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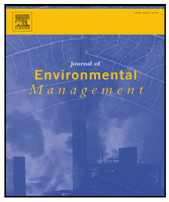

Research article

The influence of biochar position in a leach bed system anaerobically digesting chicken litter

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ABSTRACT

As a consequence of the rapidly growing poultry industry, chicken litter is becoming an abundant and problematic waste. Anaerobic digestion of chicken litter can mitigate environmental issues while producing valuable by-products. Recent studies have shown that leach bed reactor (LBR) systems are suitable for processing chicken litter and that anaerobic digestion can be enhanced using biochar. This study investigates the influence of biochar position within an LBR system on anaerobic digestion of chicken litter.

Compared to a system without biochar, application of biochar in both the LBR (mixed in with the feedstock or as a layer below the feedstock) and coupled leachate tank (LT) increased methane yield by 6 to 8% at 51 days and accelerated VFA degradation and methane production. More significant differences in methane yield were observed at shorter solid retention times. Biochar mixed in feedstock in addition to a filter in the LT performed best in terms of both methane and hydrogen sulfide production, with a 77% reduction in hydrogen sulfide yield and hydrogen sulfide contents maintained below 500 ppm. The enhanced rates of VFA degradation and methane production when applying biochar in both reactors corresponds with observed differences in the methanogen population. Biochar application in both reactors increased the abundance of *Methanobacteriales* in digestate and *Methanosarcinaceae* in leachate compared to the control. Microbial attachment and activity on biochar also increased when mixed in feedstock. Increased diversity of the methanogen population throughout the system, as well as increased activity on biochar, may have facilitated the syntrophic relationship between acetogenic bacteria and methanogens, thus accelerating VFA degradation and methane production. These results suggest mixing biochar in feedstock, in addition to a biochar filter in the LT, to enhance anaerobic digestion of chicken litter in this system.

1. Introduction

Manure management is becoming a major challenge as livestock farming expands to meet growing demands for animal-based protein due to rising global population and improving living standards (Henchion et al., 2017; Hoque et al., 2022). Poultry farming is among the fastest growing sectors of the livestock industry, as the consumption of chicken is increasing faster than other meats (Bolan et al., 2010; Bennett et al., 2018). A consequence of this growth is elevated production of chicken litter; manure, feathers and waste-feed collected on bedding material such as straw or wood chips. Chicken litter is typically applied to agricultural land as a soil conditioner and nutrient source. However, direct application can result in poor air quality (e.g. odorous gases such as ammonia) and uncontrolled greenhouse gas emissions (Bolan et al., 2010). Anaerobic digestion is a strategy that can mitigate these issues while capitalising on the relatively high methane potential of chicken manure (Kafle and Chen, 2016). This is achieved through conversion of organic matter, by microorganisms in an oxygen-free environment to biogas and digestate. Biogas is a combustible gas mixture containing methane that can be used a renewable source for electrical and thermal energy, and digestate can be applied as a more stable and less odorous organic fertiliser (Alburquerque et al., 2012). Despite these value-adding by-products and the capability to mitigate environmental issues, barriers to adoption of this strategy on farms include high capital costs, operational difficulties and low biogas yields from litter-based anaerobic digesters (Bolan et al., 2010). Further research improving anaerobic digestion of chicken litter is required to increase adoption of this strategy in the poultry sector.

Chicken litter is a dry substrate with a total solids (TS) content typically varying between 20 and 70% depending on bedding type

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and chicken age (Wedwitschka et al., 2020). To process such dry waste using conventional low-solids anaerobic digestion (<10% TS) requires dilution with large volumes of water and thus large reactors with mechanical mixing that are economically unattractive. For this reason, compact, water-efficient systems known as high-solids (>15% TS) anaerobic digesters are of interest for processing chicken litter. A high-solids digester that does not require a continuous supply of waste, known as a batch leach bed reactor (LBR), is particularly well suited for chicken litter as it is collected on an intermittent basis (Tait et al., 2021). LBRs operate by recirculating process fluid known as leachate that has percolated through the organic waste. The recirculation of leachate enhances mass transfer (slow for dry substrates) and thus biological degradation and biogas production rates by increasing moisture content and acting as a medium to redistribute microorganisms, nutrients and process intermediates (Yang et al., 2015). However, the lack of dilution compared to low-solids systems, in addition to recirculation of process liquids, can result in elevated levels of process inhibitors that adversely impact biogas production. Ammonia inhibition is of particular concern for chicken litter due to the high organic nitrogen content of chicken manure. Total-ammonia nitrogen (TAN) concentrations ranging from 1.7 to 14 g/L have been reported to cause up to 50% reduction in methane yield depending on factors such as pH, temperature and microbial acclimation (Chen et al., 2008). TAN concentrations up to 10 g/L have been observed for anaerobic digestion of chicken manure in LBRs (Bayrakdar et al., 2018). For this reason, only a limited number of studies have considered LBRs for anaerobic digestion of chicken manure and litter.

Ammonia removal techniques such as membrane extraction (Bayrakdar et al., 2018), gas stripping and precipitation (Ramm et al., 2020) have been investigated to counteract ammonia inhibition in LBRs processing chicken manure. Although these strategies can mitigate ammonia inhibition, they are generally expensive and/or produce environmentally problematic wastes (Rubežius et al., 2020). These are further barriers to adoption of anaerobic digestion on poultry farms. Balancing the carbon-to-nitrogen ratio (C/N) by co-digesting chicken manure and straw is an alternative method that has been shown to improve biogas production in an LBR without the need for ammonia removal (Zayen et al., 2021). Furthermore, the addition of straw yields superior permeability enabling sufficient percolation of leachate through chicken manure (Wedwitschka et al., 2020). These observations suggest that chicken litter is suitable for anaerobic digestion in LBRs without ammonia removal.

The addition of biochar to anaerobic digesters is another strategy to enhance high-solids anaerobic digestion of chicken litter. Biochar is a porous, carbon-rich solid residue formed through thermal decomposition of biomass such as agricultural residues. Biochar is typically used as a soil amendment so it can be applied on farms with digestate after application in anaerobic digesters. Adding biochar to acid- and ammonia-stressed, batch, high-solids anaerobic digesters (without leachate recirculation) processing chicken litter (Indren et al., 2020a,b, 2021) and manure (Kizito et al., 2022) has been shown to enhance methane yield and production rates. Enhanced methane production rates have also been observed when recirculating leachate from an LBR digesting chicken litter through a biochar filter in a secondary reactor (Collins et al., 2023b). Application of the biochar filter also significantly reduced production of hydrogen sulfide (Collins et al., 2023b) — a toxic and highly corrosive contaminant gas that can reduce the lifespan of systems converting biogas to energy (Wang et al., 2019; Tsui et al., 2022). Despite these improvements, as majority of the methane and hydrogen sulfide production occurred in the LBR, performance may be further enhanced through direct application of biochar in the LBR (Collins et al., 2023b). This motivates further research considering the influence of biochar position in LBR systems processing chicken litter.

