biocatalysts for enhancing the methane content in AD reactors irrespective of the substrate being treated (Khan et al., 2021). Further classifying within the carbonized materials, adequate addition of pyrochar (PC, a solid by-product of the slow pyrolysis process) and hydrochar (HC, a solid by-product of hydrothermal carbonization, HTC) are being predominantly reported as biocatalysts in the AD process (Khan et al., 2021; Cavali et al., 2022).

Biochar, be it PC or HC, has played a valuable role in enhancing the methane yield and digestion efficiency of AD majorly due to three reasons: (a) rapid biofilm development and microbial colonization (Sunyoto et al., 2016; Cooney et al., 2016), (b) the ability to adsorb toxic elements (Mumme et al., 2014), subsequently reduce the lag time of methanogenic activity, increased enzymatic activity, and fast establishment of DIET mechanism especially between acidogens (electron-donating microorganisms) and methanogens (electron-accepting microorganisms) during AD (Khan et al., 2021; Yun et al., 2021; Li et al., 2021), and (c) rapid, but more controlled hydrolytic, acidogenic, acetogenic and syntrophic bacterial activities that stabilize the AD process and reduce the chances of process instability (Chiappero et al., 2020; Wang et al., 2018). Biochar is also known to be an adsorbent of nitrogenous (ammonium, nitrate, and nitrite) and phosphorus compounds, which, in excess concentration, is detrimental to the AD process (Peng et al., 2023; Fagohungbe et al., 2017; Lü et al., 2016). The presence of alkali functional groups such as phenolic hydroxyl and amino groups and alkali/alkaline earth metal ions makes biochar a material with high buffer capacity that has the potential to neutralize the pH inside an AD reactor even under stressed conditions, thus improving the overall process stability (Zhao et al., 2021). Amendment of PC and HC at varied dosing rates in AD reactors treating different biomass has been reported synergistic to no effect on biogas/biomethane production (Xu et al., 2016; Choudhury et al., 2020; Zhou et al., 2020; Jang et al., 2018). Therefore, a proper clarity is still required over these claims or discrepancies; it can be summarized that the microbial impact and elemental level material flow pathways have been reported differently.

Biogas slurry has been recognized as a potential substrate for producing these carbonized materials locally within the AD biorefinery (Cavali et al., 2022; Sharma et al., 2023). Such a concept will also support establishing a circular economy (Khan et al., 2021) and overcome the existing limitations of the AD process, also improving the environmental footprint (Caiardi et al., 2022). Some recent studies have utilized biogas slurry-derived biochar (PC and HC) to treat organic wastes in AD (Alghashm et al., 2023; Xu et al., 2022). As previously discussed, these studies also observed that the biogas slurry-derived biochar also had a synergistic effect on AD, improving the methane content and yield, shortening the lag time, and boosting the VFAs oxidizing bacterial population. Even though there have been studies focused on the effect of these additives in the AD reactor, it is still unclear about the sustainable route with respect to energy output and process stability. This becomes more significant when considering that the properties of the carbon materials vastly vary and, thus, variably impact the fate of its performance while applied in AD (Jang et al., 2018; Romero-güiza et al., 2016).

Therefore, this study investigated the influence of seven carbon materials on the performance of AD reactors under mesophilic conditions in two sets of experiments.

* In the first set of experiments, the influence of five *“commercially available”* carbon materials on AD of α-cellulose were investigated to understand the behaviour and mechanism of different carbon materials in AD under similar conditions.
* Later in the second set of experiments, the effect of the amendment of *“locally prepared”* HC and PC was investigated on the performance of AD treating dry fallen leaves (DFL), fruit/vegetable wastes (FVW) and cow dung (CD) at a mixing ratio (based on total solids, TS) of 0.4:0.6:1, optimized earlier by the author’s previous work (D’Silva et al., 2022a).

By combining both the experimental sets within a single study, this research aims to provide a comprehensive understanding of carbon-material-assisted AD, bridging the gap between controlled experiments with commercial additives and applications using locally prepared biochar. The findings will offer valuable insights into the feasibility of carbon material supplementation for improving AD efficiency, methane yield, and process stability.

1. **Materials and methods**
   1. First set of experiments
      1. Collection and preparation of substrates

For the first set of experiments, α-cellulose (Merck Kft., Hungary) was used as the substrate, while the anaerobic inoculum was collected from an operational biogas plant (Zöldforrás Energia Kft., Szeged, Hungary). The TS and VS contents of the α-cellulose were 95.30% and 95.14%, and of anaerobic inoculum were 4.55% and 2.84%.

* + 1. Carbon materials used

Five different commercially available carbon materials were used in the first set of experiments, all of which were procured from Sigma-Aldrich through Merck Kft., Hungary

• Graphite powder, particle size: <20 µm (- Product code: 282863 <https://www.sigmaaldrich.com/AT/en/product/aldrich/282863?context=product>)

• Activated carbon, mesh size: 100 (149 µm) (- Product code: 161551 <https://www.sigmaaldrich.com/AT/en/product/sigald/161551?context=product>)

• Graphene, particle size: 25 µm, specific surface area: 120-150 m²/g (- Product code: 900413 <https://www.sigmaaldrich.com/AT/en/product/aldrich/900413?context=product>)

• Carbon, particle size: 2-12 µm (- Product code: 484164 <https://www.sigmaaldrich.com/AT/en/product/aldrich/484164?context=product>)

• Carbon nanotube multiwalled, carbon content >90% Dimensions: diameter (D) 110-170 nm, length (L) 5-9 µm (- Product code: 659258 <https://www.sigmaaldrich.com/AT/en/product/aldrich/659258?context=product>)

* + 1. Reactor configuration and experimental setup

The first set of experiments was carried out at the Department of Biotechnology and Microbiology, University of Szeged, Hungary. The experiment utilized glass reactors with a total volume of 160 mL, with 100 mL headspace and 60 mL working volume in triplicates. Carbon materials were added to the system at a dosing rate of 5 g/L into the respective reactors, generally reported as an optimal dosage rate for carbon conducting materials in a previous literature review (Nguyen et al., 2021). The carbon materials were added only once at the beginning of the experiment. About 190 mg of α-cellulose was added to all the reactors, except for the negative control, maintaining a concentration of 3.16 g VS/L. About 60 mL of inoculum was added to each reactor. The reactors were sealed with butyl rubber septa and aluminium caps.

Following the assembly of the reactors, anaerobiosis was achieved by sealing the bottles and purging them with N2 gas. Samples were incubated at 37 °C until the next round of sampling. The experiment was carried out for a period of 107 days in fed-batch mode, as shown in Table 2. The reactors were fed four times throughout the experiment, which was divided into four phases by the operational days of feedings, as given below:

* Phase I: 0 – 22 d
* Phase II: 23 – 57 d
* Phase III: 58 – 85 d
* Phase IV: 86 – 107 d

In the first phase, the reactors were allowed to adapt to the additives in the respective reactors. Later, when the first phase reached a gas production plateau, 3.16 g VS/L of α-cellulose was added again to start the second phase. From the second phase onwards, half of the reactors were divided into control and stressed reactors in triplicates. This was done to analyse the stress resistance effect of the additives. Oxygen was used as the stress agent in this study. For that, 100 mL of atmospheric air was injected into the 100 mL headspace of each reactor. This injection resulted in an introduced O2 quantity of 21 mL in the headspace of the reactors. The goal was to compare the stressed reactors equipped with additives to the stressed control reactor (stressed reactor without additives) and the non-stressed triplicates with the same additive.

**Table 2.** Reactor configuration and experimental design for the first set of experiments

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Reactor type | Reactor name | Carbon material dosage (g/L) | Substrate added (g VS/L) | O2 stress dosing (mL) | O2 stress dosing phases | No. of replications |
| Non-stressed | No amendment | - | 3.16 | - | - | 3 |
| Activated carbon | 5 | 3.16 | - | - | 3 |
| Graphene | 5 | 3.16 | - | - | 3 |
| Carbon | 5 | 3.16 | - | - | 3 |
| Carbon nanotube | 5 | 3.16 | - | - | 3 |
| Graphite | 5 | 3.16 | - | - | 3 |
| Stressed | No amendment | - | 3.16 | 21 | II, III, IV | 3 |
| Activated carbon | 5 | 3.16 | 21 | II, III, IV | 3 |
| Graphene | 5 | 3.16 | 21 | II, III, IV | 3 |
| Carbon | 5 | 3.16 | 21 | II, III, IV | 3 |
| Carbon nanotube | 5 | 3.16 | 21 | II, III, IV | 3 |
| Graphite | 5 | 3.16 | 21 | II, III, IV | 3 |

* + 1. Analytical methods

The proximate characteristics of moisture content (MC), TS, and VS of the slurry samples were analysed using standard methods (APHA, 2012). The methane and oxygen contents of the biogas generated in the reactors were determined using a 6890N Network GC System (Agilent Technologies, U.S.). During the measurements, 200 µL samples were injected into the instrument from the reactor head spaces using a Hamilton SampleLock gas-tight syringe. Organic acid measurements were standardized according to the feeding period. Prior to the first introduction of the substrate into the reactors, samples were obtained for analysis. This sample was designated as day 0 of the given experimental period. After six days (day 6), additional samples were collected to assess any variations in the levels of organic acids in each phase.

The quantity of organic acids (acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid and caproic acid) was determined using a Hitachi Chromaster high-performance liquid chromatography (HPLC) system. Initially, the samples were extracted from each reactor, treating the three parallels as a single sample. Subsequently, the sludge samples were centrifuged at 13,000 rpm for 10 min. During HPLC analysis, the instrument parameters set were Agilent Hi-Plex H column at a temperature of 50 °C and refractive index detector temperature of 41 °C. The eluent used was 0.01 N H2SO4 at a flow rate of 0.6 mL/min. Total VFA (TVFA) was considered for data illustration, which is the sum of acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid and caproic acid. The biomethane yield from this experiment is represented in the unit mL/g.VSinput where VSinput means the VS content of substrate excluding the VS content of inoculum.

* 1. Second set of experiments
     1. Collection and preparation of substrates

The DFL was collected from the Indian Institute of Technology (IIT) Delhi campus, New Delhi, India. Meanwhile, the FVW was gathered from the waste management collection facility within the campus. The CD was provided by the nearest dairy farm. The DFL was grinded using a grinding machine of 2.00 kW power capacity, and the FVW was finely pulverized using a pulverizer of 3.73 kW power capacity. The TS and VS contents of DFL, FVW and CD were 89.64% and 66.99%, 14.65% and 13.15%, and 22.70% and 18.46%, respectively. More information on the preparation and characteristics of the substrates to be digested is well explained in the author’s previous research work (D’Silva et al., 2022a).

* + 1. Carbon materials used

The anaerobic digestate derived from anaerobic co-digestion of DFL, FVW, and CD was collected from the previous batch experiment (D’Silva et al., 2022a); the solid particle was sieved using a double-layered cloth and dried in sunlight for about one week until the weight remained constant. The liquid portion of the digestate was stored in polyethylene bottles with airtight lids and refrigerated at 4℃. The dried material was then finely grinded for 1 to 2 min using a mixer grinder of 2 kW power capacity. The dried material was used for slow pyrolysis at 500 ℃ for a residence time of 1 h with a heating rate of 8.33℃/min. The dried material was mixed with the liquid portion of the digested slurry, maintaining a solid concentration of 15% TS for HTC. Table 1 shows the product yields from the slow pyrolysis and HTC and its comparison with existing literature. Detailed information on the methodology adopted to produce PC, HC and process water (PW) are given in the supplementary file. The general characteristics of the PW and liquid digestate are also given in the supplementary file (Table S1).

**Table 1.** Comparison of thermochemical conversion of biomass and the product yields achieved in this study with the reported values

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Thermo-chemical conversion process | Product mass yield (%) | | | Process temperature (℃) | References |
| Solid | Liquid | Gas |
| Slow pyrolysis | 25 – 35 | 20 – 30 | 25 – 35 | 300 – 650 | Villamil et al. (2020) |
| 32.83\* | | 67.17 | 500 | This study |
| Hydrothermal carbonization | 45 – 70 | 5 – 25 | 5 – 25 | 180 – 300 | Villamil et al. (2020) |
| 64.29 | 26.73 | 8.98 | 220 | This study |

\*The value shows the total yield consisting of solid and liquid products, as no separation was done.

* + 1. Reactor configuration and experimental setup

In the second set of experiments, batch assays having a total volume of 20 L and a working volume of 14 L in triplicates were set up at Biogas Production, Enrichment and Bottling Laboratory, IIT Delhi. Table 3 shows the reactor configuration and experimental design used for the study. The DFL, FVW, and CD substrates were filled inside the reactors, maintaining a TS concentration of 2 %. A separate reactor containing only CD at a TS content of 1 % was also operated along with other reactors as a control for CD substrate. According to the earlier literature, the required amount of biochar (PC and HC) was decided and optimized from the author’s review article (Khan et al., 2021). From the available literature, it was observed that a linear relationship prevails between biochar dosage and TS concentration (TS %) as in Eq. (1) (coefficient of determination, R2 = 0.86). The graph plot for determining the linear relationship is given in the supplementary file (Fig. S1).

