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Full Length Article

Enhanced biogas production from water hyacinth and cow dung with wood and faecal sludge biochar

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ABSTRACT

Water hyacinth (WH), known for its ecotoxicity and economic burden in tropical regions, can play an important role as a lignocellulosic biomass source for biogas production. Co-digesting WH with cow dung (CD) enhances biogas yield but poses challenges like process instability and excessive carbon dioxide production. To improve biogas yield from digestion of WH and CD, this study examined the impact of wood and faecal sludge biochar on the anaerobic co-digestion of CD and WH using a temperature of 37 °C for 40 days. In the controlled laboratory tests, cow dung alone produced the least methane (CH_4), but introducing 2 % wood and faecal sludge biochars significantly boosted CH_4 production by 76.8 % and 94.0 %, respectively. However, a 50 % CD-50 % WH mixture, the CH_4 increase was milder at 20 % and 37 %, respectively. Wood biochar had no significant effect while faecal sludge biochar made a statistically significant impact ($P < 0.05$). These findings offer a sustainable solution, paving the way for cost-effective and eco-friendly biogas production in regions plagued by this invasive plant. The use of faecal sludge biochar, in particular, has substantial implications for optimizing anaerobic digestion processes and reducing their environmental footprint, thereby promoting a more sustainable approach to managing WH and addressing energy needs in tropical, eutrophic regions.

1. Introduction

Global prevalence of water hyacinth (WH) in water bodies has negatively impacted water quality and biodiversity as well as impeded human activities such as navigation and hydro-electric power generation [1,2]. The current methods for controlling the growth of WH have been limited due to: the high costs associated with physical techniques; harmful environmental effects emanating from chemicals; and the time-consuming nature of biological control means [1,3]. Therefore, it could be better to shift the focus from eradication to sustainable resource utilization of WH [4]. WH offers several potential uses in phytoremediation [5], briquette production [6], animal feed, and biogas production [2]. The latter is a well-established technique used to generate energy and extract nutrients from biomass [7,8]. The biodegradability and thus energy conversion efficiency of WH is hampered by its lignocellulosic structure, which slows down the anaerobic digestion (AD) of the biomass, resulting in low biogas output [9]. Another limitation is the high accumulation of volatile fatty acids (VFA) during the digestion of WH which brings about microbial stress, acidification and ultimately inhibition of the digestion process. These limitations in the digestion of WH have been addressed through pre-treatment process and co-digestion with other highly biodegradable materials like food waste and cow dung (CD) [10]. Co-digestion of WH with CD enhances biogas production while providing an effective livestock manure management

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strategy. CD provides a suitable buffering capacity against VFAs as well as the obligate anaerobes from animal digestive systems [2], making it a good inoculum for the co-digestion of WH [11]. However, the stability of the AD process and biogas production are limited due to toxic inhibitory ammonia.

Recent studies show increasing interest in the improvement of AD using carbon-based materials such as granular activated carbon (GAC) [12], carbon nanotubes and biochar [13]. The addition of carbon-based additives, including biochar, provides a large surface area and porosity to promote microbial immobilization and metabolic interactions [14, 15]. This improves carbon dioxide sequestration [16], and boosts methane yield through direct interspecies electron transfer (DIET) and inhibitor alleviation [17]. In addition, biochar has a strong buffering ability to resist acidic/alkaline shock, frequently detected in AD systems, due to its abundant existence of acidic/alkaline functional groups and metal ions [18]. Biochar, in particular, is touted to be effective and sustainable in AD due to its low cost, environmental sustainability, capacity to utilize various biomass feedstocks, and ability to improve digestate quality [13]. Biochar, a carbonaceous solid substance formed by pyrolysis of organic feedstocks in an oxygen-deficient environment [19] has varying effects on AD depending on the feedstock and pyrolysis conditions [20] and the substrate used [13]. Studies have demonstrated the suitability of wood biochar in enhancing AD [13], however, there is a gap of research on the utilization of faecal sludge (FS) biochar in enhancing biogas production, despite its potential as an effective management strategy for hard to manage sludge. To bridge this knowledge gap, this study investigated the potential of co-digesting WH with CD, incoporating FS biochar, to enhance biogas production. The objective is to mitigate the challenges associated with the lignocellulosic structure of water hyacinth, slow anaerobic digestion, and reduced biogas output. Additionally, it aims to contribute to the field of AD by exploring the effectiveness of FS biochar, an under-researched element in enhancing biogas production. This research highlights the potential of harnessing the underutilized energy and environmental benefits of WH, presenting a more sustainable approach to address this ecological challenge and meet energy needs in affected regions.

2. Materials and methods

2.1. Sample collection

The two locations in the Inner Murchison Bay (IMB), Uganda previously identified by coordinates 0°17′28.1″ N, 32,^{0}38′31.8″ E and 0°17′27.7″ N, 32,^{0}38′32.2″ E served as collection points for WH samples. The WH locations on the IMB were selected due to their predominance in WH growth. Cow dung samples were collected from different locations within the kraal (where cattle are kept) at the College of Veterinary Medicine, Animal Resources and Bio-security, Makerere University. Subsequently, these samples were mixed to form an homogeneous composite sample of the cow dung. Faecal sludge after drying on sand drying beds was collected from Lubigi wastewater and faecal sludge treatment plant was by the grab sampling method. The dried FS was pyrolyzed at 300 °C for 12 h followed by cooling period for an additional 12 h. On the other hand, the wood charcoal samples were procured markets in Kampala specifically, Owino, Kalerwe, Kibuye, Nakawa and Kasubi markets. The markets were selected based on local availability of charcoal and potential collection of charcoal from many regions in the country. From each market, samples were collected from five randomly selected vendors. In Uganda, wood charcoal is generally manufactured in traditional earth kilns which operate at temperatures between 200 °C - 500 °C.

2.2. Storage of collected samples

The substrate samples were stored in moisture-free conditions and at 4 °C to limit chemical, physical and biological changes. The solid samples were transported to the Public Health and Environmental Engineering Laboratory (PH/EE lab), Department of Civil and Environmental Engineering, College of Engineering, Design, Art and Technology, Makerere University, in properly sealed zipper bags and carried in a coolant box, to avoid loss or gain of moisture to and from the atmosphere.

2.3. Sample preparation

Prior to preparing the samples, they were brought out of the fridge to attain room temperature. Preparation of WH samples before analysis involved trimming off roots as only leaves and stems were used in this study.The leaves and stems were crushed using a grinding mill and blended to make a homogeneous sample. Biochar preparation involved crushing to a size <2mm.