This study investigates the application of biochar in different positions within an LBR system (LBR with a leachate tank (LT)) anaerobically digesting chicken litter. More specifically, the following situations are compared: (i) a control without biochar; (ii) a system with a biochar layer below feedstock in the LBR; and systems with a biochar filter in the LT in addition to biochar in the LBR as (iii) a layer below feedstock or (iv) mixed in feedstock. The objectives of this study are to understand the influence of biochar position in this system on chemical conditions, methane and hydrogen sulfide production and methanogen populations.

2. Methodology

2.1. Experimental setup and operation

2.1.1. Experimental setup

The experimental setup employed in this study was comprised of four triplicate sets of the anaerobic digestion apparatus outlined in Fig. 1a and a heated water bath (700 L) for temperature control of the LBR systems. The anaerobic digestion apparatus enables recirculation of leachate between an LBR and coupled upflow LT, and monitoring of biogas production from each using separate gas meters. The LBRs and LTs were fabricated from PVC pipe (150 and 100 mm diameter, respectively), a threaded access coupling, caps and a gasket to form sealed vessels with working volumes of 5.3 and 3.5 L. The base of each LBR has 1.5 L of leachate storage and a plastic stand to elevate solid materials. Solid materials refers to feedstock and biochar (for applicable cases). The different cases of biochar application investigated in this study are outlined in Section 2.1.3. For all cases, solid materials were contained in a cylindrical plastic mesh basket with dual layers of geotextile membrane at the base to minimise solids entering the leachate. For cases applying a biochar filter in the LT, filter media were packed together in a cylindrical plastic mesh basket. Circulation of leachate between the bases of the LBR and LT was performed using a peristaltic pump and inline filter packed with filter wool to mitigate pump clogging. Effluent from the LT naturally flowed to the LBR for redistribution over the feedstock surface via a distribution plate. All anaerobic digesters were submerged in a temperature-controlled water bath and connected to gas flow meters developed and calibrated according to the procedure outlined by Collins et al. (2023b). Biogas samples were collected in 1 L inert multi-layer foil bags.

2.1.2. Biochar, feedstock and inoculum

Straw-based chicken litter was sourced as feedstock from a South Australian broiler chicken farm. The litter had been cleared from a broiler shed after a 10 week growth cycle and stored in a pile for two weeks before collection. Centrifuged (solid) anaerobic digester effluent was sourced as inoculum from a wastewater treatment facility treating sewage sludge in continuous stirred-tank reactors at 38 °C (SA Water, South Australia). Both chicken litter and inoculum were frozen upon collection then defrosted at 4 °C for 24 h before use. To minimise residual methane production, the inoculum was degassed at 37 °C for seven days under anaerobic conditions.

Biochar was produced for addition to anaerobic digesters. The feedstock used to produce the biochar was Douglas fir wood cubes (19 mm sides). The wood was dried for 24 h at 105 °C then loaded in 1.8 kg batches into an electric kiln (Henan Synthe Corp., Tiltable Rotary Tube Furnace). The kiln was purged with high-purity nitrogen, heated to 450 °C at a rate of 10 °C/min, then held for 90 min. The kiln rotated at 3 rpm throughout operation until cooled to below 300 °C. Kiln rotation resulted in rounded cuboid pieces of biochar with dimensions ranging from 13 to 16 mm. The biochar added to LBRs in this study was fresh; whereas, the biochar used in LTs was recycled from an experiment (unpublished work) coupling an LBR to an anaerobic biochar filter for digestion of chicken litter. The recycled biochar was inoculated using the same method and operational conditions described by Collins et al. (2023b). The recycled biochar was frozen then defrosted at 4 °C for 24 h before use. The properties of biochar, feedstock and inoculum are presented in Table 1.

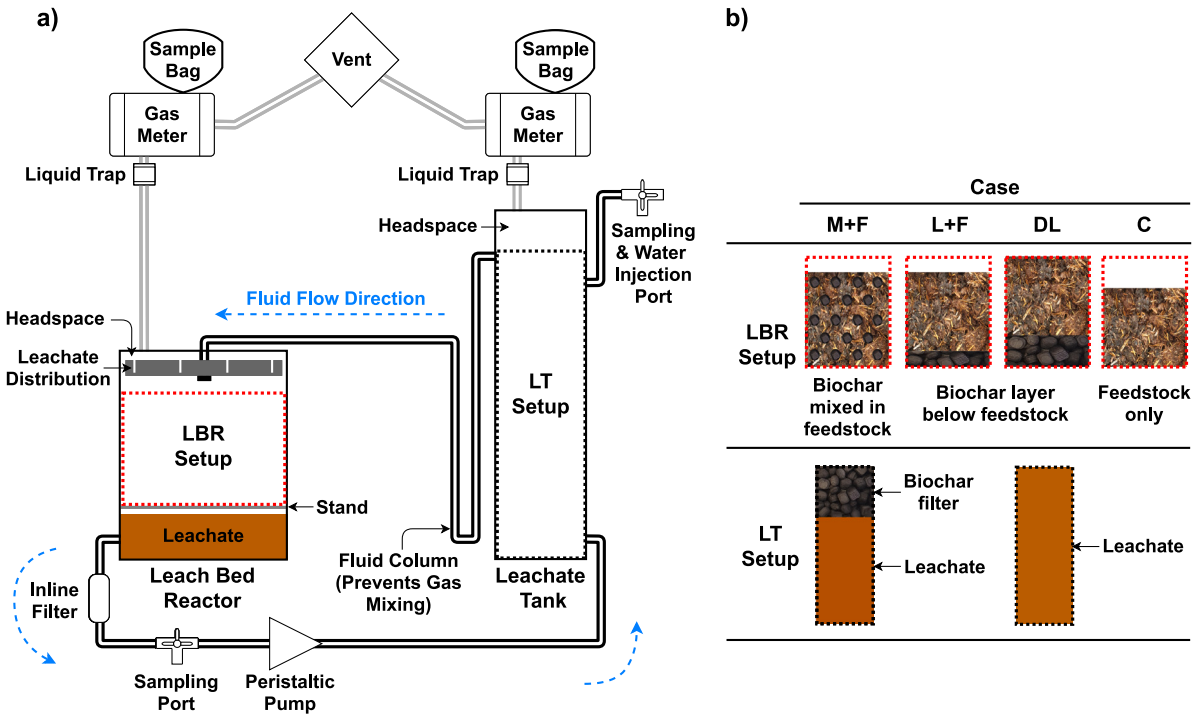

Fig. 1. Diagram of experimental setup, showing: (a) the general anaerobic digestion apparatus; and (b) the locations of biochar application investigated within the anaerobic digestion system. The cases considered are: systems applying biochar in the leach bed reactor (LBR) as a layer below feedstock (L + F), and mixed with feedstock (M + F), in addition to an external biochar filter in the leachate tank (LT); a system with only a biochar layer in the LBR (DL); and a control without biochar (C). Equal total volumes (0.8 L) of biochar were applied for all non-control cases. For cases with an external biochar filter, the total biochar volume was split equally (0.4 L) between reactors.