…….(1)

According to Eq. (1), in this present study, a TS concentration of 2 % of substrate content will require 0.85 g/L of biochar. Hence, considering the volume of the batch reactor used in the present study, 12 g of PC and HC were weighed and added to the respective reactors. The PC and HC were crushed into very fine particles using mortar and pestle and sieved using a 2 mm sieve before using them for the experiments. The nomenclature given to the reactors were CD (for the control reactor with CD substrate), DFL+FVW+CD (for the co-digestion control reactor with no biochar addition), DFL+FVW+CD+PC (for the reactor with PC), and DFL+FVW+CD+HC (for the reactor with HC and PW). To determine the biogas yields and composition from PW, the same combination of liquid digestate and PW used in the DFL+FVW+CD+HC reactor was kept in a separate reactor. After filling the reactors with an adequate quantity of substrates, liquid digestate, and biochar, as given in Table 2, the bottles were tightly sealed with rubber stoppers possessing two vents connected to leakproof stop cocks. The characteristics of liquid digestate are available in the supplementary file (Table S1). All the reactors were purged with pure nitrogen for 3 to 4 minutes to flush out residual oxygen and checked for any leakages. When it was verified that the reactors were well-sealed and leak-proof, they were arranged inside a dark room maintained at 37 ℃.

The volume of the produced biogas was determined using the water displacement method every 1 to 5 days, and the values were converted to standard temperature and pressure of 0℃ and 1.01 bara. The reactors were well-shaken on a daily basis twice a day (morning and evening) and also before and after the biogas volume determination. A calibrated Biogas Analyzer (Model: Biogas-5000, Geotech, UK) was used to analyse the composition of biogas, i.e., CH4, CO2, and other residual gases in percentage unit and H2S in ppm level. The residual gas in the biogas was assumed as ammonia after carrying out the mass balance of the residual gas content in the biogas, explained more in detail in section 3.6. Each reactor was shaken manually before and after the biogas volume determination and composition analysis. The experimental run of the reactors was extended till the 79th day, when the biogas production was entirely stopped in all the reactors.

The biogas and its composition are expressed in terms of mL/g.VSinput in which VSinput means the total VS content available inside the reactor.

**Table 3.** Reactor configuration and experimental design for the second set of experiments

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Reactor nomenclature | Reactor working volume (L) | DFL:FVW:CD ratio (based on TS) | DFL added (g) | FVW added (g) | CD added (g) | Liquid digestate added (L) | Biochar type added, dosing quantity (g) | TS maintained (%) | I/S ratio | No. of replications |
| CD | 14 | - | - | - | 308 | 13.56 | - | 1 | - | 3 |
| DFL+FVW+CD | 14 | 40:60:100 | 31 | 290 | 308 | 13.40 | - | 2 | 1:1 | 3 |
| DFL+FVW+CD+PC | 14 | 40:60:100 | 31 | 290 | 308 | 13.40 | PC, 12 | 2 | 1:1 | 3 |
| DFL+FVW+CD+HC | 14 | 40:60:100 | 31 | 290 | 308 | 13.40\* | HC, 12 | 2 | 1:1 | 3 |

\*1.5 L of process water from HTC was diluted 3 times using the liquid digestate to simulate the characteristics of hydrolysate from a reactor having a feeding rate of 2 %TS and maintain the overall VS loading since the actual feedstock used for carrying out HTC was the anaerobic digestate from a reactor treated at a feeding rate of 6 %TS

* + 1. Analytical methods

The initial characteristics of the substrates and the slurry samples from the reactors during the initial and final days were further analysed using various laboratory analyses. The proximate characteristics of the samples were analyzed using the same procedure used in the first set of experiments. The pH and total alkalinity (TA) of the slurry samples were determined using the standard methods (APHA, 2012). The total volatile acids (TVA) determination was done using the direct titration method using sulfuric acid and sodium hydroxide, developed by Di Lallo and Alberston (DiLallo et al., 1961). Total ammoniacal nitrogen (TAN) and free ammonia (FA) were calculated as per the Eqs. (2) and (3) as per the literature suitable for anaerobic conditions (Mirza et al., 2021; Ihoeghian et al., 2023),

…..(2)

……..(3)

Eqs. (2) and (3) were applied since it was estimated that the results obtained were comparable with the results carried out using standard methods during the preliminary tests with an error percentage of less than 4% (data not shown). The proximate analysis of anaerobic digestate, PC, and HC was done as per the American Society for Testing and Materials (ASTM) standards (ASTM E1755-01, (2020), ASTM D3175-20, (2020), ASTM D3173/D3173M-17a, 2017). An automated ‘Vario EL’ elemental analyzer (Make: Perkin Elmer, USA) was used for determining the elemental analysis of the samples on a dry basis (carbon, hydrogen, nitrogen, and sulfur in percentage values). The oxygen content was determined by subtracting the sum of carbon, hydrogen, nitrogen, sulfur and ash content (AC) from 100 (Baghel et al., 2021). The high heating value (HHV) was calculated using elemental analysis and AC was determined according to Channiwala & Parikh (2002), using Eq. (4),

……(4)

The micronutrients such as iron (Fe), calcium (Ca), and magnesium (Mg) were determined using atomic absorption spectrophotometer (Make: Thermo Scientific, US), and potassium (K) and sodium (Na) were analysed using a flame photometer (Make: Systonic, India). Scanning Electron Microscopy (SEM) micrographs of anaerobic digestates from reactors were taken with the help of ZEISS EVO Scanning Electron Microscope EVO 50. FTIR spectroscopy was done using NICOLET-IS-50 (Thermo Fisher Scientific, United States) using a KBr to sample ratio of 100:1. The Brunauer - Emmett - Teller (BET) surface analysis was done using BELSORP-maxII (BELSORP – series, Microtrac BEL Corp., US) following standard procedure. The phosphorus (P) content of the solid digestate was determined using the orthophosphate determination from standard methods (APHA, 2012). The germination test was carried out to determine the emergence rate (%) and mean germination time (MGT) using the Eqs. (5) and (6), following the procedure given by D’Silva et al. (2022b),

… (5)

…. (6)

Where t is the time in hours from 0 to the end of the germination test, and n is the number of seeds germinated on the day, t.

* + 1. Biodegradability index

The substrate degradation and subsequent biogas/biomethane production in the reactors were evaluated using the factor biodegradability index, calculated using Eqs. (7) & (8) by considering the elemental analysis results based on modified Buswell’s equations by Boyle (Eq. (9)) (Achinas & Euverink, 2016; Nielfa et al., 2015). Later, the biodegradability index was calculated as the ratio of experimental values of biomethane yield obtained to the calculated theoretical biomethane yield (Eq. (10)).

…….(7)

………(8)

…….(9)

* + 1. Carbon emission potential estimation

Estimating carbon emissions from anaerobic digestate, PC, and HC has been done according to Zeshan & Vishvanathan (2014), which calculates CH4, and CO2 emission potential based on the carbon content and nitrous oxide (N2O) based on nitrogen content in the material as per the Eqs. (10), (11) and (12). The calculation was made for 1 kg of material with characteristics achieved from the experiments to make it comparable.

…..(10)

…..(11)

…..(12)

where M = Total material available (kg); TS = Total solid content (%); GCF = Gas correction factor (fraction), which was assumed as 0.4 for open dumps of <5 m depth [4] DOC = Degradable organic carbon was considered equivalent to the VS content; DOCF = Fraction of DOC dissimilated was calculated from the model equation, DOCF = 0.014T + 0.28, where T is temperature in °C considered as 35°C (Zeshan & Vishvanathan, 2014), which resulted in a value of 0.77. F = Fraction of methane or carbon dioxide in the gas produced (default is 0.5); R = Methane or carbon dioxide recovered (kg). Here, methane or carbon dioxide are assumed to be released directly into the atmosphere; hence, the value is considered zero; OX = Oxidation factor (default is 0).

According to Brown et al. (2010), any fertilizers applied to the soil can emit N2O of about 1% of the nitrogen available, as suggested by IPCC. A much higher value of 1.3 – 1.7% of nitrogen available has been reported by Møller et al. (2009). Hence, in this study, an N2O emission was calculated for 1 kg of anaerobic digestate, PC, and HC using an emission factor of 1.7% nitrogen. To convert the CH4 and N2O to carbon equivalent (CO2-eq), the calculated CH4 potential was then multiplied with a factor of 28 (Subbarao et al., 2023), and for N2O, the value was multiplied with a factor of 298 (Nabuurs & Mabret, 2023).

* + 1. Ranking of the routes based on the performance using grey relational analysis

The multiple-criteria decision-making (MCDM) approach was used to select the best path for upscaling. Various MCDM techniques are available to carry out the selection (D’Silva et al., 2022b; 2021). The grey relational analysis (GRA) approach was chosen for this study because of its simple, unbiased and practical characteristics. In order to select the best pathway using the GRA approach, the parameters considered were biomethane yield, biocarbon dioxide yield, H2S production, lag phase, biodegradability index, VS removal, net energy output, emergence rate, MGT and total carbon emission equivalent. Even though pathogen removal was not within the scope of this study, pathogen removal was also considered a parameter for GRA. Since thermal treatment eliminates the pathogens, it was assumed that 100% pathogen removal occurred in HC and PC, and no removal occurred in anaerobic digestate.

The GRA approach was carried out following four major steps. The original experimental data were normalized between 0 and 1 to minimize the interference between the parameters. Further, these parameters were categorized into two: (a) larger the better and (b) smaller the better. This was done based on the requirement of minimized and maximized values. The parameters such as biomethane yield, biodegradability index, VS removal, net energy output, and emergence rate were considered for maximizing (larger the better), while the remaining parameters were considered for minimizing (smaller the better), as per the prescribed method (Panda et al., 2016).

The normalized values were then used to calculate the grey relation coefficient in the next step. A coefficient weightage of 0.50 was considered from the literature (Panda et al., 2016). Following that step, the calculated multiparametric grey relation coefficients were transformed to a single response, i.e., grey relational grade (GRG). At the end, the best strategy was optimized based on GRG values attained (higher the best).

* 1. Kinetic modelling

The experimental biogas and methane yields were fitted with the modified Gompertz kinetic model, one of the most preferred models by previous literature (Isha et al., 2021; D’Silva et al., 2022a) [3,32]. The parameters such as maximum biogas/biomethane production potential, daily biogas/biomethane production rate and lag phase were simulated from the model as reported in earlier literature (Isha et al., 2021) as shown in Eq. (13).

…….(13)

where M is the biogas yield (in mL/ g. VSinput) with respect to time t (in days), Pb is the maximum biogas/biomethane potential of the substrate (in mL/ g. VSinput), Rm is the maximum biogas/biomethane production rate (in mL/ g. VSinput), λ is the lag phase time taken for biogas/biomethane production (in days), e is the Euler's function which is equal to the value 2.7183 (Isha et al., 2021).

* 1. Statistical analysis

The average and standard deviations of the experimental data were estimated using Microsoft Excel 360 version. The cumulative yields illustrated were calculated as the summation of daily yields achieved during the experimental period. The modified Gompertz model was simulated using the least square fitting with the solver add-in option available in Microsoft Excel. The one-way ANOVA using Tukey’s post hoc test was also estimated using the Data analysis add-in available in Microsoft Excel. A confidence interval of 95% and a level of significance p < 0.05 was set for all the statistical analyses. In the second set of experiments, before using the biogas, biomethane, and biocarbon dioxide yields, the yields obtained from PW were deducted from HC-amended reactor data to make it comparable with other reactors.

1. **Results and discussion**
   1. Effect of commercially available carbon materials in AD
      1. Non-stressed (no O2 addition) reactors

Figure 1 presents the cumulative methane yield obtained from all non-stressed reactors across different phases. The cumulative methane yield in the reactor without amendment was 301.64 ±13.44 mL/g VSinput in Phase I, 337.35 ±8.51 mL/g VSinput in Phase II, 381.91 ±6.37 mL/g VSinput in Phase III, and 255.90 ±17.87 mL/g VSinput in Phase IV. An increase in methane yield was observed in Phases II and III, followed by a significant reduction in Phase IV. A similar trend was noted across all reactors (Fig. 1), except for the activated carbon-amended reactor.

In contrast to the general pattern, the activated carbon-amended reactor exhibited a gradual decrease in methane yield throughout the experiment. While this reactor achieved the highest methane yield in Phase I, its performance declined steadily in the subsequent phases. The methane yield in the activated carbon reactor was 3.72% and 14.73% lower than the non-amended reactor in Phases II and III, respectively. However, in Phases I and IV, the activated carbon reactor demonstrated significantly higher methane yield than the control reactor (one-way ANOVA and Tukey-test: p<0.05, fcrit<fvalue).

Among the other amendments, the carbon nanotube reactor exhibited the least positive effect in Phase I, yielding 287.52 ±8.18 mL/g VSinput, which was 4.68% lower than the non-amended reactor. In Phase II, the non-amended reactor displayed the highest methane yield, while in Phase III, the graphene-amended reactor performed the closest to the non-amended reactor, with a maximum methane yield of 371.88 ±4.7 mL/g VSinput.

These results were further supported by the simulated values derived from the modified Gompertz model. As shown in Table 4, all reactors exhibited an Rm value above 20 mL/g. VSinput.d and a λm value below 2 d in Phase I. Among all reactors, the activated carbon-amended reactor exhibited a comparatively slower decline in performance, especially in Phase III.