2.4. Characterization of WH and cow dung

WH and cow dung were characterized for Total Solids (TS), Total Volatile Solids (TVS), Total Suspended Solids (TSS), Total Organic Carbon (TOC), Total Nitrogen (TN). TS concentration was determined gravimetrically by taking the weight of oven dried sample at 105°C until a constant weight (for 24 h) as a fraction of the wet sample volume [21]. TVS was determined by taking the weight difference between oven-dried solids and the 2-hour muffle furnace-ignited sample at 550°C and expressed as a percentage of TS. Ash content was determined as the residue after ignition in the furnace at 550°C for 2 h [21]. pH was measured for all the samples using a pH meter (HACH, HQ 30d). TN was determined by the Kjeldahl Nitrogen method and the TOC was determined by the Loss-on-ignition method [22]. All samples for TS, TVS, TN and TOC were analysed in triplicates.

2.5. Characterization of biochar samples

Proximate analysis of the biochar samples was determined using the Thermogravimetric analyser TGA/DSC 1. Approximately 10 mg of the sample was analysed under a N_2 flow (50mL/min) and a heating rate of 25 °C/min from 25 to 105 °C for 10 min, then heated up to 900 °C, and held at 900 °C for 10 min. The flow was switched from N_2 to air for 15 min to promote complete combustion. The differences in mass loss during the heating stages allowed for calculating the percentage of moisture, volatile matter (VM), fixed carbon (FC) and ash. The pH and electrical conductivity of the biochar samples were measured using the pH and electrical conductivity meter (HACH, HQ 30d). TN was determined by the Kjeldahl Nitrogen method and the TOC was determined by the Loss-on-ignition method [22]. The percentage of sodium (Na), potassium (K) and Calcium (Ca) in the samples was determined by flame photometry (Na, K) and atomic absorption spectrophotometry (Ca) [22].

2.6. Anaerobic digestion tests

To investigate the effect of biochar addition and the ratio of cow dung to WH during anaerobic digestion, a full factorial 2^2 Design of Experiments (DOE) was performed. The two independent variables of this study were evaluated at two levels, cow dung-WH ratio (CD: WH) (50:50 – 0:100) and Biochar (BC) load (0–2 %) with 3 replicates and 3 centre points as shown in Table 1. The substrate concentrations was varied to achieve the desired CD-WH ratios. Two biochemical methane potential (BMP) setups were made using the design in Table 1 with one setup with wood biochar and the other with faecal sludge biochar.

2.7. Set-up of biochemical methane potential (BMP) reactors

The total solids and volatile solids in g/kg of the WH and cow dung were pre-determined and used in the preparation of the samples to be

Table 1

Full factorial 2^2 experimental design using Minitab.

Reactor No.	Orthogonal design		Actual value		Actual VS added (g)		
	CD: WH	BC load	CD: WH	BC load (%)	CD	WH	BC Load
R1, R5, R9	−1	−1	50:50	0	82	143.5	0
R2, R6, R10	−1	1	50:50	2	82	143.5	3.4
R13, R14, R15	0	0	75:25	1	129	71.7	2.0
R3, R7, R11	1	−1	100	0	172	0	0
R4, R8, R12	1	1	100	2	172	0	3.4

loaded in the 1000 mL bottles (used as the digesters) with an effective volume of 800 mL. The total volatile solids content for each of the reactors was maintained at 20 gVS/L in the setup. The necessary amount of WH and CD to achieve the corresponding volatile solids content was determined using the CD: WH ratio established in the DoE for each reactor. The reactors were topped up with distilled water to reach a working volume of 800 mL. The medium was mixed and the bottles tightly fixed. Nitrogen (N_2) gas was purged through the closed reactor at 0.5 bar for 1 min to create an anaerobic environment and the setup was immersed in the bucket of water to check for leakages. The reactors were then placed in a temperature controlled water bath (UNITEMP) at 37 °C (Fig. 1). This setup ensures a controlled anaerobic environment for the digestion process.

2.7.1. *Biogas monitoring*

The setup was monitored daily to observe and record any changes in the operation conditions, measure the volume of gas produced, and identify when the curve of gas volume production against time levelled off. The volume of gas generated from the reactors was determined using the water displacement technique. Monitoring continued until the daily gas production was below 1 % of the accumulated gas production as recommended by German standard VDI 4630 [23]. The quality of the gas generated was determined using the digital gas analyzer (BIOGAS 5000), which provided methane and carbon dioxide accuracy of ±0.5 % after calibration. This analysis included monitoring percentages of methane, carbon dioxide, oxygen, hydrogen sulphide and other trace gases. Connecting the needle valve of the gas collection pipe (Fig. 1) to the digital gas analyzer (BIOGAS 5000) ensured an airtight connection, preventing contamination from the surrounding environment. The percentage compositions of the constituent gases were recorded daily from day 1 to day 40. This comprehensive approach to monitoring and analysing gas production and quality enhances the reliability and precision of the experimental results.

2.7.2. *Sampling of liquid samples for pH, alkalinity and VFA analysis*

Liquid samples were extracted after measuring the quantity and quality of the gas generated in the BMP reactors on day 0 and day 40. This was done by opening the toggle valve connected to the BMP reactor and connecting a 5 mL syringe. The withdrawn samples were analysed for pH, alkalinity and VFA content. The pH was measured using the pH meter (HACH, HQ 30d) and the alkalinity was measured using Palintest Alkaphot test. For the viability of the setup, care was taken to ensure that not >10 % of the working volume was withdrawn during the entire duration of the setup. Concentrations of volatile fatty acids (VFAs), including butyric, lactic, and acetic acid, were determined using the distillation method [21]. This sampling and analysis procedure ensures the reliability of the results by maintaining the integrity of the experimental conditions.

2.7.3. *Measurements of methane generated in BMP reactor*

A displacement bottle of 1-Liter capacity was filled to approximately 80 % (800 mL) with a 5 % sodium hydroxide solution [24,25]. A gasket was placed on the mouth of the bottle and the lid was securely tightened. The displacement bottle was subjected to leak test, by connecting the needle valve of the BMP reactor to the needle valve of the displacement bottle using a 3 mm Outer Diameter (OD) tubing with compression fittings of 1/8-inch size nut ferrules (Fig. 1).