Table 1
Characteristics of biochar (fresh and recycled), chicken litter and inoculum.

Parameter	Fresh biochar		Recycled biochar		Chicken litter		Inoculum	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TS (wt%)	98.6	1.9	72.9	5.8	84.9	1.0	20.0	2.9
VS (wt%)	11.0	1.7	8.9	1.3	71.6	2.3	11.0	1.4
VS (% of TS)	11.2	2.0	12.1	0.9	84.3	2.0	55.1	2.9
Ash (% of TS)	4.0	4.0	9.6	2.6	21.9	0.5	17.6	3.8
C (% of TS)	76.25	0.13	81.29	1.20	41.11	0.80	42.73	0.17
H (% of TS)	0.45	0.17	2.17	0.49	5.37	0.04	6.33	0.08
N (% of TS)	<0.30		0.58	0.01	3.07	0.37	7.15	0.15
O[a] (% of TS)					28.6		26.2	
C/N					13.5	1.8	6.0	0.1
pH	4.03	0.01	9.41	0.01	8.30	0.03	8.25	0.04
TA (g-$CaCO_{3_{eq}}$/kg)	1.8	0.8	4.7	0.1	10.6	0.9	2.4	0.3
VFA (g/kg)	ND		ND		8.2	1.2	0.1	0.0
TAN (g-TAN/kg)	ND		ND		1.6	0.0	1.2	0.1

ND = not determined.
[a]Calculated as O = 100 − (C + H + N + Ash).

2.1.3. Reactor setup and operation

Fig. 1b outlines the different configurations of biochar application investigated in this study. These configurations include: (i) a control without biochar; (ii) a system with a biochar layer in the LBR only; (iii) a system with a biochar layer in the LBR and a biochar filter in the LT; and (iv) a system with biochar mixed with feedstock in the LBR and a biochar filter in the LT. An equal volume of biochar (0.8 L) was applied for all non-control cases, with an equal split in volume (0.4 L each) between reactors for cases that consider a biochar filter in the LT. Apart from the position of biochar within the systems, reactor setup and operation was the same for all cases.

To setup the reactors, LBRs and LTs were partially filled with 1.45 and 3.25 L of water (reverse osmosis), respectively, and peristaltic pumps were operated to remove air from the system. For cases applying biochar filters in LTs, defrosted biochar was packed in baskets (0.4 L; 100 mm diameter, 50 mm height) and placed below a plastic spacer to ensure submersion of the filter. Note that an equivalent volume of water was used for all cases. For cases using biochar in the LBR, fresh biochar was rinsed to remove loose solids (i.e. ash) then divided into 0.4 and 0.8 L batches. Biochar layers were placed in the bottom of LBR baskets (0.4 or 0.8 L; 150 mm diameter, 25 or 50 mm height) for applicable cases. For feedstock preparation, after breaking down manure chunks exceeding 50 mm, chicken litter was mixed to reduce heterogeneity. Chicken litter (240 g), inoculum (843 g), water (159 g) and biochar (0.4 L, for the applicable case) were mixed by hand to adjust the TS content to 30% and feedstock to inoculant ratio (volatile solids (VS) content based) to 2. Biochar was not included in TS and VS content calculations. Prepared substrate mixtures were then loosely packed in baskets and placed above leachate storage in LBRs. Once sealed, LBR headspace volumes were approximately 2, 1.2 and 1.6 L for the control case, the system with biochar in the LBR only, and the systems with biochar in both reactors, respectively. LTs had an approximate headspace volume of 0.5 L after sealing and connection to the LBR for drainage of excess water. Reactor temperature was maintained at 37 °C

through submersion in a temperature-controlled water bath. Anaerobic conditions were achieved by nitrogen purging the LBRs and LTs prior to connecting to gas meters. Leachate was set to recirculate between reactors hourly for 15 s at a rate of 1.33 mL/s, resulting in a LT hydraulic retention time of 5.8 days. Leachate samples (25 mL) were collected semi-weekly via sampling ports at the base of the LBR and top of the LT, and water was re-injected into the LT sampling port to replace sampled leachate. Biochar, digestate and leachate samples were collected after experiment termination at 51 days.

2.2. Biogas analysis

Biogas production from LBRs and LTs was continuously monitored using gas meters. Measured volumes were corrected to dry gas at 0 °C (Richards et al., 1991). The composition of sampled biogas – methane (CH_4), carbon dioxide (CO_2) and hydrogen sulfide (H_2S) contents – were analysed three times per week using a portable gas monitor (Geotech, Biogas5000) to determine methane and hydrogen sulfide yields. The hydrogen sulfide yields presented do not include production prior to Day 4 as analysis of hydrogen sulfide content was prevented by elevated hydrogen (H_2) levels (>10 000 ppm) that exceeded the limits of the gas analyser. Experimental methane yields were compared against the theoretical methane yield calculated for the substrate using the Buswell equation (Buswell and Mueller, 1952; Li et al., 2013).

2.3. Physical and chemical analyses

Characterisation of biochar, chicken litter and inoculum was conducted prior to the experiment. Proximate analysis was performed using a thermogravimetric analyser (Mettler Toledo, TGA-DS3+). This involved drying samples at 105 °C to determine TS content (Bridgewater et al., 2017), and ashing feedstock and inoculant at 550 °C (Bridgewater et al., 2017), or heating biochar under nitrogen purge at 950 °C before ashing (Aller et al., 2017), to determine VS and ash contents. Samples were also freeze-dried and analysed for elemental composition (carbon, hydrogen and nitrogen) using an elemental analyser (PerkinElmer, 2400 Series II).