**Fig. 1** Cumulative methane yield from all the non-stressed (no O2 added) reactors in the various phases of the experiments.

* + 1. Stressed (O2 addition) reactors

Figure 2 shows the cumulative methane yields from all reactors at different phases. In Phase I the reactors followed very similar profiles as the non-stressed reactors discussed in section 3.1.1. As a general feature, the O2 introduction into the reactors has led to declined methane production in all reactors containing added carbon components in Phase II. As the reactors acclimated to the altered conditions, better methane yields were obtained. Notably, the methane yields dropped substantially in all reactors during Phase IV, except those operating with support from activated carbon.

Reactors supplemented with activated carbon exhibited outstanding results among the additives with an average gas production of 304.86 ±40.17 mL/g VSinput. Activated carbon was followed by carbon, graphene, carbon nanotube and graphite with average gas productions of 291 ±65.30 mL/g VSinput, 288.50 ±39.33 mL/g VSinput, 281.53 ±70.89 mL/g VSinput and 277.89 ±41.38 mL/g VSinput, respectively. In the Phases involving reactors supplemented with O2 (Phases II-IV), the addition of carbon materials triggered more methane production than that of the control reactor. The largest difference was achieved by the addition of activated carbon in Phase II, i.e., 52.76% higher methane production relative to the not amended, O2 stressed reactor. Surprisingly the lowest methane augmentation was also achieved in the reactors containing activated carbon in Phase III when methane production was elevated only by 0.78% relative to the control. Similarly unstable performance of the activated carbon has been noted in section 3.1.1. Activated carbon is a thoroughly studied carbon material, which may act through multiple mechanisms (neutral pH, adhesive surface for microbial attachment, DIET mechanism, etc.), while the other tested carbon materials primarily act via the DIET mechanism (Nguyen et al., 2021).

The O2 stress effect on the performance was evident from the values estimated with the modified Gompertz model. The data fit well with the modified Gompertz model in Phase I (no O2 stress) for both sets of reactors. Upon O2 addition in Phase II, the performance became disturbed in all stressed reactors. It could be interpreted that the microbes within the reactors required an adjustment period, especially in Phase II. Once they adapted to the stressed conditions, their activities improved, which yielded better methane in Phase III. Supplying a limited amount of O2 apparently enhances the hydrolytic and acidogenic activities, thereby improving the TVA concentration within the reactors (Fu et al., 2023; Nguyen et al., 2018). This improves the methane production provided the TVA production to consumption rate is appropriate in the reactors. Longer exposure to O2 or elevated TVA concentrations and associated pH drop may bring about adverse influence on methanogens’ activity. This may explain the reduced methane yields in Phase IV. This is also supported by the higher λm values achieved in Phase IV (Table 4).

**Fig. 2** Cumulative methane yields from the stressed (O2 added) reactors at different Phases.

**Table 4.** Simulated values of experimental results using modified Gompertz model for all the reactors during four phases

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Reactor type | Reactor name | Phase | Experimental methane yield (mL/g. VSinput) | Pm (mL/ g. VSinput) | Rm (mL/ g. VSinput. d) | λm (d) | R2 |
| Non-stressed reactors | No amendment | I | 301.64 | 302.17 | 22.81 | 1.91 | 0.99 |
| II | 337.35 | 345.08 | 43.48 | 7.16 | 0.99 |
| III | 381.91 | 383.62 | 45.19 | 5.12 | 0.99 |
| IV | 255.90 | 262.23 | 18.13 | 5.68 | 0.99 |
| Activated carbon | I | 415.04 | 416.04 | 30.10 | 1.88 | 0.99 |
| II | 324.79 | 328.21 | 18.82 | 10.49 | 0.99 |
| III | 325.66 | 328.03 | 40.39 | 4.58 | 0.99 |
| IV | 281.30 | 282.61 | 29.49 | 6.33 | 0.99 |
| Graphene | I | 329.35 | 338.91 | 30.41 | 1.99 | 0.99 |
| II | 312.56 | 312.75 | 35.29 | 6.48 | 0.99 |
| III | 371.88 | 372.26 | 44.67 | 5.72 | 0.99 |
| IV | 295.84 | 298.81 | 21.89 | 6.15 | 0.99 |
| Carbon | I | 301.59 | 318.55 | 28.47 | 1.57 | 0.98 |
| II | 302.76 | 303.54 | 36.47 | 6.74 | 0.99 |
| III | 347.32 | 353.67 | 43.32 | 5.26 | 0.99 |
| IV | 265.44 | 279.11 | 19.99 | 5.83 | 0.99 |
| Carbon nanotube | I | 287.52 | 294.14 | 30.37 | 1.71 | 0.98 |
| II | 325.36 | 327.19 | 46.62 | 6.50 | 0.99 |
| III | 348.29 | 358.59 | 42.65 | 5.51 | 0.99 |
| IV | 260.50 | 271.80 | 18.26 | 5.90 | 0.99 |
| Graphite | I | 318.72 | 320.48 | 20.26 | 1.56 | 0.99 |
| II | 328.66 | 330.89 | 11.20 | 6.22 | 0.99 |
| III | 360.57 | 362.05 | 38.62 | 7.76 | 0.99 |
| IV | 265.27 | 266.61 | 19.64 | 5.77 | 0.99 |
| Stressed reactors | No amendment | I | 316.47 | 319.00 | 13.60 | 7.88 | 0.99 |
|  | II | 207.27 | 207.50 | 5.24 | 18.43 | 0.99 |
|  | III | 275.16 | 275.84 | 4.58 | 3.13 | 0.99 |
|  | IV | 196.44 | 196.50 | 2.66 | 9.81 | 0.99 |
| Activated carbon | I | 369.95 | 370.75 | 10.14 | 7.99 | 0.99 |
|  | II | 316.62 | 317.00 | 4.20 | 2.94 | 0.99 |
|  | III | 277.31 | 281.51 | 17.74 | 1.84 | 0.99 |
|  | IV | 269.08 | 269.08 | 15.22 | 10.56 | 0.99 |
| Graphene | I | 319.97 | 322.19 | 9.72 | 12.39 | 0.99 |
|  | II | 277.93 | 278.00 | 6.61 | 1.27 | 0.99 |
|  | III | 322.20 | 323.92 | 4.77 | 8.51 | 0.99 |
|  | IV | 237.39 | 237.39 | 22.77 | 8.30 | 0.99 |
| Carbon | I | 305.57 | 307.74 | 6.38 | 11.83 | 0.99 |
|  | II | 250.61 | 250.70 | 6.48 | 1.06 | 0.99 |
|  | III | 379.51 | 379.66 | 13.98 | 8.33 | 0.99 |
|  | IV | 234.13 | 234.13 | 15.89 | 6.97 | 0.99 |
| Carbon nanotube | I | 287.86 | 289.47 | 10.41 | 7.65 | 0.99 |
|  | II | 248.47 | 249.58 | 6.650 | 0.96 | 0.99 |
|  | III | 381.16 | 381.39 | 20.66 | 8.22 | 0.99 |
|  | IV | 217.90 | 217.90 | 13.26 | 5.11 | 0.99 |
| Graphite | I | 324.60 | 326.71 | 13.91 | 7.64 | 0.99 |
|  | II | 246.35 | 247.00 | 1.67 | 5.28 | 0.99 |
|  | III | 301.80 | 303.17 | 19.88 | 4.32 | 0.99 |
|  |  | IV | 239.62 | 239.62 | 7.785 | 11.00 | 0.99 |

* + 1. Comparison of TVFA production in non-stressed and O2-stressed reactors

In the following subsection, we compare the TVFA production in Phase III of non-stressed and O2-stressed reactors.

The TVFA production levels indicate the reaction mechanism that has taken place in the reactors. For the first two phases, there was no discernible variation between the reactors during the initial two feedings (Table 5).

A notable difference was observed between the oxygen-stressed and non-stressed reactors in the third phase. For non-stressed reactors, the overall volatile fatty acid content ranged between 0.5 – 2.4 g/L, with the majority being acetic acid. Meanwhile, the range was between 4 – 11.3 g/L in the stressed (see in Table 5), amendment, and the predominant constituent was determined as acetic acid.

It is speculated that the O2 stress introduced at the start of Phase II did not lead to obvious changes during this period. It is also noteworthy that there was no substantial difference between the O2-stressed reactor without amendment (4.05 g/L) and its counterpart (4.10 g/L). The TVFA concentration in the activated carbon-amended reactors indicated a balanced performance, whereas all the other carbon-amended reactors showed a rise in TVFA concentrations. This is interpreted as adding O2 could support elevated hydrolysis and acidogenesis. The porous structure of activated carbon could adsorb excess TVFAs. The other carbon materials could control the pH less efficiently, which negatively affected the methanogenic activity. In Phase IV, all the carbon-amended reactors performed poorly in comparison to reactors amended with activated carbon.

The role of carbon materials in anaerobic digestion (AD) is widely studied, particularly in enhancing electron transfer, microbial attachment, and buffering effects. Our findings align with previous research but also highlight important differences in performance trends over time.

Activated carbon, known for its high surface area and adsorptive properties, initially improved methane production but showed a gradual decline after Phase I, especially in the non-stressed reactors. This contrasts with studies reporting sustained enhancement via direct interspecies electron transfer (DIET) and pH stabilization (Zhang et al., 2020; Liu et al., 2019). However, under O₂-stressed conditions, activated carbon outperformed other materials in Phase II and IV, likely due to its buffering effect and ability to adsorb inhibitory compounds (Nguyen et al., 2021).

In contrast, graphene-amended reactors exhibited the highest methane yield in Phase III, indicating its strong DIET-promoting effect. This supports findings from Zhao et al. (2022), where graphene improved syntrophic interactions and electron transfer efficiency. However, similarly to the findings by Zhao et al., (2022), the long-term effectiveness of graphene apparently depends on microbial adaptation, as its impact declines over extended operation.

Graphite-amended reactors performed less effectively than activated carbon and graphene, particularly under O₂ stress. This aligns with previous studies indicating that graphite has lower conductivity and surface area, which limits its DIET capability compared to more conductive carbon materials (Zhang et al., 2019).

Carbon nanotubes, despite their strong electrical conductivity, had the least positive effect in Phase I, with methane yields 4.68% lower than that of the control reactor. This trend is consistent with Li et al. (2020), who noted that nanotubes can exhibit cytotoxic effects, potentially hindering microbial growth and biofilm formation, especially in the initial stages.

The general decline in methane production in Phase IV is likely due to the duration of the experiment rather than to the impact of O₂ stress. In our previous experience (data not shown) a similar trend was observed in fed-batch mode after Phase III, suggesting that micronutrient limitations or the accumulation of inhibitory compounds may play a significant role in performance reduction. This highlights the need for further investigation into nutrient supplementation strategies or potential inhibitory byproducts to maintain long-term reactor efficiency.

**Table 5**. Amount of total acids in the reactors during Phase II and Phase III.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | TVFA\* (g/L) | | | | |
|  | Phase II | | Phase III | | |
| *Carbon additives* | *Non-stressed reactors* | *O2 stressed reactors* | *Non-stressed reactors* | *O2 stressed reactors* |
| No amendment | 1.01 | 0.69 | 4.10 | 4.05 |
| Activated carbon | 0.66 | 0.62 | 8.06 | 8.56 |
| Graphite | 0.71 |  | 3.00 | 11.34 |
| Graphene | 0.98 | 0.81 | 2.18 | 10.78 |
| Carbon | 0.58 | 0.85 | 2.53 | 10.98 |
| Carbon nanotube | 0.71 | 0.61 | 2.41 | 9.01 |

\* T*VFA* indicates the sum of *acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid and caproic* acid.

* 1. Characterization of the anaerobic digestate feedstock used and locally produced pyrochar and hydrochar

Characterization of anaerobic digestate, PC and HC gives insights into the possible mechanisms during its application as an additive in AD or as a biofertilizer. The anaerobic digestate was light brown in colour (Fig. 4a) while PC's appearance seemed dark grey to blackish (Fig. 4b) and the HC was dark brown (Fig. 4c). It indicates that the severity of carbonization along with solid yield (Table 1) occurred more for PC than for HC.

The results of various analytical characterizations of anaerobic digestate feedstock, PC, and HC produced are shown in Table 6. The ultimate analysis of anaerobic digestate agrees with the elemental analysis of anaerobic digestate derived from high solids anaerobic digestion of corn straw, cattle manure and sludge (Chang et al., 2020). However, the proximate analysis results differed from other literature, that reported higher ash content over fixed carbon (Oliveira et a., 2023; Miliotti et al., 2020). In the present study, the proportion of ash content was lower and fixed carbon was higher. In comparison to digestate, the proportion of ash content increased from 25.58% to 29.10% in the HC case and reached the value of 33.57% in PC, but it never exceeded the proportion of fixed carbon content. Anaerobic digestate typically have higher fixed carbon and low ash content (Miliotti et al., 2020), as observed in this study. Nevertheless, this depends upon the substrates used for the AD process and its characteristics that ultimately decide the fundamental constituents of anaerobic digestate. The utilization of CD and DFL in the AD process containing lignocellulosic content could be the reason for increased fixed carbon content. The lignocellulosic content of DFL and CD used in this study were 35% and 52%, respectively (D’Silva et al., 2022a). Further, during the carbonization process, the remaining volatile matter escapes in the form of gas during slow pyrolysis and gets dissolved into the water in the case of HTC. Therefore, it can be observed that the carbon, hydrogen and nitrogen proportions enhanced for HC and PC compared to anaerobic digestate feedstock.