The transfer of gas from the BMP bottle to the displacement bottle occurred gradually by slowly opening both the needle valve of the BMP reactor and the needle valve of the displacement bottle. The biogas displaced the sodium hydroxide out of the displacement bottle via toggle valve. The volume of the 0.05 M sodium hydroxide solution displaced was equivalent to the volume of methane generated in the reactor since the sodium hydroxide absorbed the carbon-dioxide directly.

2.8. *Kinetic model*

The experimental BMP results were fitted to the modified Gompertz model estimates kinetic parameters as predictor variables (Eq. (1)). This model offers the best fitting by accurately simulating the cumulative production of methane [26,27].

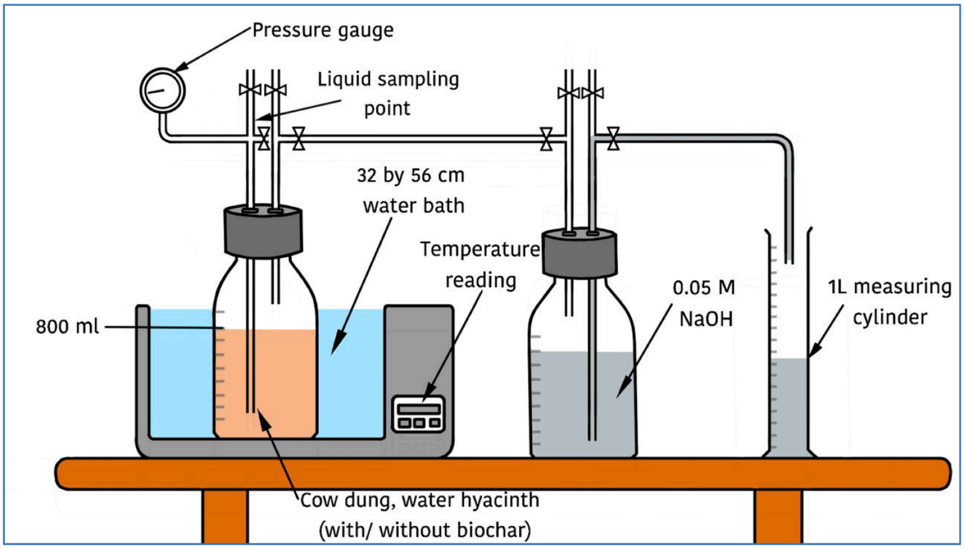


Fig. 1. Experimental Set up of the reactor bottles in a water bath.

$$BMP(t) = BMP_{max} \cdot exp\left\{ - exp\left[\frac{\mu_m \cdot e}{BMP_{max}} \ (\lambda - t) + 1 \right] \right\} \tag{1}$$

Where $BMP(t)$ represents the cumulative methane yield at time t (day) and BMP_{max} stands for maximum methane yield, both expressed in mL CH_4 /gVS. μ_m states for the methane production rate in mL CH_4 /gVS. day, λ stands for lag phase in days and e is the expotential of 1.

2.9. Data analysis

Statistical analysis was carried out using Microsoft Excel Professional Plus 2019 version 1809 for Windows and Minitab 27 software. Descriptive statistics including means and standard deviations were used for parameters characterising the water hyacinth, cow dung and biochar samples as well as to analyse biogas production from the BMP setups. For the DOE factorial design and analysis of response variables were performed with the Minitab 27 software. The effect of the independent variables CD: WH and BC load over the response variables was performed by analysis of variance (ANOVA) and linear regression, both at a confidence level of $p < 0.05$. The kinetic analysis was performed using the R-statistical software v.4.2.2.

3. Results and discussion

3.1. Characterization of WH and cow dung

The WH and cow dung feedstocks were characterized to determine their desirability for AD and to predict their performance once they were co-digested. It can be seen that the WH had a higher Moisture content, TN, TOC than the cow dung whereas the cow dung exhibited a higher TS, VS and pH (Table 2). Despite the WH having a higher TOC and TN concentration than the cow dung, it had a slightly lower C/N ratio than the cow dung (Table 2).

Regarding the composition of the feedstocks, both WH and cow dung had a high VS as a percentage of the TS of 83.8 ± 0.51 % and 84.1 ± 2.44 % respectively, indicating that most components of the substrates were organic matter susceptible to degradation by microorganisms. This confirmed their suitability as feedstocks for the anaerobic digestion process. It should be noted however that not all the VS of the feedstock is degradable as VS is divided into two fractions of biodegradable VS and refractory VS [28] therefore in as much as VS content is a primary indicator of probable methane potential, it is not conclusive until the variations in organic matter composition of the substrates are taken into account [29]. The C/N ratio of WH was found to be below the optimal range of 16–30 recommended for effective metabolic processes [30–33] unlike the one for cow dung which lay in the optimal range. The low C/N ratio of WH suggests that the mono-digestion of WH could pose a potential risk of ammonia inhibition which would likely cause the pH to exceed 8.5 and impact the activity of methanogens [34].

Table 2
Characteristics of WH and cow dung substrates.

Parameter	Unit	Water hyacinth (WH) Mean±SD	Cow dung (CD) Mean±SD
Total solids (TS)	%	8.44±0.25	17.3 ± 0.7
Total solids (TS)	g/kg	84.0 ± 2.5	173±3.1
Volatile solids (VS)	% of TS	83.8 ± 0.5	84.1 ± 2.4
Moisture content (% wet sample)	% wet sample	91.6 ± 0.25	82.7 ± 0.3
Total Nitrogen (TN)	%	3.20±0.04	2.50±0.07
Total organic carbon (TOC)	%	48.1 ± 0.2	44.7 ± 0.3
pH		7.76	8.38
C/N ratio		15.0 ± 0.1	17.9 ± 0.4

3.2. Characterization of wood biochar and faecal sludge biochar

The biochars were characterized to predict their performance once added to the substrates during co-digestion (Table 3). It can be seen that the FS biochar had a higher ash content, TN, TOC, pH and EC than the wood biochar whereas the wood biochar exhibited a higher FC, VM (Table 3). The higher FC content in wood biochar indicates a higher potential for carbon sequestration and stability during anaerobic digestion while the higher TOC content in faecal sludge biochar suggests a higher potential for organic carbon utilization and biogas production in anaerobic reactors. The FS biochar presented higher ash content compared to the wood biochar which can be attributed to the higher amount of inorganic matter in their substrate concentrated in the biochar during pyrolysis [16]. The pH of both biochars was slightly alkaline, however the faecal sludge biochar presented a higher pH as compared to the wood biochar.