Aqueous samples of chicken litter and inoculum were prepared for analysis of pH, total alkalinity (TA), TAN and total VFA concentration by homogenising 2 g of sample in 30 mL of MQ water, then centrifuging at 4000 rpm for 15 min. The pH of supernatant was measured using a calibrated pH probe (Mettler Toledo, InLab Expert Pro). TAN was analysed using colorimetric analysis (Forster, 1995). TA and total VFA concentration were measured by titrating 20 mL of supernatant against 0.1 N sulfuric acid. Titration to an end-point pH of 4.4 indicated TA (Bridgewater et al., 2017), and titration between pH 5 and 4.4 indicated total VFA concentration (Sun et al., 2017). To measure biochar pH, 1 g of crushed sample was placed in 20 mL of MQ water, shaken for 1 h, rested for 30 min, then stirred continuously during pH measurement (Singh et al., 2017). To determine TA, crushed biochar (0.5 g) was placed in 10 mL of 1 M hydrochloric acid, shaken for 2 h, rested overnight then back-titrated with 0.5 M sodium hydroxide to an end-point pH of 7 (Singh et al., 2017).

Leachate samples collected semi-weekly were also analysed for pH, TA, TAN and total VFA concentration using the same procedures outlined for chicken litter and inoculum characterisation. The leachate samples were immediately cooled to 4 °C at collection then centrifuged at 4000 rpm for 15 min prior to analysis. Free-ammonia nitrogen (FAN) concentrations were calculated based on measured pH, TAN concentrations and digestion temperature (Hansen et al., 1998). Additionally, the VFA composition of leachate samples was analysed weekly between Days 6 and 34 using an Agilent 5977B/5890B GCMS system. Samples were prepared by acidifying a 1 mL aliquot of supernatant with phosphoric acid, centrifuging at 13 400 rpm for 10 min, then filtering through a 0.2 μm syringe filter (Sartorius, Minisart). Compound separation was undertaken using a COL-Elite-FFAP capillary column (Perkin Elmer, 30 m × 0.25 mm ID × 0.32 μm phase thickness) with helium carrier gas at a flow of 2 mL/min. Samples (1 μL) were injected in split mode (50:1) with injection temperatures of 250 °C. The oven temperature was held at 50 °C for one min, before a 10 °C/min ramp to 240 °C and a final hold of 5 min. The mass spectrometer scanned from m/z 50–400 at approximately three scans per second. Data interpretation was undertaken using Agilent Chemstation software. A seven-point calibration curve and reproducibility validation for C2–C7 volatile fatty acids was constructed using a certified volatile free acid mix (Supelco, CRM46975). A three-point calibration check was analysed with each sample batch.

2.4. Microbial analysis

The microbial analysis in this study provides an end-of-experiment snapshot of the four key orders and families (*Methanobacteriales*, *Methanomicrobiales*, *Methanosaetaceae* and *Methanosarcinaceae*) that are well established as known contributors to methanogenesis during anaerobic digestion. The methanogen population of chicken litter, inoculum, digestate, leachate (LT effluent) and used biochar were analysed. Biochar, digestate and leachate samples were collected at experiment end. Leachate samples (45 mL) were centrifuged for 15 min at 4000 rpm to obtain a pellet for DNA extraction. Biochar samples were collected from the different positions investigated for biochar application. Eight samples of biochar were collected (for each replicate) from different locations throughout the layer, filter or feedstock and rinsed with MQ water upon collection to remove attached sludge. A representative sample was obtained by shaving 1 mm off one side of each the eight samples, crushing with mortar and pestle, then mixing together. To break apart biofilms, 250 mg of this representative sample was placed in 0.5 mL of phosphate-buffered saline and sonicated using 15 s pulses for 2 min. DNA was then extracted from biochar, chicken litter, digestate and leachate samples according to PowerSoil DNA isolation kit (Qiagen, Germany) instructions with elution of recovered DNA in 100 μL of elution solution, followed by precipitation and resuspension of DNA to reduce inhibiting substances. The quantity of DNA extracted from samples was analysed using a Nanodrop Spectrophotometer (NanoDrop Technologies, Wilmington, USA). To determine the relative abundance of key methane-producing families and orders in the extracted DNA, quantitative polymerase chain reaction (qPCR) was conducted using a thermal cycler (CFX Connect, Bio-Rad Laboratories, Hercules, CA) in accordance with a previously outlined procedure (Collins et al., 2023b).

The surface of biochar samples collected after experiment termination were observed using a scanning electron microscope (Quanta 450, FEI). Biochar samples were collected for each of the different positions investigated for biochar application from one replicate per case. Three samples were collected from different locations throughout the feedstock, layer or filter, rinsed with MQ water to remove attached sludge, then placed in fixative (4% paraformaldehyde/1.25% glutaraldehyde in phosphate-buffered saline, 4% sucrose, pH 7.2). Samples were prepared according to a previously outlined procedure (Collins et al., 2023b). Dried samples were coated with a surface layer of carbon.

3. Results and discussion

3.1. Effect of biochar position on methane generation

Fig. 2 presents the cumulative and daily methane yields for the total system, as well as the individual contributions of the LBR and LT, for the control case without biochar and systems applying biochar in different positions. For each subfigure, the four lines represent the mean and the bars the range of biological replicates for the four cases considered. Mean total cumulative methane yields of 178, 179, 188 and 192 mL CH_4/g-VS were observed at 51 days (Fig. 2A1) for the control and systems with biochar applied as a layer in the LBR only, biochar