As the thermochemical treatment enhanced, the carbon content increased. The HC showed slightly higher hydrogen content than the feedstock anaerobic digestate. But it was much lower than that of PC. Nitrogen also showed an increase, which was more evident for PC. Decarboxylation resulted in a very sharp decrease in the oxygen content, which was the lowest for the PC. The sulfur content did not have any considerable changes with anaerobic digestate, and PC reported similar values of 0.36, and a slight decrease was observed for HC. Increased fixed carbon and ash content raised the HHV, by 36.40% for HC and 51.06% for PC.

The BET analysis showed that the volatilization of matter during the carbonization opened pores on the material's surface in both the char samples compared to the anaerobic digestate feedstock. The specific surface area of HC and PC were 7.20 and 14.79 m2/g, respectively. The BET surface area of HC and PC reported in the selected literature were between 3 and 12 m2/g (HTC of cellulose and anaerobic digestate of maize silage at 190℃ for 6 h, slow pyrolysis of different anaerobic digestates at 500℃ for 2 h) (Cong et al., 2022; Mumme et al., 2011; Correa Rodriguez et al., 2017). Variations likely stem from differences in operating conditions. For example, Correa Rodriguez et al. (2017) achieved the BET surface area of 8 m2/g at 250℃ for 3 h using an anaerobic digestate from an AD plant treating corn silage, grass silage and cattle manure at a mixing ratio of 40:30:30. Usually, chars from slow pyrolysis and HTC exhibit varied BET surface area, a higher value for PC than HC. The variation could be most probably due to differences in the porosity formation mechanism happening in the material during the processes. As volatile matter reduces, voids form, increasing porosity in PC. However, the possible mechanism during HTC might differ from that of slow pyrolysis. The anaerobic digestate constituents, such as available hemicellulose and cellulose, might have solubilized in pressurized hot water, resulting in the formation of amorphous microspheres through polymerization of intermediate hydrolysis products (Yu et al., 2023; Sevilla et al., 2009). At the same time, constituents with complex structures, such as lignin, contribute more to the solid product of the HTC, i.e., hydrochar (Dinjus et al., 2011). This would result in the adsorption of condensed compounds in the liquid on its surface, reducing the porosity. In this study, there was no nitrogen content in the PW and an increase in nitrogen content was observed in the HC, which will be further discussed in section 3.6. Another aspect of the lower porosity could be that this was analyzed directly for BET surface area without further treatment using solvents. This would mean that there might be the presence of tarry products on the surface during condensation that would have clogged the pores of the HC (Miliotti et al., 2020). The O/C and H/C atomic ratios of the digestate, HC, and PC were 0.69 and 0.75, 0.28 and 0.65, 0.47 and 0.65, respectively. Therefore, HC and PC obtained in this study are comparable with peat and lignite (Ribeiro et al., 2018). The contents of macronutrients and trace elements in anaerobic digestate, PC and HC are explained more in detail in sections 3.6 and 3.7.2.

|  |  |  |
| --- | --- | --- |
| A bowl of brown powder  Description automatically generated  (a) | A black powder in a glass dish  Description automatically generated  (b) | A bowl of dirt in a glass  Description automatically generated  (c) |

**Fig.3 (a-c)** The pictorial view of (a) anaerobic digestate feedstock, (b) pyrochar and (c) hydrochar

**Table 6.** Characteristics of anaerobic digestate feedstock used, pyrochar and hydrochar

|  |  |  |  |
| --- | --- | --- | --- |
|  | Anaerobic digestate | Pyrochar | Hydrochar |
| *Physical* | | | |
| pH | 6.98 ± 0.14 | 9.39 ± 0.25 | 8.21 ± 0.07 |
| *Proximate analysis* | | | |
| Moisture (%) | 8.23 ± 0.05 | 3.91 ± 0.12 | 5.23 ± 0.31 |
| Total solids (%) | 91.77 ± 0.05 | 96.09 ± 0.12 | 94.77 ± 0.31 |
| Volatile solids (%) | 35.98 ± 0.64 | 15.79 ± 0.56 | 17.76 ± 1.22 |
| Fixed carbon (%) | 30.21 ± 1.34 | 51.96 ± 0.78 | 47.91 ± 1.52 |
| Ash content (%) | 25.58 ± 0.91 | 33.57 ± 0.36 | 29.10 ± 0.11 |
| Fuel ratio | 0.84 ± 0.05 | 4.94 ± 0.34 | 2.71 ± 0.26 |
| *Ultimate analysis* | | | |
| Carbon (%) | 35.75 ± 0.18 | 51.98 ± 1.18 | 45.70 ± 0.25 |
| Hydrogen (%) | 4.48 ± 0.02 | 6.48 ± 0.07 | 5.23 ± 0.16 |
| Nitrogen (%) | 0.99 ± 0.05 | 1.18 ± 0.06 | 1.86 ± 0.10 |
| Sulfur (%) | 0.36 ± 0.05 | 0.36 ± 0.01 | 0.33 ± 0.03 |
| Oxygen (%) | 33.11 ± 1.23 | 6.54 ± 0.87 | 17.72 ± 0.49 |
| *Atomic ratio* | | | |
| O/C ratio | 0.69 ± 0.03 | 0.28 ± 0.01 | 0.47 ± 0.03 |
| H/C ratio | 0.75 ± 0.00 | 0.65 ± 0.02 | 0.65 ± 0.02 |
| (O+N)/C ratio | 0.72 ± 0.03 | 0.29 ± 0.01 | 0.51 ± 0.03 |
| N/C ratio | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.03 0.00 |
| N/H ratio | 0.02 ± 0.00 | 0.01 ± 0.00 | 0.03 ± 0.00 |
| N/O ratio | 0.03 ± 0.00 | 0.21 ± 0.04 | 0.12 ± 0.01 |
| *Energy content* | | | |
| HHV (MJ/kg) | 12.63 ± 0.31 | 25.81 ± 0.66 | 19.86 ± 0.06 |
| *Physisorption isotherm* | | | |
| BET surface area (m2/g) | 2.24 | 14.79 | 7.20 |
| Total pore volume (cm2/g)) | 0.03 | 0.12 | 0.07 |
| Average pore diameter (nm) | 21.37 | 32.06 | 40.55 |
| *Macronutrients and trace elements* | | | |
| Iron (mg/g) | 820.21 ± 1.23 | 1243.42 ± 17.21 | 954.01 ± 12.37 |
| Calcium (mg/g) | 1.02 ± 0.05 | 3.42 ± 0.11 | 2.53 ± 0.14 |
| Magnesium (mg/g) | 0.32 ± 0.02 | 0.71 ± 0.04 | 0.54 ± 0.01 |
| Potassium (mg/g) | 0.43 ± 0.02 | 0.76 ± 0.02 | 0.47 ± 0.03 |
| Sodium (mg/g) | 124.11 ± 1.34 | 312.36 ± 2.22 | 224.03 ± 2.12 |
| Phosphorusa (mg/g) | 0.31 ± 0.07 | 0.17 ± 0.04 | 0.24 ± 0.06 |

aas orthophosphate

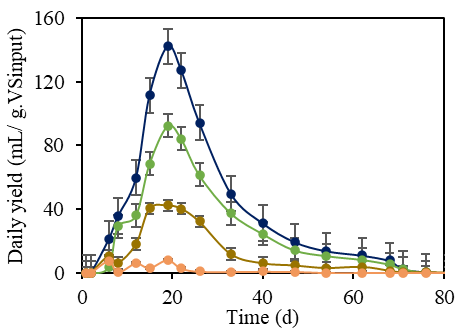
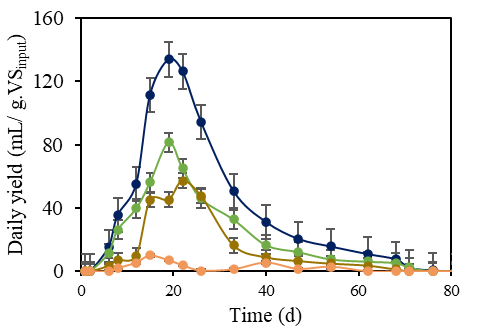
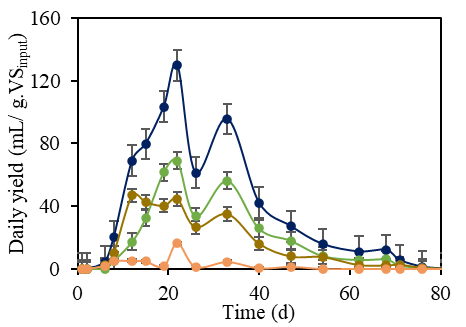
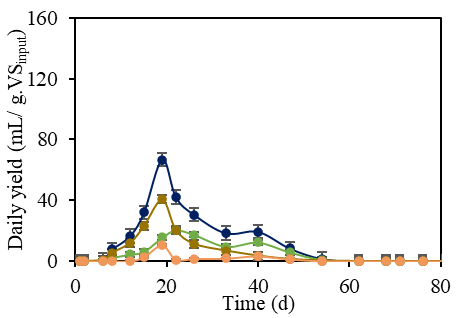
* 1. Effect of locally prepared carbon materials in AD

Figure 4(a-d) presents daily yields of biogas, biomethane, biocarbon dioxide, and residual gas. Biogas production began on day 4, while biomethane initiation in the control reactor was delayed until day 6, indicating late methanogenic activity (Fig. 4a). In all the other reactors, the biomethane yield was initiated from day 4 (Fig. 4(b-d)) and seemed to be higher in the reactor DFL+FVW+CD+PC, achieving 11.63 mL/g.VSinput. Biogas and biomethane production increased significantly within the first 10 days. Later, on the 20th day of operation, the biogas yields crossed 120 mL/g.VSinput in all the reactors except the reactor CD. The adequate organic loading rate (> 20 g. VS/L) required for a CD-based AD reactor (Hamzah et al., 2023) was not available in the CD reactor, which might be the reason for lower methane yields. DFL+FVW+CD+PC and DFL+FVW+CD+HC exhibited more stable biogas and biomethane yields than DFL+FVW+CD, with the latter producing 2.06% more biogas. In contrast, a pronounced dip in biogas and biomethane yields was observed in the reactor DFL+FVW+CD during the 26th day of operation. Biogas yields were statistically insignificant (p > 0.05, f < fcrit, ANOVA).

Methane production in DFL+FVW+CD+PC and DFL+FVW+CD+HC started earlier than in CD and DFL+FVW+CD (Fig. 5a-d). Biomethane yields in DFL+FVW+CD+PC and DFL+FVW+CD+HC followed a similar trend to biogas yields. In contrast, a sinusoidal trend was observed in the DFL+FVW+CD reactor, denoting the instability in the methanogenic activity. The methane yields were also shown to be non-significant statistically, identical to the biogas yield. In general, the daily yields (biogas, biomethane and biocarbon dioxide) had no statistical significance (p > 0.05 and f < fcrit (one-way ANOVA)).

Figure 5(a-d) shows cumulative biogas yields and composition. The results showed that the DFL+FVW+CD+HC outranked all the other reactors with the highest methane yield of 479 ± 64 mL/g. VSinput followed by DFL+FVW+CD+PC with a methane yield of 411 ± 43 mL/g. VSinput. Interestingly, the reactors, apart from the control reactor, showed similar profiles that influenced the statistical results using one-way ANOVA. However, as the day progressed, the observed data showed statistical significance (p < 0.05 and f > fcrit (one-way ANOVA)). The biogas yield showed statistical significance for the experimental data between days 24 and 79. It was between 17 and 79 d for methane yield and days 20 and 79 for carbon dioxide yield. Tukey’s test proved that the data of the DFL+FVW+CD reactor differed from the DFL+FVW+CD+PC and DFL+FVW+CD+HC. At the same time, no statistical significance was found between DFL+FVW+CD+PC and DFL+FVW+CD+HC. Table 7 compares the results achieved in DFL+FVW+CD+PC and DFL+FVW+CD+HC reactor with other literature. The PC and HC with PW enhanced methane production and digestion efficiency over CD and DFL+FVW+CD.