The faecal sludge biochar presented higher concentrations of the alkali and alkali earth metals (AAEMS), such as Na, K, and Ca as compared to the wood sludge biochar and these have been reported in previous studies to contribute to the buffering capacity of biochars in anaerobic digestion [35,36]. This is correlated by the high pH and ash content presented by the faecal sludge biochar as compared to the wood biochar which could have served to reduce VFA accumulation in the biochar amended reactors. Additionally, the presence of the AAEMs has been shown to promote in-situ carbon-dioxide sequestration by chemical sorption [20]. Chemical sorption of carbon-dioxide results in formation of carbonates which have been reported to enhance methane formation through hydrogenotrophic methanogens [37].

3.3. Comparison of methane yield from wood and faecal sludge biochar setups

Figs. 2 and 3 show the average BMP curves obtained by each additive within the factorial design for the co-digestion of WH and cow dung for the wood biochar and faecal sludge biochar setups respectively. In the wood biochar setup, all reactors exhibited a lag phase of 4 days which is similar to other WH-based co-digestion studies [38,39]. The volumes of methane gas were found in a range of 84.04 – 196.80 mLCH_4/gVS. Initially, 50 % CD:50 %WH with 0 % BC had higher methane yield than all the other ratios for the first 15 days, but was superseded by 50 % CD:50 %WH + 2 % BC, 100 % CD + 2 % BC and 75 % CD:25 %WH + 1 % BC each with methane volumes of 122.29 ± 19.56, 148.58 ± 14.97, and 196.80 ± 1.86 mLCH_4/gVS, respectively (Fig. 2). In comparison, the final methane volumes in the faecal sludge biochar setup were found to be in a range of 90.43 – 175.25 mLCH_4/gVS with 50 % CD:50 %WH + 2 % BC, 100 % CD + 2 % BC and 75 % CD:25 %WH + 1 % BC each with methane volumes of 144.82 ± 20.33, 175.25 ± 5.29, 157.60 ± 59.25 mLCH_4/gVS respectively (Fig. 3).

Overall, the 100 % CD + 0 % BC had the least yield for both setups

Table 3
Characteristics of biochars.

Parameters	Unit	Wood biochar Mean ± SD	Faecal sludge biochar Mean ± SD
Volatile Matter	%	21.7 ± 1.0	12.8 ± 1.4
Moisture content	%	8.07±0.23	36.5 ± 1.1
Ash content (%)	%	29.4 ± 3.8	40.5 ± 6.9
Fixed carbon (FC)	%	40.9 ± 2.7	10.2 ± 8.2
Total organic carbon (TOC)	%	15.9 ± 0.3	28.1 ± 1.3
Total Nitrogen (TN)	%	0.89±0.05	1.57±0.09
Potassium (K)	%	0.72±0.01	0.61±0.09
Calcium (Ca)	%	0.25±0.02	4.87±0.19
Sodium (Na)	%	0.04±0.00	0.42±0.00
pH		7.69	8.66
EC	dS/m	1.646	3840

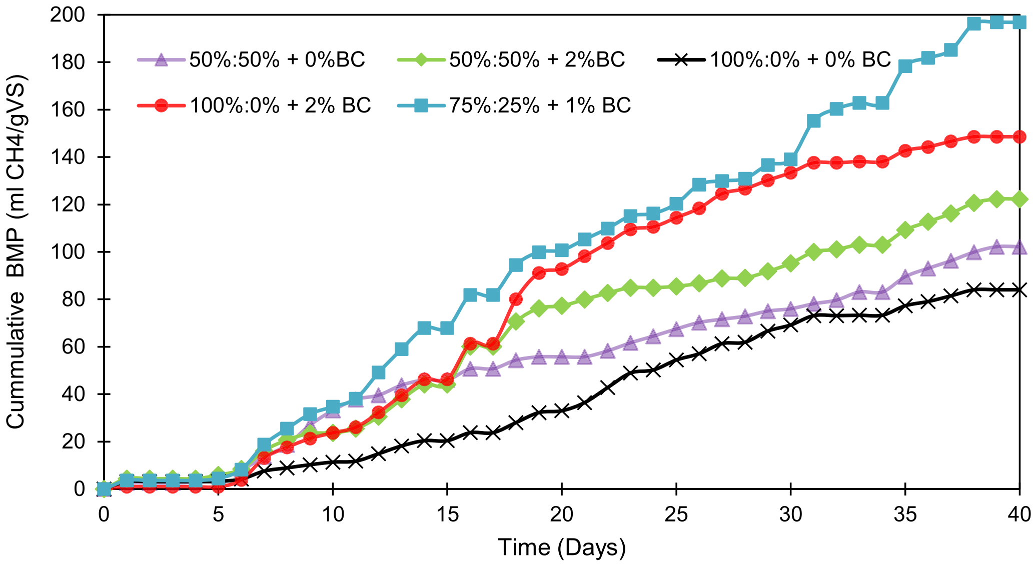


Fig. 2. Cumulative biogas production vs retention time for Wood BC setup.

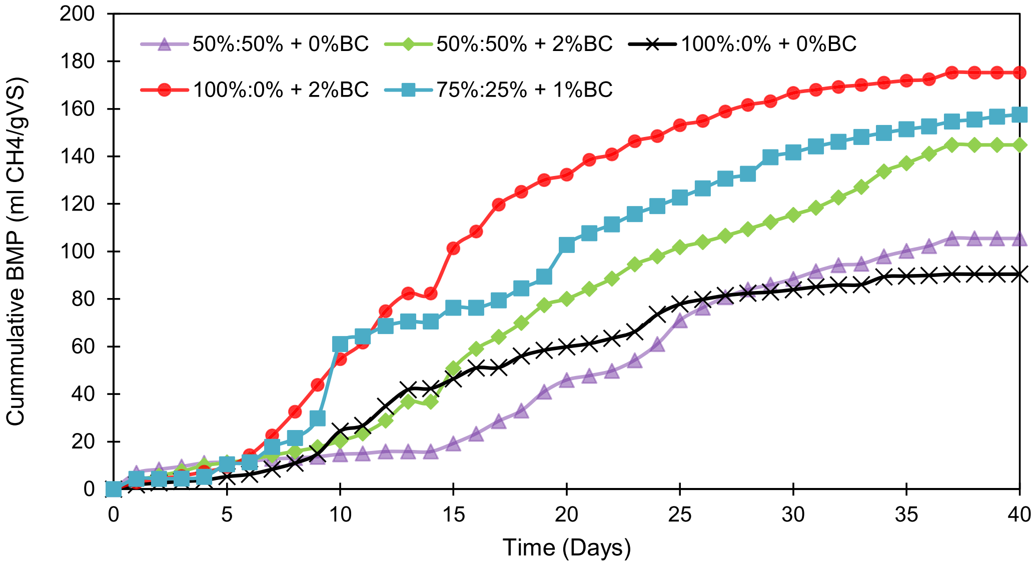


Fig. 3. Cumulative biogas production vs retention time for faecal sludge BC setup.