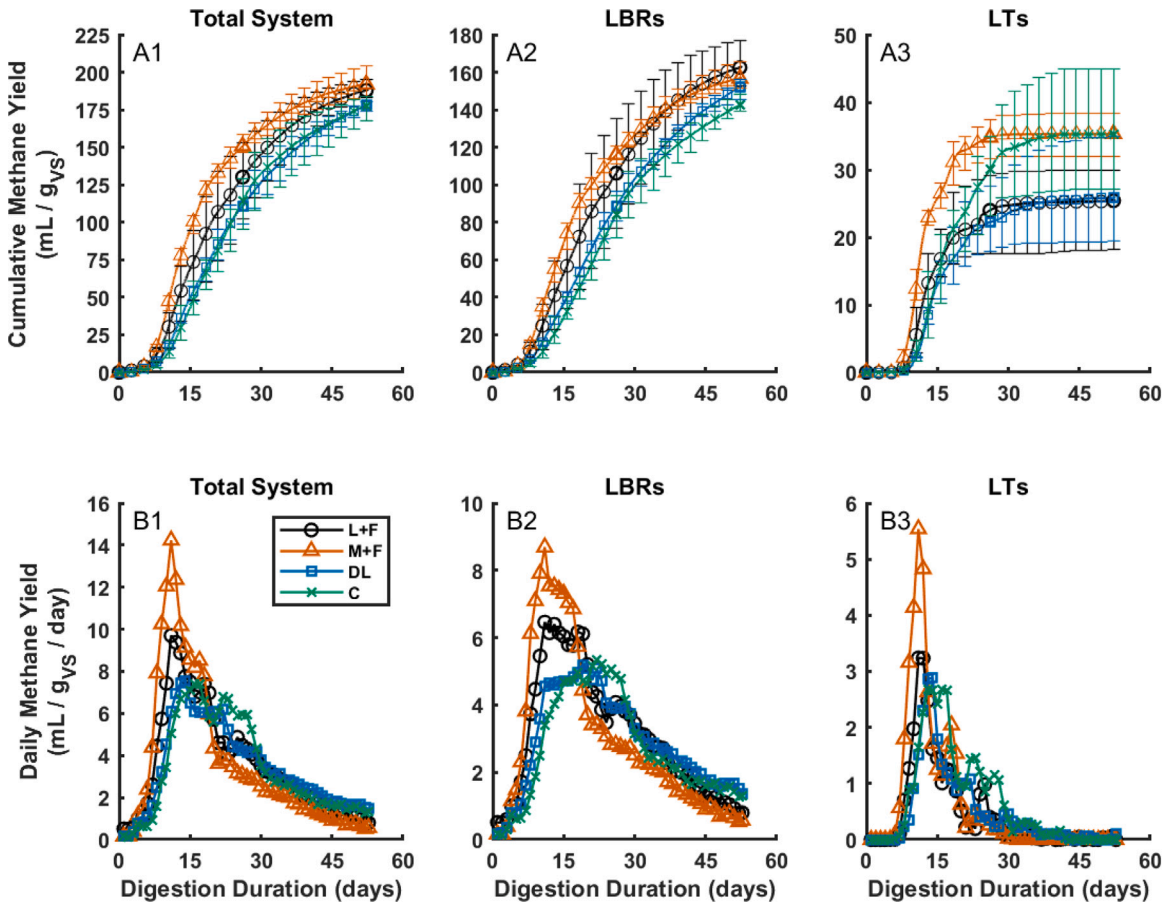

Fig. 2. The cumulative (A1 to A3) and daily (B1 to B3) methane yields for the total system (A1, B1) and the individual contributions from the LBR (A2, B2) and coupled LT (A3, B3). The yields are normalised by the initial volatile solids content of feedstock and inoculant. Data is shown for a control system without biochar (C), a system with a biochar layer in the LBR only (DL), and systems with an external biochar filter in addition to biochar mixed with feedstock (M + F), or a biochar layer (L + F), in the LBR. The lines represent the mean and the range bars the variation of biological replicates. Range bars for the daily methane yield have been provided in the Supplementary Material. Note the difference in *y*-axis scale between subfigures.

applied as a layer in the LBR with an LT filter, and biochar mixed in feedstock with an LT filter, respectively. This is a 6 to 8% increase in methane yield compared to the control when applying biochar in both reactors; whereas, applying a layer of biochar in the LBR alone did not increase methane yield. As the application of biochar in both reactors enhanced the rate of methane production (refer to Fig. 2A1), more prominent differences in cumulative methane yield compared to the control were observed throughout the experiment. Considering the case with biochar mixed in feedstock in addition to a biochar filter in the LT, at Days 20, 25, 30 and 35, the mean cumulative methane yield was 70, 37, 22 and 15% higher than the control, respectively. Therefore, the benefit of biochar application in both reactors in terms of enhanced methane production increases if shorter solid retention times are implemented when processing chicken litter in this system.

The individual contributions of the LBR and LT to the cumulative methane yield for the control and systems applying biochar in different positions is presented in Fig. 2A2 and A3. The LBR contributed 80, 86, 87 and 82% of the total methane yield for the control case, and systems with biochar applied as a layer in the LBR only, biochar applied as a layer in the LBR with an LT filter, and biochar mixed in feedstock with an LT filter, respectively. For the latter three cases, an increase of 7, 14 and 10% in mean LBR cumulative methane yield compared with the control was observed at Day 51 (refer to Fig. 2A2). The application of biochar in both reactors enhanced the rate of LBR methane production, and thus the rate of methane generation in the total system. For the case with biochar mixed in feedstock in addition to an LT filter, faster methane production in the LT (refer to Fig. 2A3) also contributed to faster production in the total system. Although less of a contribution to the total methane yield, a 30% higher mean cumulative methane yield (35 compared with 25 mL CH_4/g-VS) was observed at 51 days in the LTs for the control and system with biochar mixed in feedstock in addition to an LT filter (refer to Fig. 2A3). Potential explanations for the observed differences are discussed further in Section 3.3. It should also be noted that similar mean peak methane contents of 62 to 64% in the LBR and 74 to 75% in the LT were observed for all cases (refer to Figure SM2 in the Supplementary Material).

Daily methane production from the total system, LBR and LT is shown in Fig. 2B1 to B3 for the control and systems applying biochar in different positions. Range bars have not been included in these subfigures to improve clarity; however, they have been provided in the Supplementary Material (Figure SM3). Clear differences in the total daily methane production profiles (Fig. 2B1) were observed between the control and systems applying biochar in both reactors. For the systems applying biochar in both reactors, higher peaks in mean daily methane production of 14.2 (case with biochar in feedstock) and 9.7 mL CH_4/g-VS/d were observed at Day 11, followed by a rapid decline in the rate of methane production. For the control, a delayed and lower peak in mean daily methane production of 7.4 mL CH_4/g-VS/d was observed at Day 16, followed by extended methane production at similar, but lower, rates. The behaviour of the case with biochar in the LBR alone was closer to that of the control with peak mean daily methane production of 7.6 mL CH_4/g-VS/d at 14 days. Similar