To identify any difference in the kinetic parameters in the respective reactors, the model fitting values obtained using the modified Gompertz model are given in Table 8. The coefficient of determination (R2) values for the experimental and model fitting results were around 0.99, implying that the modified Gompertz model fits the experimental biomethane yield profile well. For the reactors DFL+FVW+CD, DFL+FVW+CD+PC and DFL+FVW+CD+HC reactors, the λm was reduced to 9.67%, 42.46%, and 40.52% compared to the control reactor. Meanwhile, the Rm values increased by 66.89%, 76.09%, and 80.14% compared to the control reactor. In short, it could be interpreted that PC was more successful in reducing the lag phase; meanwhile, HC with PW enhanced the methane production yield. It could also be depicted that the principal mechanism undergone could be entirely different in both reactors, which could have resulted in variations in the microbial composition and activity. The methane production yields for DFL+FVW+CD with a lag period of 9.34 d showed that the reactor produced a small amount of methane in the early stage. However, the lower lag period in reactors DFL+FVW+CD+PC and DFL+FVW+CD+HC explains the rapid initiation of methanogenic activity, providing much earlier methane formation in the respective reactors. In these reactors, following the microbial growth phases, a moderate methane yield was obtained during the lag phase till reaching the log period, which experienced rapid methane production. The PW contributed to the biogas and biomethane yields in DFL+FVW+CD+HC. A separate biomethane potential experiment of process water used in this study yielded 38.52 mL/g. VSinput of biogas and 29.14 mL/g. VSinput of biomethane. The daily and cumulative yields of biogas and its composition during AD of PW are given in the supplementary file, Fig. S2 (a, b). It is already reported in the literature that the soluble volatiles present in the form of volatile acids (acetic acid, butyric acid etc.) in the PW are the source of readily available feed for the microbes and produce biogas, preferably high in methane content (Roy et al., 2022; Erdogan et al., 2015). High concentrations of TVA were found in the PW in the present study. In the case of the reactor DFL+FVW+CD, the sinusoidal curve suggests methanogen inhibition due to excess VFAs, stabilizing once VFA production and consumption balanced. This could be correlated with the increased CO2 production during the unstable phase, which is the by-product of VFA production during acidogenesis.



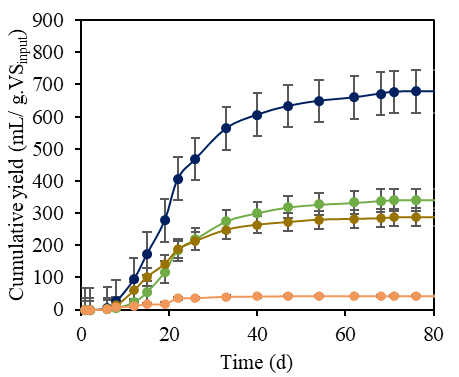
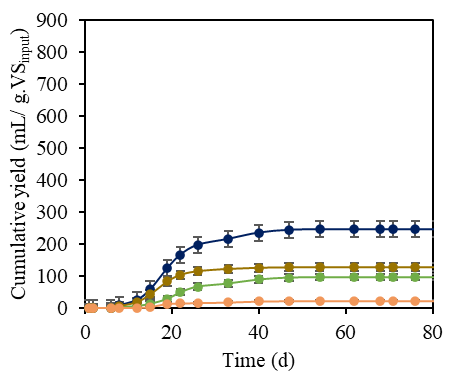
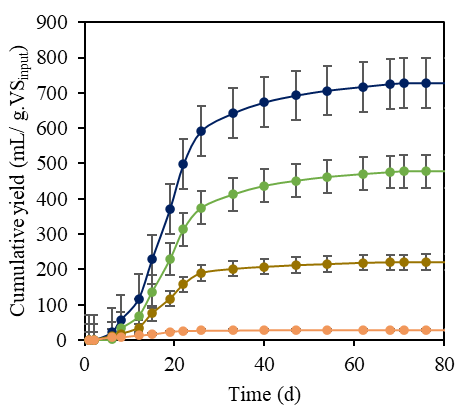
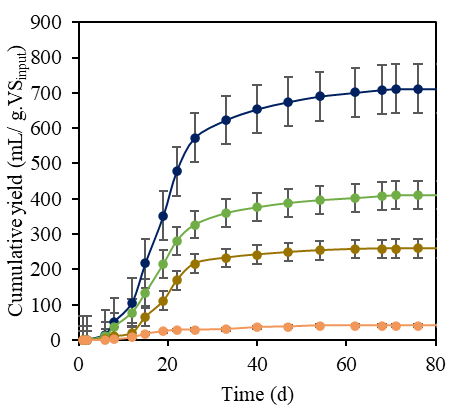
(b)

(c)

(d)

(a)

**Fig.4 (a-d)** Daily biogas production and its composition in the reactors (a) CD, (b) DFL+FVW+CD, (c) DFL+FVW+CD+PC and (d) DFL+FVW+CD+HC



(a)

(b)

(c)

(d)

**Fig.5 (a-d)** Cumulative biogas yield and its composition achieved in the reactors, (a) CD, (b) DFL+FVW+CD, (c) DFL+FVW+CD+PC, and (d) DFL+FVW+CD+HC

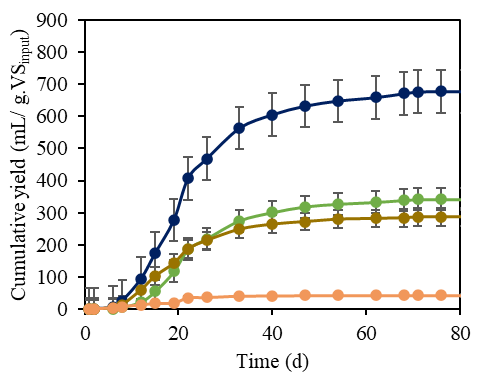
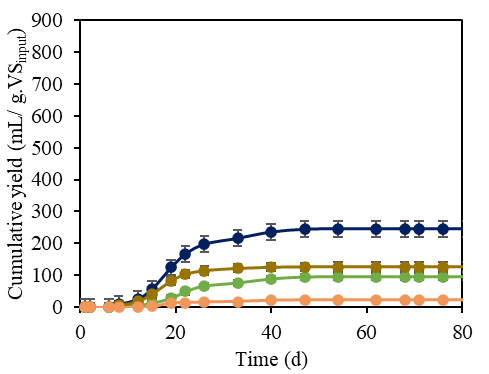
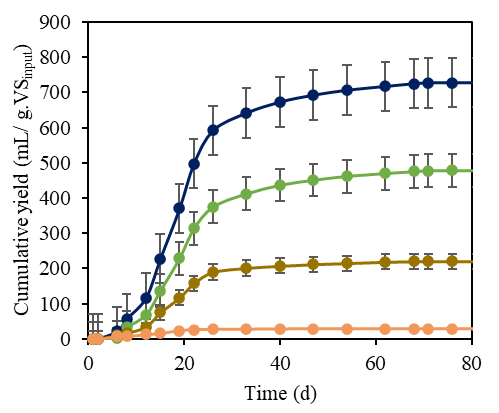
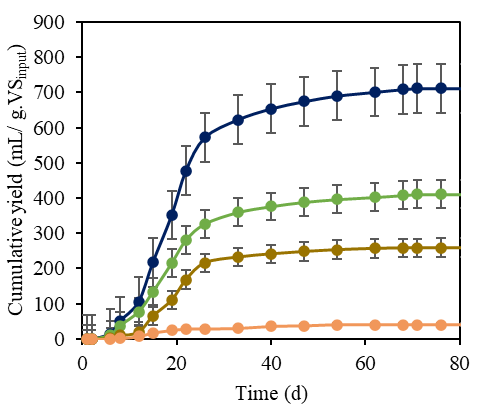
**Table 7.** Comparison of the effects of digestate-derived pyrochar on methane production from AD of solid organic wastes.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Biochar type, dosage | Operational solids concentration (% TS), Temperature (℃) | Digestive substrates | Methane yield (mL/ g VS) | | % increase | References |
| Without addition | With addition |
| PC, 6 g/L | 20, 37 | Kitchen waste | 308.60 | 341.00 | 10.50% | Li et al., (2022) |
| PC, 0.85 g/L | 2, 37 | DFL, FVW and CD | 342.73 ± 38.12 | 411.21 ± 43.21 | 19.98% | This study |
| HC, 4 g/L | 5, 35 | Sewage sludge and swine manure | 206.3 | 308.4 | 33.11% | Xu et al., (2020) |
| HC, 10 g/L | 5, 35 | Food waste and sewage sludge | 104.82 | 133.11 | 21.25% | Xu et al., (2022) |
| HC, 0.85 g/L | 2, 37 | DFL, FVW and CD | 342.73 ± 38.12 | 478.56 ± 63.47 | 39.63% | This study |

**Table 8.** Experimental results, kinetic parameters, theoretical estimations, and biodegradability index of the reactors for biomethane production in the reactors.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Reactor name | Experimental biomethane yield (mL/ g. VSinput) | Predicted biomethane yield (mL/ g. VSinput) | Pm (mL/ g. VSinput) | Rm (mL/ g. VSinput. d) | λm (d) | T90 (d) | Teff (d) | R2 | Error (%) | Theoretical biomethane production (mL/ g. VSinput) | Biodegradability index (%) |
| CD | 96 ± 7 | 95.65 | 95.67 | 4.79 | 10.34 | 35 | 25 | 0.99 | 1.68 | 219.42 | 43.58 |
| DFL+FVW+CD | 343 ± 38 | 335.59 | 345.66 | 14.47 | 9.34 | 40 | 31 | 0.99 | 1.95 | 506.47 | 67.67 |
| DFL+FVW+CD+PC | 411 ± 43 | 424.11 | 424.28 | 20.04 | 5.95 | 34 | 29 | 0.99 | 2.86 | 506.47 | 81.19 |
| DFL+FVW+CD+HC | 479 ± 64 | 477.73 | 479.32 | 24.12 | 6.15 | 35 | 28 | 0.99 | 1.21 | 566.73 | 84.44 |

* 1. Process parameters



(a)

(b)

(c)

(d)

Table 9 shows the initial and final characteristics of the slurry samples from all the reactors. The initial pH in all the reactors was in the range of 6.21 – 7.05. Mixing DFL and FVW with CD reduced the initial pH in the reactors other than the control reactor (only with CD). It could be because the pH of FVW was lower than 6.00 being an easily soluble substrate causing the release of H+ ions (D’Silva et al., 2022a). In the case of the reactors amended with HC and PC, there was a slight increase in the pH compared to the co-digestion reactor without any amendment resulting from higher pH values of PC and HC.

The TVA concentration in the initial slurry collected from the reactors recorded 1334 ± 234 mg/L in CD, 1964 ± 312 mg/L in DFL+FVW+CD, 2127 ± 327 mg/L in DFL+FVW+CD+PC, and 2356 ± 342 mg/L in DFL+FVW+CD+HC. At the end of the experiments, the TVA concentrations noted were 1674 ±196 mg/L in CD, 2742 ± 278 mg/L in DFL+FVW+CD, 1263 ± 246 mg/L in DFL+FVW+CD+PC, and 1673 ± 326 mg/L in DFL+FVW+CD+HC. Usually, in anaerobic digesters, the rapid hydrolysis leads to rapid production of TVA in the reactors. Excess TVA accumulation, if not consumed by methanogens, lowers pH, inhibits methanogenic activity, and increases CO2 production, potentially leading to digester failure, as observed in failed full-scale digesters (Gandhi et al., 2022; Vats et al., 2019). The failed full-scale digesters have been reported to have a lower pH and enhanced TVA concentration (Tyagi et al., 2021; Isha et al., 2020). Higher methane yields in PC and HC-amended reactors suggest enhanced methanogenic activity, likely due to trace element availability (iron, sodium, magnesium, potassium, calcium) in their ash content, which supports microbial growth (Wang et al., 2023; Ortner et al., 2014; Zhang et al., 2012). Oxides and zero-valent forms of iron are known to stimulate microbial activity, enhancing biogas production and VS reduction (Domrongpokkaphan et al., 2021; Zhang et al., 2020; Ugwu et al., 2020). The TVA concentration in DFL+FVW+CD was higher, indicating that methanogenic activity was inhibited probably due to the inefficient biochemical process that occurred during the reactions, which might have led to a reduced methane production yield. The situation was different in the DFL+FVW+CD+HC reactor, which was already infused with VFA-concentrated PW, leading to an early proliferation of acetogenic and methanogenic activity. The reason for enhancement in methane production might also be due to the buffering capacity of the PC and HC, which could be explained using the parameter total alkalinity. Earlier in one study, it was reported that PC can enhance methanogenic activity, while HC can contribute to methane production as a substrate with the available VS content (Mumme et al., 2014). The study observed no apparent effect of PC on biogas production, whereas HC increased the methane yield by 32% (Mumme et al., 2014). However, in that study, PC had shown a significant ammonium adsorption capacity, which is crucial in AD process stability and will be discussed later in this section.

Bicarbonates, the primary form of TA, buffer pH (6.8–7.2) by reacting with ammonia and CO2 to form ammonium bicarbonate. VFA production consumes TA, reducing buffering capacity and potentially destabilizing the system (Kovács et al., 2013; Dague et al., 1968). Thus, an accumulation of VFA damages the system's buffering capacity and reduces pH.

The TVA to TA ratio (ideally <1) is a more reliable indicator of AD stability than TA alone, as it reflects both VFA formation and buffering capacity. As previously explained, it can give a better idea of the formation of VFA and the simultaneous consumption of bicarbonate alkalinity during the process. When this ratio is equal to 1 or greater than one, chances are very high that the buffering capacity within the digester remains too low, and its failure is impending (Chynoweth & Mah, 1971; Sanders et al., 1965). It can be seen from Table 8 that the TVA to TA ratio in the initial and final samples in all the reactors was well below the value of 1, indicating sufficient buffering capacity was held within the reactors during the digestion period. The buffering capacity of the system will be further defined using the TAN and CO2 concentrations.