(84.04 ± 46.5 and 90.43 ± 47.09 mLCH$_4$/gVS) and this was found to be lower than the methane yield range observed in a WH and cow dung co-digestion study by [40] (85.9 – 224 mLCH$_4$/gVS). It was also observed that 50 % CD + 50 % WH + 0 % BC produced a higher methane yield in both setups (102.2 ± 68.8 and 105.47 ± 9.09 mLCH$_4$/gVS) as compared to 100 % CD + 0 % BC. This suggests that co-digestion of cow dung and WH resulted in increased methane production as compared to mono-digestion of cow dung. This could be attributed to the addition of the WH substrate which was pre-treated to very fine particles thus providing enhanced solubilization of the substrate due to increased surface area [10,41]. The performance of 50 % CD + 50 % WH + 0 % BC in this study was similar to that established in a recent study where CD/WH ratio of 50 %:50 % yielded 93.2 mL CH$_4$/gVS [40].

From Figs. 2 and 3, the 2 % wood and faecal sludge BC load addition to 50 %CD:50 %WH and 100 % CD resulted in an increase in the methane yield. However, addition of the wood biochar resulted in 20 % and 77 % higher methane yield while the faecal sludge biochar resulted in 37 % and 94 % higher methane yield from the 50 %CD:50 %WH and 100 % CD mixtures respectively. These findings indicate that, while both forms of biochars improved AD, their effect is not the same and depends on the feedstocks used in AD as well as those used in BC production and this is similar to other studies [13]. While the wood BC presented a higher FC content as compared to the faecal sludge BC, the faecal sludge BC presented higher N, K and Ca content which could have provided nutrients for the microorganisms thereby facilitating a higher methane production. The high pH of the faecal sludge BC could have served to increase the pH in the amended reactors as compared to the wood BC setup (Fig. 6) thus providing more optimum conditions for the action of methanogenic bacteria.

The increase in methane yield could be attributed to adsorption of inhibitors such as VFAs which would result in a more stable system for the growth of microorganisms (Fig. 7). [16] investigated the influence of BC samples from wood pellets, sewage sludge and rice husks on methane production from anaerobic digestion of waste activated sludge. In this study, it was observed that the rice husk BC amended reactors had the greatest increase in methane content (67 %) compared to the control reactors, followed by the sewage sludge BC (37 %) and the wood pellet BC (23 %). The increase in methane content was attributed to capture of carbon-dioxide from biogas by physical adsorption through electrostatic forces and chemical sorption by AAEMs. Accordingly, in a study to investigate the effect of biochar made from wood pellets, wheat straw, and sheep manure on high-solids anaerobic digestion of poultry litter, the addition of wood pellet biochar increased methane yield by 32 %. However, addition of biochar made from wheat straw or sheep manure had a negative impact on digester performance, which was attributed to the high ash content of the substrates, inhibiting the methane generation [41].

3.4. Comparison of biogas quality from wood and faecal sludge biochar setups

Biogas consists primarily of methane (CH_4) and carbon dioxide (CO_2), but its quality is mostly dependent on CH_4 composition [42]. The average percentage composition of methane, carbon dioxide and oxygen in the biogas produced by day 40 are shown in Figs. 4 and 5 for the wood and faecal sludge biochar setups respectively. In both setups, it was observed that the 75 %CD:25 %WH + 1 %BC, had the highest methane percentage composition of the mix ratios considered. The 50 %CD:50 % WH + 0 % BC showed the lowest methane percentage composition (Figs. 4 and 5).

The enhanced methane yield could be a result of conversion of carbon dioxide to methane by aceticlastic methanogens through direct interspecies electron transfer (DIET) [17,43]. It has been reported that biochar offers surfaces for immobilization and colonization of microorganisms which promotes the electron transfer between syntrophic microorganisms [44]. Additionally, the conductive nature of the biochars, demonstrated by the high conductivity (Table 3), could also promote DIET during AD however the capacity of stimulating DIET has been reported to be related to the redox characteristics of the biochars [16] and no clear positive impact of high EC on methane production has been observed in other studies [45]. However, it has been suggested that the EC of biochar amended reactors can strengthen the electron transfer and interspecies electron transfer mechanism from electron donors to acceptors [44]. Electron transfer between these syntrophic microorganisms enables them to overcome the energy barriers involved in the reactions for converting acetate and volatile fatty acids to methane [46].

3.5. Kinetic parameters

Tables 4 and 5 show the values of BMPmax, μm and λ obtained by fitting experimental BMP for the co-digestion of cow dung and water hyacinth with wood and faecal sludge biochar into the modified Gompertz model respectively. The BMPmax was generally increased with the addition of biochar in both experiments. The addition of 2 % wood biochar to 50 %CD:50WH and 100 %CD increased BMPmax by 13.6 % and 45.2 %, respectively. However, the addition of 2 % faecal sludge biochar to the same CD:WH ratio increased BMPmax by 18.4 % and 87.5 %, respectively. The addition of biochar also shortened the methanogenic lag phase and raised the maximum methane production rates in both setups and this has been attributed to positive role of biochar as electron transfer mediator in DIET interactions [47]. Accordingly, In a study to assess the application of eco-compatible biochar in anaerobic digestion to relieve acid stress, it was observed that the addition of 0.5–1 mm biochar to 4, 6 and 8 g/L glucose shortened the methanogenic lag phase by 11.4 %, 30.3 % and 21.6 % and raised the maximum methane production rate by 86.6 %, 21.4 % and 5.2 %, respectively [48]. Similar findings were made in a study of anaerobic digestion of olive mill wastewater in the presence of biochar where the addition of biochar reduced the lag phase for methanogenesis and increased the maximum rate of biogas generation [49].