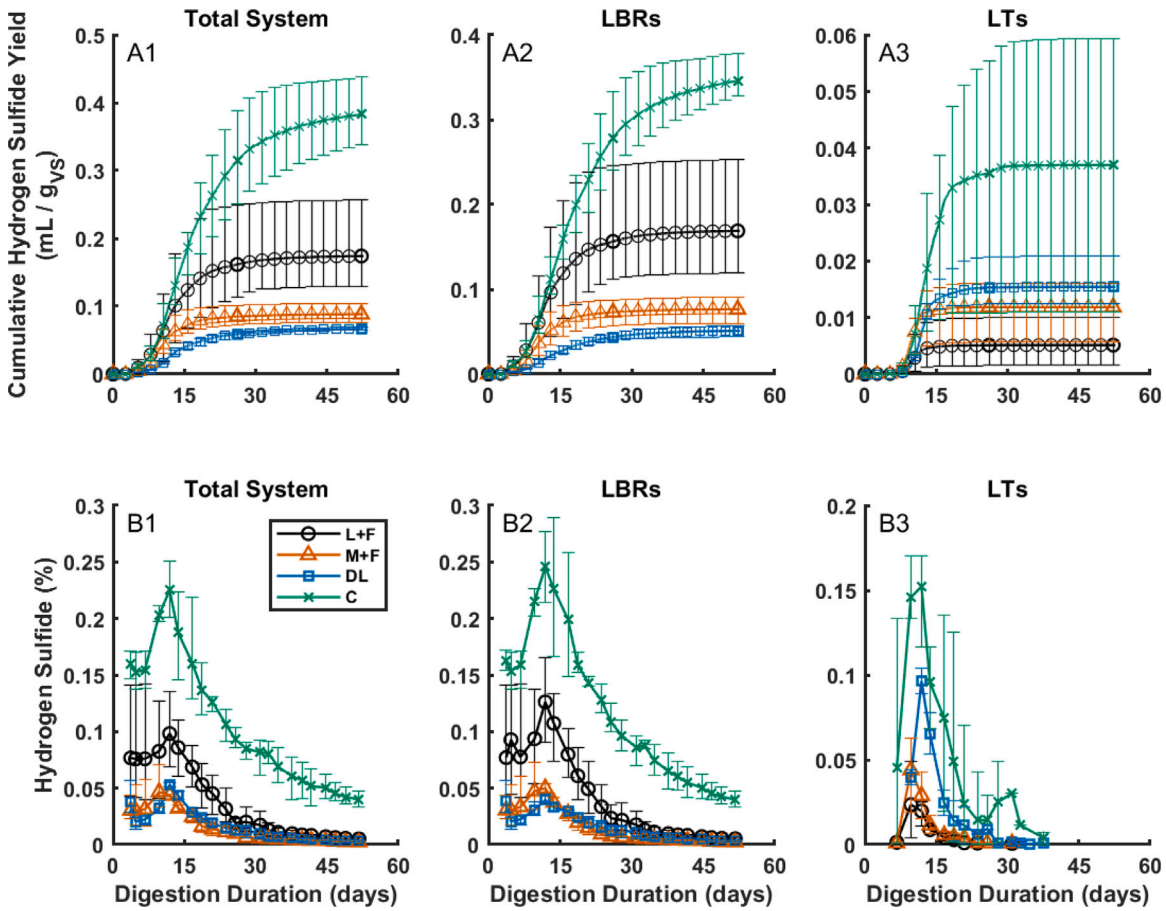

Fig. 3. The hydrogen sulfide yield (A1 to A3) and content (B1 to B3) throughout anaerobic digestion for the total system (A1, B1), LBR (A2, B2) and coupled LT (A3, B3). The hydrogen sulfide content for the total system (B1) is determined as a volume-weighted average. The yields are normalised by the initial volatile solids content of feedstock and inoculant. Data is shown for a control system without biochar (C), a system with a biochar layer in the LBR only (DL), and systems with an external biochar filter in addition to biochar mixed with feedstock (M + F), or a biochar layer (L + F), in the LBR. The lines represent the mean and the range bars the variation of biological replicates. Note the difference in *y*-axis scale between subfigures.

trends were observed for LBRs (Fig. 2B2) and LTs (Fig. 2B3) with earlier and higher rates of methane production occurring in both the LBR and LT when applying biochar in both reactors. Of the two cases applying biochar in both reactors, higher mean peak methane yields were observed in both the LBR and LT for the case that mixed biochar in feedstock. This suggests that mixing biochar with feedstock in addition to a biochar filter in the LT maximises the enhancement of methane production rate when anaerobically digesting chicken litter in this system. However, if the intention is to re-use biochar for anaerobic digestion of further chicken litter batches, separating biochar mixed with feedstock is impractical. Under these circumstances, the more practical option to enhance methane production rate is the application of a biochar layer below feedstock with a filter in the LT.

In this study, methane yields ranging from 178 to 192 mL CH_4/g-VS were observed in 51 days for anaerobic digestion of chicken litter in an LBR coupled to an LT with and without (the control) the application of biochar in various positions throughout the system. These methane yields are 33 to 36% of the theoretical methane yield (533 mL CH_4/g-VS). Experimental methane yields are expected to be much lower than the theoretical yield as the applied model does not account for non-biodegradable matter and lignocellulosic materials are difficult to degrade (Nielfa et al., 2015; Kafle and Chen, 2016). Furthermore, a reduction in methane yield is expected for high-solids anaerobic digestion (Li et al., 2013). Other studies co-digesting different ratios of chicken manure and straw in LBRs have produced methane yields of 133 mL CH_4/g-VS (90% manure) in 36 days (Wedwitschka et al., 2020) and 175 to 194 mL CH_4/g-VS (40 to 69% manure) in 35 days (Zayen et al., 2021). A comparable methane yield of 150 mL CH_4/g-VS was observed within 36 days for the control in this experiment. For the same feedstock and system used in this study, methane yields of 162 mL CH_4/g-VS have been observed for two cases; a control without leachate recirculation (90 days), and application of a biochar filter (0.8 L) in the LT only (51 days) (Collins et al., 2023b). Comparison of the control cases between the two studies demonstrates that leachate recirculation without biochar application significantly reduces solid retention time, with 90% of the methane yield obtained in 41 days compared to 66 days if leachate is not recirculated. Furthermore, all cases in the two studies that applied biochar in the LT enhanced methane production rate compared with the controls; however, enhanced methane yield was only observed for cases also applying biochar in the LBR. Overall, these results indicate that biochar should be applied in both the LBR and LT to enhance both methane production rate and yield when digesting chicken litter in this system.

3.2. *Effect of biochar position on hydrogen sulfide production*

Hydrogen sulfide production was monitored from Day 4 of the experiment. The cumulative hydrogen sulfide yields for the total system, LBR and LT are presented in Fig. 3A1 to A3. Compared with the control, the mean total cumulative hydrogen sulfide yield was reduced by 83, 55 and 77% when applying biochar as a layer in the LBR only, biochar as a layer in the LBR with an LT filter, and biochar mixed in feedstock with

an LT filter, respectively. Furthermore, the mean cumulative hydrogen sulfide yield was reduced by 85, 51 and 78% in the LBR, and 59, 68 and 86% in the LT, respectively. The reduction in hydrogen sulfide yield when applying biochar corresponds to observed differences in hydrogen sulfide content (Fig. 3B1 to B3). Mean peak hydrogen sulfide contents of 2460, 400, 1260 and 500 ppm were observed for the control case, and systems with biochar applied as a layer in the LBR only, biochar applied as a layer in the LBR with an LT filter, and biochar mixed in feedstock with an LT filter, respectively. For the same cases, mean peak hydrogen sulfide contents of 1520, 960, 240 and 440 ppm were observed in the LT, respectively.