The values of TAN observed initially in the reactors were 1154 ± 322 mg/L for CD, 1246 ± 281 mg/L for DFL+FVW+CD, 1754 ± 518 mg/L for DFL+FVW+CD+PC and 1728 ± 331 mg/L for DFL+FVW+CD+HC. The initial TAN concentration could be due to the dissociation of nitrogenous compounds, especially in CD, and also the presence of trace concentrations already in the liquid digestate used for preparing the initial slurry. The final values of TAN in the reactors were 1595 ± 305 mg/L in CD, 2165 ± 590 mg/L in DFL+FVW+CD, 1257 ± 370 mg/L in DFL+FVW+CD+PC and 1656 ± 561 mg/L in DFL+FVW+CD+HC. The dissociation of ammonia/ammonium ions is more favourable under higher pH levels, as was the case for PC and HC-amended reactors. Hence, the free ammonia concentration was higher in HC, and PC-amended reactors compared to co-digestion reactor.

It is known that ammonia/ammoniacal nitrogen is a by-product of the deamination of amino acids produced from protein degradation during AD (Meegoda et al., 2018). When biochar is added to an AD reactor, ammonia adsorption is the prominent phenomenon observed (Cai et al., 2022). A raw char's average ammonia adsorption capacity is also reported to be around 50 mg/g (Cai et al., 2022). Also, in the present study, the calcium content of PC and HC were 3.42 ± 0.11 mg/g and 2.53 ± 0.14 mg/g, respectively, a major constituent of ash content generally observed in biochar (Rahmat et al., 2023). Therefore, about 41.01 mg and 30.36 mg of calcium are additionally present inside the reactors amended with PC and HC, which can generally increase the chance of forming CaCO3 (Kang et al., 2020). When this is linked with the CO2 concentration in the biogas and its partial pressure in headspace, there is a high chance that an interaction of ammonium/ammonia ions with bicarbonates in the closed carbonate system, that might have taken place within the reactors, as reported in a previous study (van Langerak & Lettinga, 1998), more than the case of calcium and CO2 interaction.

Ammonia/ammonium ions in HC and PC reactors likely reduced CO2 in the headspace by forming ammonium bicarbonate, a common reaction in anaerobic digesters (Isha et al., 2021). Moreover, these ammonium bicarbonates settle down as solids (Sutter et al., 2017) and only decompose back to CO2, TAN and water at a slow rate at ambient temperature (< 45 ℃) and increase the rate as the temperature goes beyond 60℃ (Streekstra & Livingston, 2020). The initial and final pH found in the respective reactors also support the formation of bicarbonates in an aqueous closed carbonate system (Table 8). Thus, there is a high chance that the exact mechanism might have occurred more in the HC-amended and the PC-amended reactor but at a slower rate.

Theoretical estimations suggest that with the partial pressure occurring within the reactors daily, a total of 0.23 moles in CD, 0.50 moles in DFL+FVW+CD, 0.45 moles in DFL+FVW+CD+PC and 1.31 moles in DFL+FVW+CD+HC of CO2 were solubilized within the respective reactors. Considering that an equal amount of TAN is consumed for producing ammonium bicarbonate, 273 mg TAN/L in CD, 607 mg TAN/L in DFL+FVW+CD, 539 mg TAN/L in DFL+FVW+CD+PC and 1594 mg TAN/L in DFL+FVW+CD+HC are consumed. Lower CO2 reduction observed in the PC amended reactor may be due to the influence of H2S production and subsequent adsorption that might have occurred on the surface of the PC, compared to HC. Hence, it is essential to verify the concentration of H2S and subsequent adsorption that might have occurred in PC and HC-amended reactors.

**Table 9.** Estimated process parameters during the initial and final phases observed in the reactors

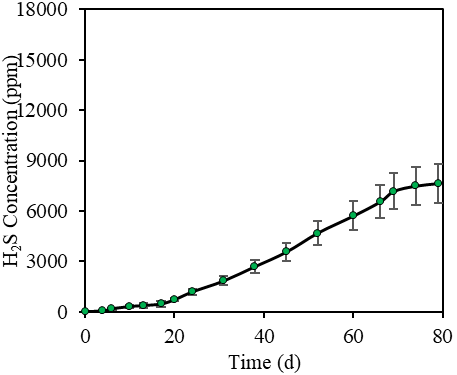
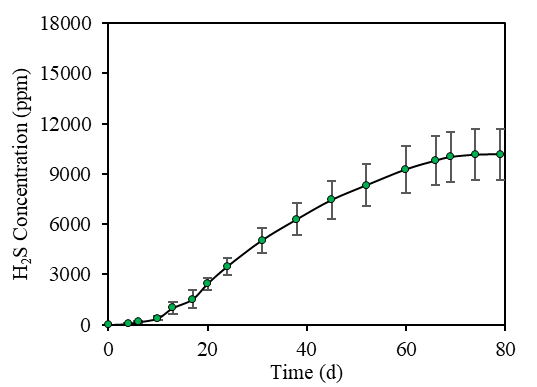
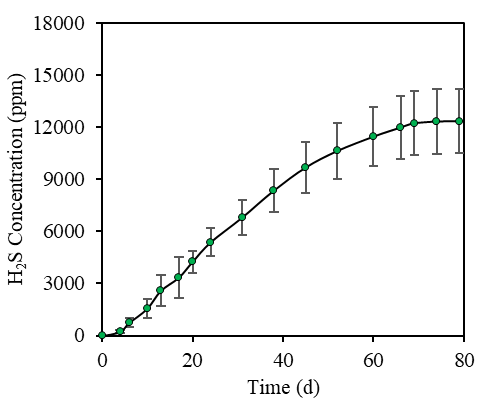
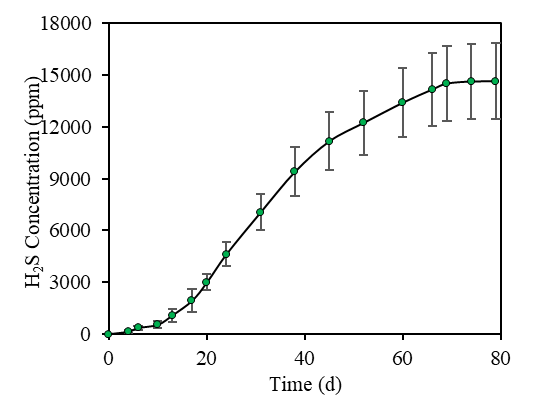
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Reactor nomenclature | Initial VS (g/L) | pH | | EC (µS/cm) | | TDS (mg/L) | | TVA (mg/L) | | TA (mg/L as CaCO3) | | TVA/TA ratio | | TAN (mg/L) | | FAN (mg/L) | | VS removal (%) |
| Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final |
| CD | 4.06 | 7.05 ± 0.25 | 7.09 ± 0.01 | 2365 ± 526 | 2243 ± 436 | 1350 ± 96 | 1230 ± 112 | 1334 ± 234 | 1674 ±196 | 4532 ± 236 | 4234 ± 261 | 0.29 ± 0.04 | 0.39 ± 0.02 | 1154 ± 322 | 1595 ± 305 | 25 ± 9 | 52 ± 18 | 32 ± 2 |
| DFL+FVW+CD | 8.27 | 6.21 ± 0.22 | 6.49 ± 0.02 | 2740 ± 113 | 2550 ± 126 | 1860 ±123 | 1530 ± 102 | 1964 ± 312 | 2742 ± 278 | 5921 ± 165 | 4963 ± 124 | 0.33 ± 0.04 | 0.55 ± 0.04 | 1246 ± 281 | 2165 ± 590 | 4 ± 2 | 33 ± 4 | 68 ± 5 |
| DFL+FVW+CD+PC | 8.27 | 6.91 ± 0.36 | 7.17 ± 0.21 | 3260 ± 134 | 2270 ± 156 | 2020 ± 193 | 1820 ± 167 | 2127 ± 327 | 1263 ± 246 | 6342 ± 266 | 5231 ± 197 | 0.33 ± 0.04 | 0.24 ± 0.03 | 1754 ± 518 | 1257 ± 370 | 31 ± 13 | 52 ± 12 | 81 ± 3 |
| DFL+FVW+CD+HC | 14.23 | 6.66 ± 0.13 | 7.62 ± 0.04 | 3430 ± 178 | 2190 ± 182 | 2240 ± 177 | 1960 ±153 | 2356 ± 342 | 1673 ± 326 | 7313 ± 237 | 5631 ± 312 | 0.32 ± 0.03 | 0.30 ± 0.04 | 1728 ± 331 | 1656 ± 561 | 13 ± 5 | 118 ± 18 | 79 ± 4 |

3.5. Profile of hydrogen sulfide production

Figure 6 (a-d) shows the cumulative evolution of H2S within the respective reactors. The cumulative concentrations achieved within the reactors during the experimental run were 12531 ± 1853 ppm in the CD reactor, 14634 ± 2195 ppm in the DFL+FVW+CD reactor, 7632 ± 1145 ppm in the DFL+FVW+CD+PC and 10178 ± 1527 ppm in the DFL+FVW+CD+HC reactor. This would mean that CD substrate was the major contributor to the H2S production due to its higher proportion (Table 3) and high elemental sulfur content availability (D’Silva et al., 2022a). PC and HC had an H2S-reducing effect in the respective reactors. Compared to the co-digestion reactor, the PC-amended reactor removed 47.86% of H2S, and HC amended reactor removed 30.45%. Researchers have suggested different mechanisms behind this reducing effect. Chemisorption of different species (TAN, H2S, CO2 etc.) onto the surface of PC and HC is one way that may occur during the process. Chemisorption occurs when these molecules are reacted with the functional groups such as carboxylic, carbonyls, phenol, ether, lactone, and pyrone, which are usually present in PC and HC, observed in this study as well. The reaction occurs when the molecule gets adhered to the surface of the PC or HC by weak van der Waal forces. Moreover, H2S diffusion into the aqueous medium occurs under alkaline conditions (greater pKa value = 7.2) and dissociates into HS− and H+ (Kanjanarong et al., 2017, Xu et al., 2014). Another mechanism that could happen is that the thiol (-SH bonds) group that is formed on the carbon structure is further reacted with oxygen-based functional groups, resulting in the formation of sulfur oxide species (S0 and SO42-) (Xu et al., 2014). Usually, SO42− is formed on the surface of the biochar where oxygen is sufficient, while elemental sulfur (S0) is formed in biochar pores due to limited oxygen (Kanjanarong et al., 2017). The cumulative yield of H2S concentration from PW fed reactor and FTIR peaks of anaerobic digestate, PC, and HC are given in the supplementary file (Fig. S3 & S4).

With the amount of PC and HC fed into the reactors being relatively lower, chemisorption and thiol oxidation mechanisms may have limitations in this study. However, chemisorption could be the main reason behind the lagged H2S production in the PC-amended reactor compared to other reactors. Initially, the chemisorption of H2S might have taken place in the presence of PC with a highly alkaline nature. It is known that when biomass is combusted at higher temperatures under inert conditions, the organic compounds get decomposed and increase in ash and trace element contents, giving a basic pH nature to its surface (Mireles et al., 2019). Hence, it can be generalized that chemisorption can occur with the dissociated HS- ions in the presence of PC.

Another factor could be the presence of iron in PC and HC. Generally observed in its oxide form in any biomass, there is a high chance that the dissociated H2S under higher pH conditions might have reacted with the iron available in PC and HC to form iron sulfide and water. The higher alkaline nature and iron content in PC might have supported H2S reduction in PC amended reactor compared to HC amended reactor. Similar events of sulfide interaction with iron have been reported while digesting sewage sludge (Shakeri et al., 2017). Oliveria et al. (2020) found that the maximum H2S adsorption capacity of raw PC is about 160 mg H2S/g biochar, and a maximum removal of more than 98% was achieved while treating sulfide-laden wastewater using the addition of PC in anaerobic treatment technology. In another study, Choudhary and Lansing (2020) observed that adding unmodified PC can reduce up to 91% of H2S if added to an AD reactor treating CD. The study also observed that the mechanism of sulfide removal in a biochar-amended AD reactor is similar to an external biochar-based biogas scrubbing unit columns.



(a)

(b)

(c)

(d)

**Fig.6 (a-d)** The cumulative hydrogen sulfide concentrations produced in the reactors, (a) CD, (b) DFL+FVW+CD, (c) DFL+FVW+CD+PC, and (d) DFL+FVW+CD+HC

* 1. Volatile solids mass removal

Volatile solids conversion to biogas production during anaerobic digestion will reduce the VS in the digested slurry and reflect the digestion efficiency. Establishing a suitable environment will holistically support the overall AD reactions, delivering stable operation and adequate substrate degradation, resulting in enhanced methane yield as witnessed, subsequently reducing the VS content in the digested slurry (D’Silva et al., 2022a; Gaur et al., 2017). The VS content in the substrate while fed into the reactors in the initial stage was in the ranges of 4.06 g/L (CD) to 14.32 g/L (DFL+FVW+CD+HC) (Table 9). The fed VS content was reduced gradually as the days progressed and converted to biogas, achieving a removal efficiency between 32 ± 2 and 81 ± 3 %. A substantial VS reduction was seen in the reactors fed with PC and HC (Table 9). The maximum VS removal in the reactors was observed in the order of DFL+FVW+CD+PC > DFL+FVW+CD+HC > DFL+FVW+CD > CD. The results were much higher than the author’s previous study that reported VS removal of 55% during co-digestion of DFL, FVW and CD at 6%TS and 37°C (D’Silva et al., 2022a) and observed during the AcoD of activated sludge, anaerobic granular sludge and CD that reported a maximum VS removal of 57 % (Gaur et al., 2017). As previously conveyed, the biochemical reactions during the hydrolysis and acidogenesis contributed to VS reduction in the reactors. Hence, the reduced VS will be evident in the reactors, especially in the DFL+FVW+CD, DFL+FVW+CD+PC and DFL+FVW+CD+HC. It should be specified that the initial VS loading in the reactor DFL+FVW+CD+HC was much higher than in other reactors due to the supplementation of PW. Despite that, the reactor could give a stable performance and produce a much better biogas quality with respect to methane content. In addition, the supplementation of PC and HC with PW might have synergistically influenced the microbial activity that led to the utilization of VS and VFAs for biogas production, bridging the gap between VFAs production and consumption, buffering the pH inside the system (Gaur et al., 2017).