3.6. Statistical analysis of models for wood and faecal sludge biochar setups

For analysing the factorial design, the cumulative BMP was designated as the response variable for both wood and faecal sludge biochar setups. The independent variables were the CD:WH ratio and BC loading. In the wood biochar setup, it was observed that the CD:WH ratio was statistically significant ($p < 0.05$) for the response, indicating that CD:WH ratio has an effect on the methane yield. However, BC load had no significant effect on the response. Furthermore, the interaction of CD:WH ratio and BC load was statistically significant implying a combined effect of the CD:WH ratio and BC load on the cumulative methane yield. This is similar to a study where the influence of wood biochar on anaerobic digestion of WH was investigated, where both inoculum to substrate ratio (ISR) and interaction were significant but the BC load had no significant effect on the response [13]. However, in the FS biochar

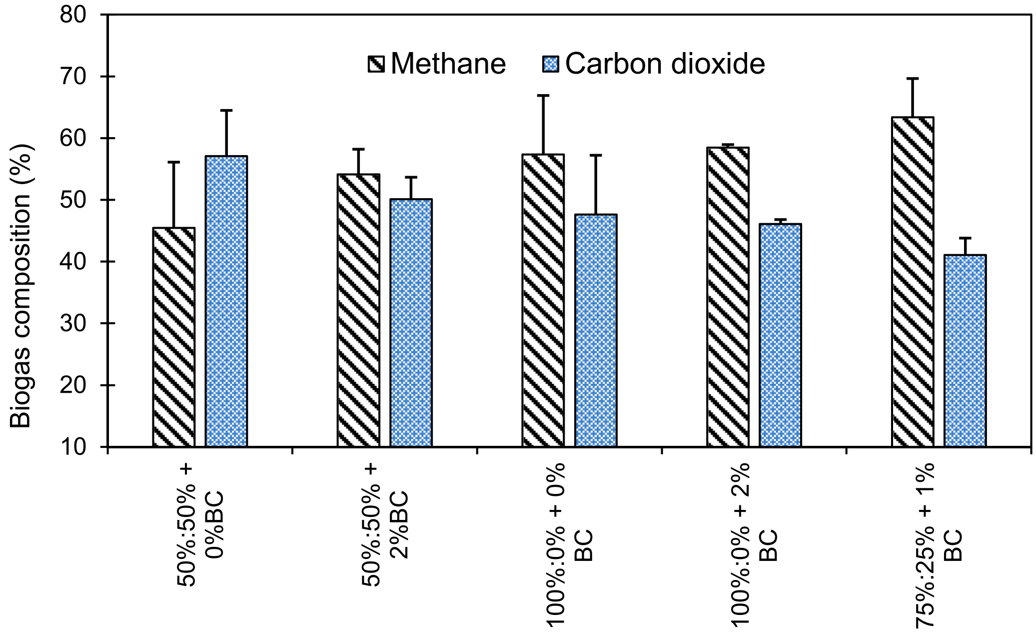


Fig. 4. Biogas composition by percentage for wood BC setup.

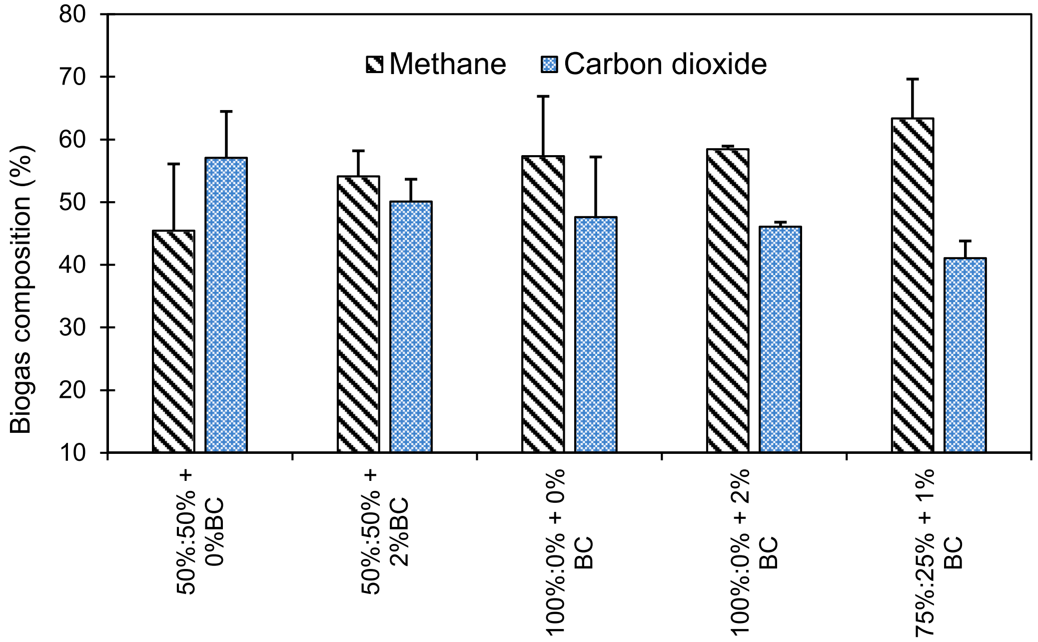


Fig. 5. Biogas composition by percentage for faecal sludge BC setup.

Table 4
Kinetic parameters for the AD of cow dung, water hyacinth and wood biochar.

CD:WH (%)	BC (%)	BMP_{Exp} (mL CH_4 /gVS)	BMP_{max} (mL CH_4 /gVS)	μ_m (mL CH_4 /gVS)	λ (days)	R^2
50:50	0	60.1	64.0	1.86	5.81	0.972
50:50	2	71.9	72.7	2.76	4.58	0.986
100:0	0	49.3	62.9	1.87	8.29	0.995
100:0	2	87.4	91.3	4.29	7.46	0.997
75:25	1	115.8	138.0	3.75	4.94	0.988

Table 5
Kinetic parameters for the AD of cow dung, water hyacinth and feacal sludge biochar.

CD:WH (%)	BC (%)	BMP_{Exp} (mL CH_4 /gVS)	BMP_{max} (mL CH_4 /gVS)	μ_m (mL CH_4 /gVS)	λ (days)	R^2
50:50	0	105.5	139.2	4.16	8.96	0.983
50:50	2	144.8	164.8	5.48	6.04	0.995
100:0	0	90.4	94.1	4.38	4.94	0.995
100:0	2	175.3	176.4	9.78	4.75	0.998
75:25	1	157.6	167.1	6.37	3.54	0.986

CD:WH - Cow dung to Water hyacinth ratio, BC - biochar; BMP_{Exp} - experimental methane yield; BMP_{max} - maximum methane yield; μ_m - methane production rate; λ - lag phase; R^2 - coeffecient of determination.

setup, both the CD:WH ratio and biochar loadings had a significant effect ($p < 0.05$) on the cumulative methane yield (Table 6). However, their interaction was insignificant ($P = 0.0791$) indicating that the effect of the CD:WH ratio on the cumulative methane yield did not depend on the levels of the biochar loadings. The difference in the performance of the CD:WH ratio and BC in the two setups could be attributed to the difference in the physical and chemical properties of the wood and FS biochar.