The results demonstrate that biochar application, regardless of position in the system, significantly reduced hydrogen sulfide production. The best performing cases in terms of reduced hydrogen sulfide yield compared to the control were application of biochar (0.8 L) as a layer in the LBR only, and biochar mixed with feedstock in the LBR in addition to a biochar filter in the LT (0.4 L each reactor). In terms of biogas quality, hydrogen sulfide contents at or below 500 ppm were observed in the LBR for both cases; however, not applying a biochar filter in the LT resulted in hydrogen sulfide levels up to 960 ppm compared with 440 ppm. As 500 ppm is the suggested tolerance limit for some combined heat and power units (Choudhury and Lansing, 2021), this indicates the potential of biochar application in both the LBR and LT. For the same feedstock and system but with biochar application (0.8 L) in the LT only, peak hydrogen sulfide contents up to 4520 ppm in the LBR and 188 ppm in the LT have been observed (Collins et al., 2023b). Direct application of biochar in the LBR in this study resulted in significantly lower hydrogen sulfide content and yield. This further supports the use of biochar in both reactors. Comparison of the two studies also suggests that increasing biochar volume in the LBR and LT decreases hydrogen sulfide production in the respective reactor. Further research could investigate the influence of the ratio of biochar to feedstock and/or leachate on hydrogen sulfide reduction. It should also be noted that this study considered biochar produced at 450 °C compared to 600 °C. Biochars produced at different temperatures have been observed to influence anaerobic digestion and inhibitor levels (Zhang et al., 2019; Sugiarto et al., 2021; Collins et al., 2023a). Therefore, further research could consider the role of biochar production temperature on hydrogen sulfide reduction in this type of system.

For the case considering a biochar layer in the LBR in addition to a biochar filter in the LT (0.4 L each reactor), it is unclear why there was lower impact on hydrogen sulfide reduction in the LBR. Compared to the case applying a thicker biochar layer in the LBR only, the lower volume of biochar in the LBR may reduce the capacity for adsorption of hydrogen sulfide gas. Furthermore, if leachate channelling (bypassing large portions of the biochar layer) were to occur, the thicker layer of biochar in the LBR (50 mm, 3–4 biochar cubes thick) would ensure greater contact between leachate and biochar than the case with the thinner layer (25 mm, 1–2 biochar cubes thick). At the basic pH levels observed for leachate in this experiment (discussed in Section 3.4), dissolved hydrogen sulfide exists primarily as bisulfide ions that may react with the biochar surface to form sulfurous compounds (Yan et al., 2018). Therefore, increased contact between biochar and leachate could explain enhanced removal via this mechanism. Similarly, the increased reduction of hydrogen sulfide in the LBR when mixing an equal volume of biochar (0.4 L) in feedstock may be due to enhanced contact with feedstock and leachate that promoted mechanisms other than gas adsorption. As discussed in Section 3.2, biochar has also been reported to influence interactions between methanogens and sulfate-reducing bacteria that can both increase methane yield and reduce hydrogen sulfide production (Oliveira et al., 2020; Tsui et al., 2022). Promotion of this mechanism (if present) would be expected with greater contact between biochar, feedstock and inoculum. Further research is required to clarify the mechanisms responsible for biochar induced hydrogen sulfide reduction in this system, and the influence of biochar position on these mechanisms.

3.3. Effect of biochar position on chemical parameters

The chemical conditions of leachate extracted from the LBR and LT were monitored throughout anaerobic digestion. Fig. 4 shows changes in pH, TA, total VFAs concentration, and VFAs to TA ratio from Day 3 to 47. The variation in pH is presented in Fig. 4A1 and A2. In the LBR, initial pH ranging from 6.1 to 6.4 was observed for all cases. A slight rise in pH occurred by Day 6 for cases applying biochar in both the LBR and LT, followed by a rapid rise to 7.2 (biochar layer) and 7.5 (biochar in feedstock) by Day 10. The control and case with biochar only in the LBR had slight declines in pH by Day 6, followed by a rise to 6.5 and 7.0 by Day 10, respectively. A pH exceeding 7.0 was not observed in the control until Day 13; whereas, all cases applying biochar exceeded a pH of 7.6 at this time. In the LT, the pH was initially higher in systems with biochar filters due to the release of substances from submerged biochar (e.g. ash). At Day 6, pH minima of 6.3, 6.7, 7.3 and 7.0 were observed for the control and cases with biochar applied as a layer in the LBR only, biochar applied as a layer in the LBR with an LT filter, and biochar mixed in feedstock with an LT filter, respectively. Cases without a biochar filter did not exceed a pH of 7.0 until Days 10 (biochar in LBR only) and 13 (control). From Day 16, the pH of samples from the LBR and LT for all cases was maintained between 7.5 and 7.9. The faster rise in pH to conditions more suited for methane production when applying biochar in both the LBR and LT corresponds with observations of earlier methane production for these cases. Mixing biochar with feedstock in addition to the LT filter resulted in the fastest rise in pH and thus fastest methane production.

Differences in the TA of leachate samples depending on biochar position in the system were observed throughout anaerobic digestion as shown in Fig. 4C1 and C2. In both the LBR and LT, similar mean TA levels were observed throughout anaerobic digestion for the control and system applying a biochar layer in the LBR in addition to a filter in the LT. Compared to these cases, higher TA levels were observed when mixing biochar in feedstock in addition to a biochar filter in the LT, and lower TA levels were observed when applying a biochar layer in the LBR only. An elevated TA compared to the control could be explained by biochar introducing alkali and alkaline-earth metals from ash to the system (Wang et al., 2017); however, for the same feedstock and system, an increase in leachate TA was not observed when applying biochar compared to glass and polystyrene as filter media in the LT (Collins et al., 2023b). This suggests that the influence of alkali and alkaline earth-metals from biochar on the system is negligible compared with alkaline substances introduced from the feedstock and inoculum. The higher TA observed when mixing biochar in feedstock may indicate that increased contact between biochar and feedstock enhances feedstock breakdown and transfer of alkaline substances to leachate. This is supported by higher mean TAN and FAN concentrations (refer to Figures SM6 and SM7 in the Supplementary Material) in the first 20 days for this case. The lower TA levels observed when applying biochar layers below feedstock could be explained by: (i) contact between the biochar layer and feedstock having less impact on feedstock degradation; and (ii) the biochar layer below feedstock filtering out more alkaline substances (e.g. salts) from leachate than biochar mixed in feedstock. Increased filtering of alkaline substances from leachate for biochar layers compared to biochar mixed in feedstock could be attributed to all leachate passing through and thus contacting the concentrated layer of biochar. This filtering effect may increase with layer thickness, which corresponds with the observed differences in TA in this experiment. However, it is unclear why submerged biochar filters in the LT do not seem to have this filtering effect. Further research would be needed to clarify the differences in TA based on biochar position in this system.