* 1. Elemental mass balance

Fig.7 (a-d) shows the elemental level mass balance of all the reactors. From Fig. 7(a), it can be inferred that only 10.45% of carbon and 7.40% of hydrogen have contributed to the biogas produced (in the form of either CH4 or CO2), and the remaining were present in the slurry either in the form of soluble forms (VFA) or unutilized VS present in solid digestate in the reactor CD. This also is in agreement with the reduced VS mass conversion observed in the reactor.

This vastly changed in the case of the other three reactors (Fig. 7b-d). The carbon conversion to biogas in the reactors DFL+FVW+CD, DFL+FVW+CD+PC, and DFL+FVW+CD+HC were 42.43%, 41.77%, and 70.43%, respectively. In the case of hydrogen, the percentage of conversion was 34.84% for DFL+FVW+CD, 37.68% for DFL+FVW+CD+PC, and 64.84% for DFL+FVW+CD+HC. Likewise, nitrogen and sulfide conversions seem to align with the claims portrayed in section 3.3 concerning ammonia-bicarbonate interaction and sulfide dissociation phenomenon. Sulfide adsorption was confirmed in DFL+FVW+CD+PC reactors as the solid digestate had a higher elemental sulfur proportion than DFL+FVW+CD and DFL+FVW+CD+HC reactors. The residual gas was assumed to be NH3 since the possibility of ammonia production was confirmed, as discussed in section 3.3. It was well associated with the elemental mass balance of nitrogen in the respective reactors. In the DFL+FVW+CD+HC reactor, there was an excess influence of nitrogen since HTC was also carried out using liquid digestate to maintain the reactor's solid concentration (15%TS). During HTC, nitrogen seems to be adsorbed to the solid portion from the liquid, reducing nitrogen in process water. During HTC, the nitrogen gets released initially from the biomass to the liquid; however, as the porous structures are formed, the soluble nitrogen gets adsorbed to the surface of the HC (Alhnidi et al., 2020).

**Carbon (100%)**

CD = 46.68 g

LD = 15.40 g

Total = 62.08 g

0.001 g H (0.02% Hin)

0.001 g N (0.02% Nin)

2.75 g C (4.42% Cin)

0.46 g H (7.36% Hin)

**Nitrogen (100%)**

CD = 2.15 g

LD = 2.80 g

Total = 4.95 g

**Hydrogen (100%)**

CD = 2.05 g

LD = 4.20 g

Total = 6.25 g

**Ash (100%)**

CD = 14.85 g

LD = 0.70 g

Total = 15.55 g

**Sulfur (100%)**

CD = 3.26 g

LD = 2.80 g

Total = 4.66 g

3.74 g C (6.03% Cin)

0.01 g H (0.02% Hin)

0.45 g S (9.66% Sin)

**Ash (79.16% Ashin)**

Total = 12.31 g

**Nitrogen (24.04% Nin)**

Total = 1.19 g

**Carbon (46.12% Cin)**

Total = 28.63 g

**Hydrogen (20.32% Hin)**

Total = 1.27 g

**Sulfur (3.43% Sin)**

Total = 0.16 g

**Anaerobic Digestion of CD**

(Feeding rate:1% TS, Temperature: 37℃)

**Liquid slurry and other losses**

Carbon = 26.96 g C (43.43% Cin)

Hydrogen = 4.50 g H (72.00% Hin)

Sulfur = 4.05 g S (86.91% Sin)

Nitrogen = 3.76 g N (75.95% Nin)

Ash = 3.24 g (20.83% Ashin)

**CH4**

**CO2**

**H2S**

**NH3**

(a)

**Carbon (100%)**

DFL = 11.38 g

FVW = 14.38 g

CD = 33.19 g

LD = 15.40 g

Total = 87.84 g

0.001 g H (0.02% Hin)

0.001 g N (0.02% Nin)

20.13 g C (22.92% Cin)

3.36 g H (33.38% Hin)

**Nitrogen (100%)**

DFL = 0.52 g

FVW = 0.61 g

CD = 1.53 g

LD = 2.80 g

Total = 6.08 g

**Hydrogen (100%)**

DFL = 1.52 g

FVW = 2.28 g

CD = 1.46 g

LD = 4.20 g

Total = 10.01 g

**Ash (100%)**

DFL = 6.13 g

FVW = 29.64 g

CD = 14.85 g

LD = 0.70 g

Total = 51.31 g

**Sulfur (100%)**

DFL = 0.39 g

FVW = 0.76 g

CD = 3.26 g

LD = 2.80 g

Total = 5.81 g

17.14 g C (19.51% Cin)

0.09 g H (1.44% Hin)

1.47 g S (29.69% Sin)

**Ash (52.60% Ashin)**

Total = 26.99 g

**Nitrogen (9.91% Nin)**

Total = 0.60 g

**Carbon (14.54% Cin)**

Total = 12.78 g

**Hydrogen (10.08% Hin)**

Total = 1.01 g

**Sulfur (6.46% Sin)**

Total = 0.32 g

**Anaerobic Co-digestion of DFL, FVW and CD**

(Feeding rate:2% TS, Temperature: 37℃)

**Liquid slurry and other losses**

Carbon = 37.79 g C (43.02% Cin)

Hydrogen = 5.59 g H (55.84% Hin)

Sulfur = 4.21 g S (72.46% Sin)

Nitrogen = 5.47 g N (89.96% Nin)

Ash = 24.32 g (47.39% Ashin)

**CH4**

**CO2**

**H2S**

**NH3**

(b)

**Carbon (100%)**

DFL = 11.38 g

FVW = 14.38 g

CD = 46.68 g

LD = 15.40 g

PC = 7.13 g

Total = 94.97 g

0.0006 g H (0.01% Hin)

0.0029 g N (0.05% Nin)

24.20 g C (25.48% Cin)

4.03 g H (37.21% Hin)

**Nitrogen (100%)**

DFL = 0.52 g

FVW = 0.61 g

CD = 2.15 g

LD = 2.80 g

PC = 0.14 g

Total = 6.22 g

**Hydrogen (100%)**

DFL = 1.52 g

FVW = 2.28 g

CD = 2.05 g

LD = 4.20 g

PC = 0.78 g

Total = 10.83 g

**Ash (100%)**

DFL = 6.13 g

FVW = 29.64 g

CD = 14.85 g

LD = 0.70 g

PC = 4.03 g

Total = 55.34 g

**Sulfur (100%)**

DFL = 0.39 g

FVW = 0.76 g

CD = 3.26 g

LD = 1.40 g

PC = 0.04 g

Total = 5.85 g

15.47 g C (16.29% Cin)

0.05 g H (0.46% Hin)

0.77 g S (13.16% Sin)

**Ash (53.56% Ashin)**

Total = 29.64 g

**Nitrogen (13.65% Nin)**

Total = 0.85 g

**Carbon (16.26% Cin)**

Total = 15.44 g

**Hydrogen (9.78% Hin)**

Total = 1.06 g

**Sulfur (17.82% Sin)**

Total = 0.77 g

**Anaerobic Co-digestion of DFL, FVW, CD and PC**

(Feeding rate:2% TS, Temperature: 37℃)

**Liquid slurry and other losses**

Carbon = 39.86 g C (41.97% Cin)

Hydrogen = 5.68 g H (52.45% Hin)

Sulfur = 4.32 g S (73.85% Sin)

Nitrogen = 5.37 g N (86.33% Nin)

Ash = 25.70 g (46.44% Ashin)

**CH4**

**CO2**

**H2S**

**NH3**

(c)

**Carbon (100%)**

DFL = 11.38 g

FVW = 14.38 g

CD = 33.19 g

LD = 15.40 g

HC = 5.82 g

PW = 7.08 g

Total = 100.74 g

0.0008 g H (0.01% Hin)

0.0035 g N (0.06% Nin)

48.42 g C (48.05% Cin)

8.07 g H (64.35% Hin)

**Nitrogen (100%)**

DFL = 0.52 g

FVW = 0.61 g

CD = 1.53 g

LD = 2.80 g

HC = 0.14 g

PW = -0.23 g

Total = 6.07 g

**Hydrogen (100%)**

DFL = 1.52 g

FVW = 2.28 g

CD = 1.46 g

LD = 4.20 g

HC = 0.63 g

PW = 1.86 g

Total = 12.54 g

**Ash (100%)**

DFL = 6.13 g

FVW = 29.64 g

CD = 14.85 g

LD = 0.70 g

HC = 4.03 g

PW = 7.63 g

Total = 62.43 g

**Sulfur (100%)**

DFL = 0.39 g

FVW = 0.76 g

CD = 3.26 g

LD = 1.40 g

HC = 0.04 g

Total = 6.01 g

22.55 g C (22.38% Cin)

0.06 g H (0.48% Hin)

1.02 g S (16.97% Sin)

**Ash (57.10% Ashin)**

Total = 35.65 g

**Nitrogen (13.67% Nin)**

Total = 0.83 g

**Carbon (14.59% Cin)**

Total = 14.70 g

**Hydrogen (16.16% Hin)**

Total = 0.54 g

**Sulfur (7.82% Sin)**

Total = 0.47 g

**Anaerobic Co-digestion of DFL, FVW, CD, HC and PW**

(Feeding rate:2% TS, Temperature: 37℃)

**Liquid slurry and other losses**

Carbon = 15.07 g C (14.95% Cin)

Hydrogen = 4.19 g H (44.64% Hin)

Sulfur = 4.52 g S (75.21% Sin)

Nitrogen = 5.23 g N (86.16% Nin)

Ash = 26.78 g (42.90% Ashin)

**CH4**

**CO2**

**H2S**

**NH3**

(d)

**Fig. 7 (a-d)** Elemental level mass balance of the reactors (a) CD, (b) DFL+FVW+CD, (c) DFL+FVW+CD+PC and (d) DFL+FVW+CD+HC

(Cin =Carbon input, Hin = Hydrogen input, Nin = Nitrogen input, Sin = Sulfur input, Ashin = Ash input)

* 1. Energy output yield

Table 10 shows the estimated energy output yield from all four reactors. The amount of biogas produced, methane content, and subsequent VS reduction prominently impact possible energy output yield from AD reactors (Isha et al., 2021; D’Silva et al., 2022a). This is why the energy output yield from all the reactors was ranked in the order of DFL+FVW+CD+HC> DFL+FVW+CD+PC > DFL+FVW+CD > CD. In the DFL+FVW+CD reactor, the energy output yield was 1268.26 kJ. As the PC was introduced into the reactor (DFL+FVW+CD+PC), the energy output yield was about 1524.09 kJ. Further, the introduction of HC and process water into the reactor (DFL+FVW+CD+HC) increased the dissolved volatiles in the reactor but improved volatile mass conversion, subsequently enhancing the biogas produced and methane content, resulting in an energy output yield of 3050.24 kJ. The high energy output yield obtained in DFL+FVW+CD+PC and DFL+FVW+CD+HC may be due to the early hydrolysis and improved degradation of the respective substrates fed, as previously discussed.