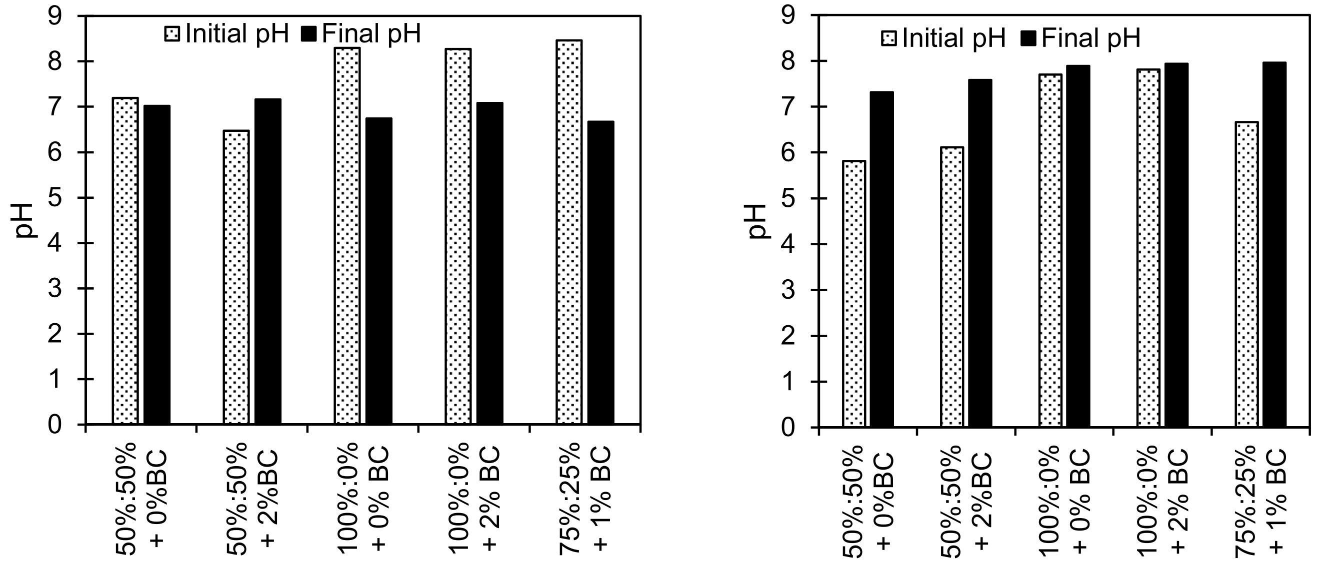
Table 6
Analysis of variance for the factorial regression model for the setups.

Term	**Wood biochar setup**		**Faecal sludge biochar setup**	
	F-value	**p-value**	**F-value**	**p- value**
CD: WH	6.002	0.00294	4.448	0.0129
BC	2.264	0.13400	28.490	0.0000
CD: WH *BC	6.198	0.01361	3.115	0.0791

3.7. *Liquid sample monitoring for wood and faecal sludge biochar setups*

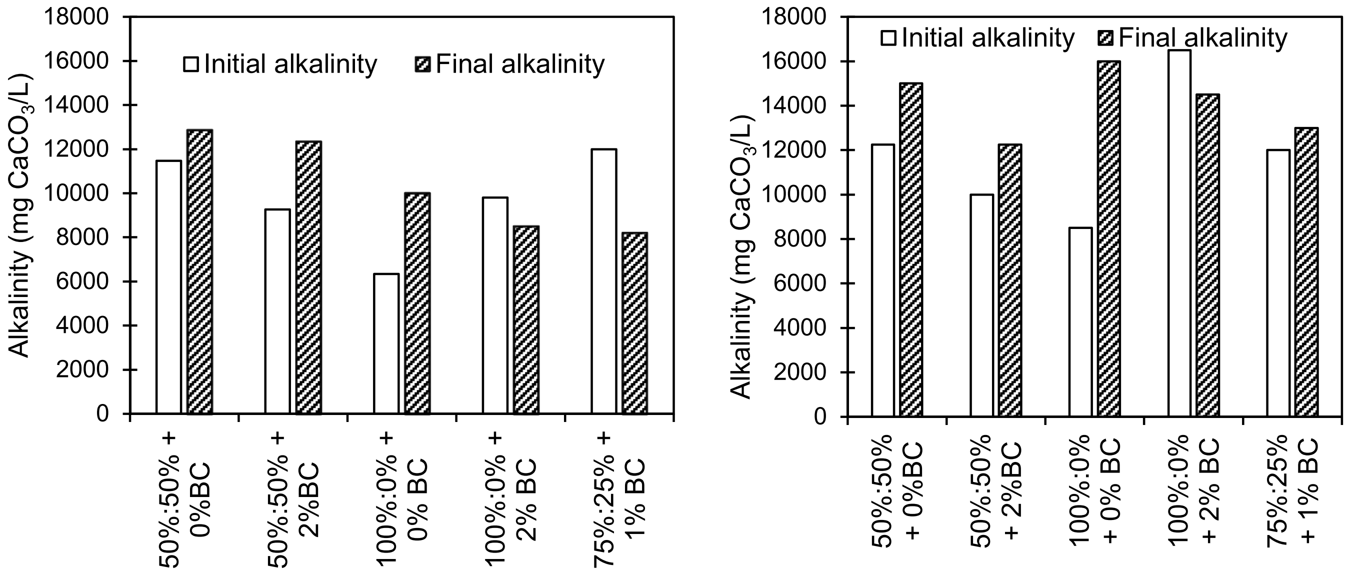
The pH and alkalinity were measured at the beginning and the end of the digestion period without pH adjustment (Fig. 6) in order to assess the impact of addition of BC on the pH and alkalinity. The accumulated VFAs at the end of digestion are shown in Fig. 7.