**Table 10.** Energy output yield available from all the reactors

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Reactor configuration | Biogas produced (mL) | Biomethane produced (mL) | Methane content (%) | Total energy available (kJ) | Energy output (kJ) | Heat output (kJ) | Electricity output (kJ) | Electricity output (kWh) |
| CD | 13926 | 5400 | 38.78 | 192.20 | 172.98 | 96.10 | 76.88 | 0.02 |
| DFL+FVW+CD | 78499 | 39595 | 50.44 | 1409.18 | 1268.26 | 704.59 | 563.67 | 0.16 |
| DFL+FVW+CD+PC | 82435 | 47582 | 57.72 | 1693.43 | 1524.09 | 846.72 | 677.37 | 0.19 |
| DFL+FVW+CD+HC | 144833 | 95228 | 65.75 | 3389.15 | 3050.24 | 1694.58 | 1355.66 | 0.38 |

* 1. Quality analysis of anaerobic digestate, pyrochar and hydrochar as biofertilizer
     1. *Surface morphology*

Figure 8 (a, b, and c) shows the SEM image of the surface morphology of the digested slurry from the reactors DFL+FVW+CD, DFL+FVW+CD+PC, and DFL+FVW+CD+HC collected at the end of the experimental results. It can be seen that, in the reactor DFL+FVW+CD (Fig. 8a), there are fewer voids or cavities available, whereas in reactors DFL+FVW+CD+PC (Fig. 8b) and DFL+FVW+CD+HC (Fig. 8c) more visible cavities and porous structures are observed. Some scientific reasons for these differences could be the varied microbial activity and accessibility to feedstock. In the reactor, DFL+FVW+CD, the inability of bacterial population to utilize only the soluble and hemicelluloses available in the material, which was free from the lignocellulosic structure. However, this might not have been the case for the reactors DFL+FVW+CD+PC and DFL+FVW+CD+HC. The addition of PC and HC might have resulted in enhanced microbial growth and population. Literature suggests that the porous structure of PC and HC acts as an adhesive surface to which microbes can easily attach and colonize, along with acting as a pH buffering agent (Khan et al., 2021). Most of the studies have reported a high relative abundance of microbial populations that is crucial for methane production in biochar-amended AD reactors (Ihoeghian et al., 2023; Xu et al., 2023; Wang et al., 2021). Xu et al. (2022) observed an increase in the relative abundance of *Syntrophomonas* and *Methanosaeta* in a digestate-derived HC-amended AD reactor treating sewage sludge and food waste. The inferences made here directly correlate with the results discussed in earlier sections.

|  |  |
| --- | --- |
| **(a)** | **(b)** |
| A map of the world  Description automatically generated with low confidence  **(c)** | |

**Fig.8 (a-c)** Morphological images of anaerobic digestate derived from reactors (a) DFL+FVW+CD, (b) DFL+FVW+CD+PC, (c) DFL+FVW+CD+HC

* + 1. *Germination test*

As anaerobic digestate is known for its applicability as a potential organic fertilizer with good nutrient availability, Vaish et al., (2022) conducted a pot experiment using anaerobic digestate derived from AD of organic fraction of municipal solid wastes, sewage sludge, and flower waste which improved the growth rate of the Brinjal (*Solanum melongena L.*) plant. Similarly, the liquid digestate, solid anaerobic digestate, PC, and HC of the present study were subjected to a germination test. The emergence rates achieved for anaerobic digestate, PC, and HC were 70 ± 10%, 93 ± 6%, 77 ± 6%, and 87± 6 %, respectively. Meanwhile, the MGT required for liquid digestate, solid digestate, PC, and HC were 27 ± 1 h, 19 ± 3 h, 29 ± 2 h, and 32 ± 1 h. The results are aligned with the compost-based fertilizer prepared from dry fallen leaves, fruit/vegetable wastes and cow dung (D’Silva et al., 2022b). It can be seen that the emergence rate and mean germination rate of anaerobic digestate were 17.7 % higher and 5.37 d lower than the PC and 7.13 % and 2.95 d lower than HC-based fertilizer. It does not imply that the quality of the PC and HC were lower than the anaerobic digestate. This variation could be due to the slow-releasing nature of nutrients and trace elements in PC and HC. Biochar, in general, is known to have slow-release nature of nitrogen, phosphorus and other micronutrients compared to commonly available organic fertilizers including anaerobic digestate and compost (Nogués et al., 2023; Wu et al., 2021; Stefaniuk et al., 2015) and the ash content containing trace elements that improve the nutrient uptake capacity of the seeds (Ibeto et al., 2023). The ash content, macronutrients, and micronutrients present in the digestate, PC, and HC are shown in Table 6.

Sharma et al. (2023) developed a biochar-based slow-release fertilizer from anaerobic digestate recovered from AD-treating cow dung. The study observed a slow release of nitrogen and phosphorus and high nutrient uptake while conducting a germination test using wheat seedlings. A similar phenomenon might have occurred in the present study in which anaerobic digestate might have released the nutrients much earlier than PC and HC, which could have given a better result for anaerobic digestate. Basinas et al. (2023) reported that biochar produced from digestate at 500 °C improved soil fertility over long-term application due to its positive attributes of a highly alkaline nature and reasonable cation exchange capacity (72.2 cmol/kg). In a previous study, Gronwald et al. (2016) compared the performance of PC and HC as soil-nourishing agents and found that both chars have their enhancing properties. The study concluded that PC could act as a potential C-sequestrating pathway. At the same time, the faster mineralization attribute of HC can make it a mid-term fertilizer with a slow nutrient-release property. The summarizations were backed by another study by Monlau et al. (2016). However, the quality of this biofertilizer can be varied according to the properties of the char used and its production process conditions applied (Ding et al., 2016). Hence, more intensive investigations have to be thrown in from this perspective to confirm the results and standardize them.

* + 1. *Carbon emission estimation during biofertilizer application*

Figure 9 shows the estimated carbon equivalent emission using raw anaerobic digestate, PC, and HC as biofertilizers. The carbon emission depends on the undestroyed VS in the raw anaerobic digestate, PC and HC. These VS undergo natural degradation during the application, producing CH4, CO2, and N2O emissions (Li et al., 2023). The total carbon emission equivalent of anaerobic digestate, PC, and HC were 1.90, 1.21, and 1.35 kg CO2-eq/kg of material, respectively. It was estimated that CH4 is the major contributor to the overall carbon equivalent emission compared to CO2 and N2O in all three cases. The higher VS content makes anaerobic digestate vulnerable to carbon emissions, reducing the overall carbon-neutral characteristic of AD (Zeshan & Visvanathan, 2014). Applying slow pyrolysis or HTC reduces the VS content in the solid product, reducing the possible carbon emission while applying them as biofertilizers. Compared with previous studies, the results were on the higher side, the primary reason being that most of the studies considered only CH4 and N2O emissions but not the possible direct CO2 emissions. Also, the factor used for the carbon equivalent of methane in these studies was 21 or 25, whereas an updated factor of 28 was used in this present study. The nearest value reported that is comparable with the results is 1.135 kg CO2-eq/kg of material for the landfilled waste generated in the region of Alytus (Lithuania) (Miliūte et al., 2010). Apart from that, Zeshan & Visvanathan (2014) reported a value between 0.139 – 0.568 kg CO2-eq/kg of material for different digestate management scenarios, Liamsanguan & Gheewala (2008) reported a value of 0.63 kg CO2-eq/kg material, and Gunamantha (2012) obtained a value of 0.20 kg CO2-eq/kg material.

**Fig.9** Estimated carbon emission during biofertilizer application

* 1. Ranking the performance of the reactors using the GRA method

Table 11 represents the results of the grey relational analysis. The DFL+FVW+CD+HC outranked other reactors, followed by DFL+FVW+CD+PC and DFL+FVW+CD, with GRG values of 0.45, 0.71, and 0.80. The biomethane yield, biodegradability index, VS removal, net energy output, emergence rate, and total carbon emission have contributed positively to the GRG value, contributing to the higher GRG value for the DFL+FVW+CD+HC.

These parameters were also found to be beneficial for DFL+FVW+CD+PC, which resulted in ranking it next to the HC-amended reactor. Meanwhile, the lower methane yield, higher biocarbon dioxide yield, and total carbon equivalent emission made the DFL+FVW+CD reactor rank at the bottom. Thus, DFL+FVW+CD+HC was the best route according to the grey relational analysis for upscaling. However, the GRG values of DFL+FVW+CD+HC and DFL+FVW+CD+PC reactors were very similar, meaning there can be improvement in both cases if HC and PC dosing rates are optimized further. Also, there is a need for long-term evaluation of these strategies to confirm the results. This calls for further studies to address these research gaps.

**Table 11.** Grey relation analysis results of ranking the DFL+FVW+CD, DFL+FVW+CD+PC and DFL+FVW+CD+HC

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Reactor Nomenclature | Biomethane yield | Biocarbon dioxide yield | H2S production | Lag phase (d) | Biodegradability index (%) | VS removal (%) | Net energy output | Emergence rate (%) | MGT (h) | Total carbon emission (kg CO2-eq/kg material) | Pathogen removal (%) |
| DFL+FVW+CD | 342.73 | 287.15 | 14634.00 | 9.34 | 67.67 | 68 | 1268.26 | 93.33 | 18.49 | 1.9 | 0 |
| DFL+FVW+CD+PC | 411.21 | 259.08 | 7632.00 | 5.95 | 81.19 | 81 | 1524.09 | 76.67 | 28.86 | 1.21 | 100 |
| DFL+FVW+CD+HC | 478.56 | 220.78 | 10178.00 | 6.15 | 84.44 | 78 | 3050.24 | 86.67 | 31.54 | 1.35 | 100 |
| MIN | 342.73 | 220.78 | 7632.00 | 5.95 | 67.67 | 68 | 1268.26 | 76.67 | 18.49 | 1.21 | 0 |
| MAX | 478.56 | 287.15 | 14634.00 | 9.34 | 84.44 | 81 | 3050.24 | 93.33 | 31.54 | 1.9 | 100 |
| *Normalization* | | | | | | | | | | | |
| DFL+FVW+CD | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.00 | 1.00 | 0 | 0 |
| DFL+FVW+CD+PC | 0.50 | 0.42 | 1.00 | 1.00 | 0.81 | 1.00 | 0.14 | 0 | 0.21 | 1.00 | 1.00 |
| DFL+FVW+CD+HC | 1.00 | 1.00 | 0.64 | 0.94 | 1.00 | 0.77 | 1.00 | 0.60 | 0 | 0.80 | 1.00 |
| MIN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MAX | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| *Deviation Sequence* | | | | | | | | | | | |
| DFL+FVW+CD | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0 | 0 | 1.00 | 1.00 |
| DFL+FVW+CD+PC | 0.50 | 0.58 | 0 | 0 | 0.19 | 0 | 0.86 | 1 | 0.79 | 0 | 0 |
| DFL+FVW+CD+HC | 0 | 0 | 0.36 | 0.06 | 0 | 0.23 | 0 | 0.40 | 1 | 0.20 | 0 |
| MIN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MAX | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| *Grey Relational Coefficient* | | | | | | | | | | | |
| DFL+FVW+CD | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 1.00 | 1.00 | 0.33 | 0.33 |
| DFL+FVW+CD+PC | 0.50 | 0.46 | 1.00 | 1.00 | 0.72 | 1.00 | 0.37 | 0.33 | 0.39 | 1.00 | 1.00 |
| DFL+FVW+CD+HC | 1.00 | 1.00 | 0.58 | 0.89 | 1.00 | 0.68 | 1.00 | 0.56 | 0.33 | 0.71 | 1.00 |
| *Grey Relational Grade* | | | | | | | | | | | |
| DFL+FVW+CD | 0.45 | | | | | | | | | | |
| DFL+FVW+CD+PC | 0.71 | | | | | | | | | | |
| DFL+FVW+CD+HC | 0.80 | | | | | | | | | | |
| *Ranking* | | | | | | | | | | | | |
| DFL+FVW+CD | | 3 | | | | | | | | | | |
| DFL+FVW+CD+PC | | 2 | | | | | | | | | | |
| DFL+FVW+CD+HC | | 1 | | | | | | | | | | |

1. **Conclusion**

This study evaluated the potential of commercially available and locally prepared carbon materials in AD to enhance methane yield in two sets of experiments. The first experiment demonstrated that activated carbon-amended reactors performed consistently better than other carbon amendments, even under oxygen-stressed conditions. In the second experiment, the addition of PC or a combination of HC with PW positively influenced co-digestion, reducing the carbon dioxide content in biogas. The PC-supplemented reactor yielded 411.21 ± 43.21 mL CH4/g VSinput, while the HC + PW reactor achieved a higher yield of 478.56 ± 63.47 mL CH4/g VSinput. The underlying mechanisms varied, as evidenced by biogas composition, kinetic modeling, process parameters, and mass balance analysis. The PC and HC exhibited improved nutrient availability over conventional anaerobic digestate, suggesting potential as slow-release fertilizers. Additionally, a potential reduction in carbon emissions (29–36%) was observed with the digestate-to-char approach compared to raw anaerobic digestate. The GRA ranking indicated that the DFL+FVW+CD+HC reactor performed optimally (GRG = 0.80), followed by the DFL+FVW+CD+PC reactor (GRG = 0.71). While the digestate-to-char approach demonstrates potential for enhancing AD benefits, additional research is necessary to optimize process efficiency, nutrient leaching, and agronomic application of PC and HC, assess economic feasibility, and evaluate long-term stability before large-scale implementation.

**Data availability statement**

Data will be made available on request.

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**CRediT authorship contribution statement**

**Tinku Casper D' Silva**: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Validation, Writing – original draft. **Noémi N. Horváth-Gönczi:** Conceptualization, Methodology, Investigation, Formal Analysis, Validation, Writing – original draft. **Ottó Ribár:** Investigation, Formal analysis, Writing – original draft. **Sameer Ahmad Khan**: Investigation, Writing – review & editing. **Ram Chandra**: Conceptualization, Visualization, Supervision, Funding acquisition, Project administration, Resources. **Virendra Kumar Vijay**: Supervision, Funding acquisition, Resources. **Zoltán Bagi:** Supervision, Project administration, Funding acquisition, Resources, Writing – review & editing. **Kornél L. Kovács**: Conceptualization, Methodology, Supervision, Writing – review & editing, Funding acquisition, Resources. **Bhushan P. Gandhi**: Writing – review & editing. **Kirk T. Semple**: Supervision, Writing – review & editing, Resources.

**Conflict of 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.

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