The pH of the 100 % CD ratio was maintained in the optimum range of 6.5 to 7.5 and this could be because cow dung has been reported to have a high buffering capacity which is supported by its high pH value in Figs. 6A and 6B [50]. Addition of the wood biochar resulted in a drop in both pH and alkalinity for both the 50 %WH:50 %CD + 0 %BC and 100 %CD:0 %WH + 0 % BC (Figs. 6A and 6C), while the faecal sludge biochar resulted in an increase in the pH and alkalinity for the same mixtures (Figs. 6B and 6D). This could attest to the greater buffering capacity offered by the faecal sludge biochar which is correlated to its high pH, ash content and concentration of AAEMs in Table 3. The alkaline property of biochar has been considered to raise CH_4 content by



A : pH at beginning and end for wood BC setup

B: pH at beginning and end for faecal sludge BC setup



C: Alkalinity at beginning and end for wood BC setup

D: Alkalinity at beginning and end for faecal sludge BC setup

Fig. 6. pH (A and B) and alkalinity (C and D) at beginning and end of the experiments.

reacting hydrogen sulphide and CO_2 with alkaline substances in ash thereby regulating the pH in the AD system [51,52].

It was also observed that the addition of both forms of BC greatly reduced the concentration of the VFAs accumulated at the end of digestion (Fig. 7). However, the faecal sludge BC amended reactors displayed lower VFA concentrations as compared to the wood biochar amended reactors thereby further attesting to its greater buffering capacity. The buffering capacity of biochar has been attributed in previous studies to the surface functional groups, such as amine which adsorb H^+, and the inorganic materials such as Ca, K, Na, Fe and Si. The alkalinity of biochar has been generally represented by Eq. (2) (Ca and C_XH_XCOOH are selected as representative of AAEMs and VFAs respectively) [35].

$$CaCO_3 + 2\ C_xH_xCOOH \rightarrow [C_xH_xCOOH]2Ca + H_2O + CO_2 \tag{2}$$

4. Conclusions

This study concludes that water hyacinth and cow dung are excellent substrates for anaerobic digestion due to their high organic matter content, favourable C/N ratios, and high moisture levels. Additionally, wood and faecal sludge biochar can effectively enhance anaerobic digestion processes, with wood biochar contributing to system stability and faecal sludge biochar aiding in nutrient supplementation and carbon dioxide sequestration.

The addition of 2 % biochar, whether from wood or faecal sludge, significantly boosts methane yield, but the extent of the increase depends on the specific feedstock used. Faecal sludge biochar is found to be more effective in increasing methane yield, primarily due to its higher pH, ash content, and percentage of alkali and alkali earth metals

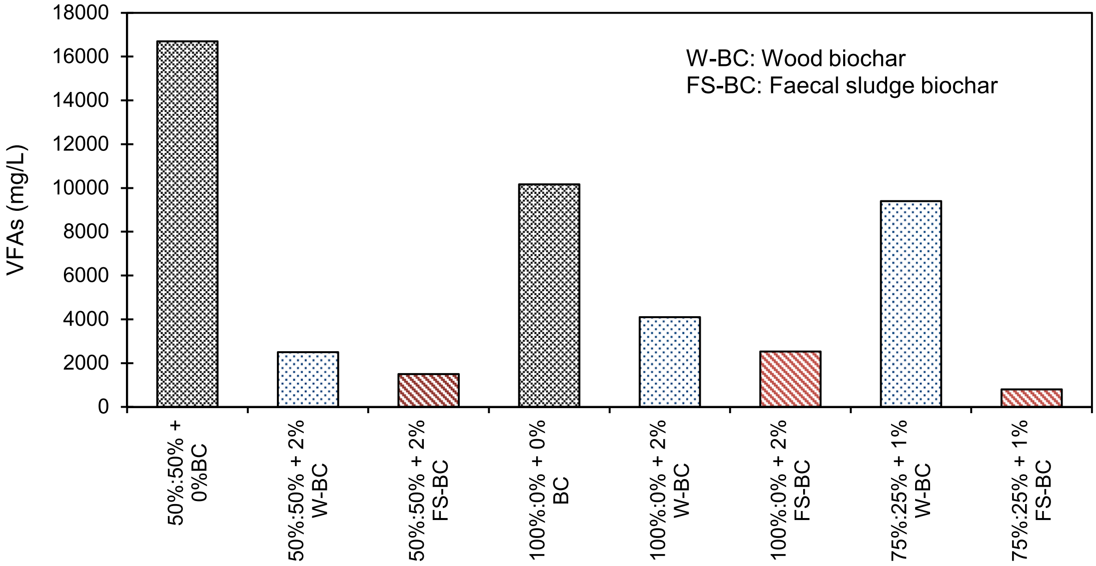


Fig. 7. Volatile Fatty acids (VFAs) accumulated at the end of digestion.

(AAEMs).

The study emphasises the necessity for further investigation, particularly into the influence of pyrolysis temperature on biochar properties, microbiological interactions between different biochar types and the substrates, and the field performance of biochar in methane yield. While laboratory tests indicate significant potential for optimizing anaerobic digestion using biochar, further research is required to explore these findings in practical applications.

Financial interests

I declare that I have no financial interests or relationships with any organization or entity that could be perceived as influencing the conduct or reporting of this research.

Non-Financial interests

I declare that I have no non-financial interests that could be perceived as influencing the conduct or reporting of this research. This includes personal relationships, academic competition, or any other factors that might compromise the integrity of the research.

Collaborations and affiliations

I acknowledge that all contributors to this research have been appropriately acknowledged in the manuscript. I have disclosed all relevant affiliations and collaborations that could be perceived as influencing the research.

Originality

I confirm that the manuscript is original and has not been published previously, in whole or in part, and is not currently under consideration for publication elsewhere. All authors have been properly credited for their contributions.

CRediT authorship contribution statement

Robinah N. Kulabako: Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization. **Swaib Semiyaga:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis, Conceptualization. **Rodney S. Tumwesige:** Writing – original draft, Software, Methodology, Formal analysis, Conceptualization. **Collin Irumba:** Writing – original draft, Methodology, Formal analysis, Conceptualization. **Miria I. Opio:** Writing – review & editing, Formal analysis, Conceptualization. **Musa Manga:** Writing – review & editing, Methodology, Conceptualization. **Vianney Tumwesige:** Writing – review & editing, Conceptualization. **Jessica Quintana-Najera:** Writing – review & editing, Formal analysis, Conceptualization. **Andrew B. Ross:** Writing – review & editing, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The author declares 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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Data availability

Data will be made available on request.

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