The total VFAs concentration in leachate extracted from LBRs and LTs was monitored semi-weekly between Days 3 and 47 as shown in Fig. 4B1 and B2. Higher mean total VFAs concentrations were observed in leachate collected from both reactors for the control system until Day

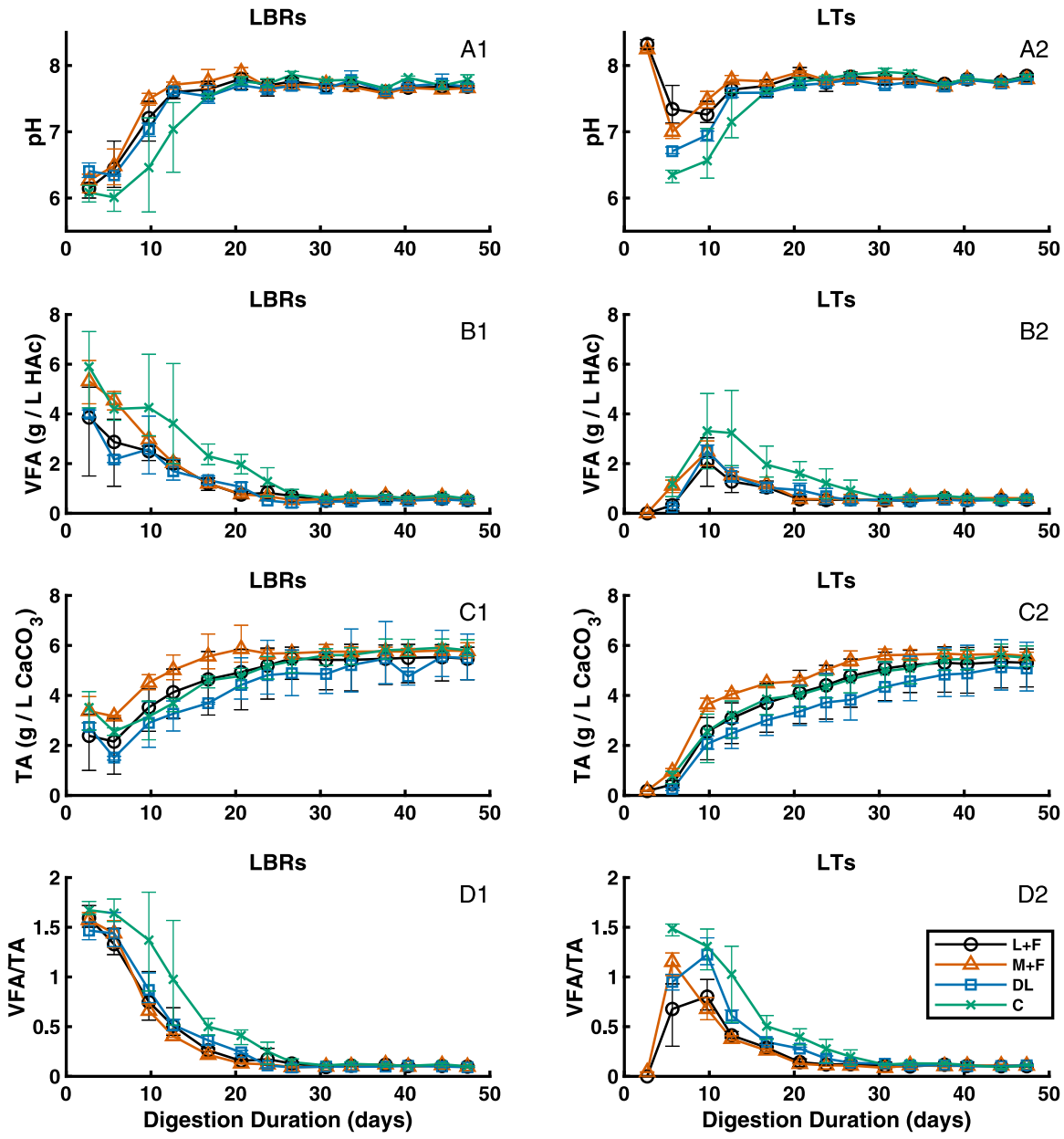

Fig. 4. The pH (A1, A2), total VFA concentration (B1, B2), total alkalinity (C1, C2), and VFA to total alkalinity ratio (D1, D2) of leachate samples extracted throughout anaerobic digestion from the LBR (A1 to D1) and coupled LT (A2 to D2). Data is shown for a control system without biochar (C), a system with a biochar layer in the LBR only (DL), and systems with an external biochar filter in addition to biochar mixed with feedstock (M + F), or a biochar layer (L + F), in the LBR. The lines represent the mean and the range bars the variation of biological replicates.

31. The enhanced LBR methane yield and production rate observed for cases applying biochar, despite lower VFA levels in leachate, indicate higher VFA degradation rates and conversion to methane in the LBR prior to leaching. Faster VFA degradation has also been observed for the same system and feedstock when applying biochar in the LT only (Collins et al., 2023b). Therefore, biochar application, regardless of position in the system, enhances the rate of VFA conversion and methane production in the LBR. Comparing the different biochar application cases, elevated mean total VFAs concentrations were observed in LBR leachate prior to Day 13 when applying biochar in both reactors despite more rapid methane production in the LBR. This may indicate that application of biochar in both reactors enhances hydrolysis and VFA production, thereby increasing methane yield. Furthermore, the higher mean total VFA concentrations for the case mixing biochar in feedstock likely indicates that increased biochar contact with feedstock maximised hydrolysis and VFA production. The elevated VFA levels in leachate for the control and case with biochar mixed in feedstock also explains the higher methane yields observed in the LT for these cases.

The composition of VFAs in leachate samples was also analysed weekly between Days 6 and 34. The mean concentrations of acetate, propionate, butyrate, isobutyrate, isovalerate, and summation of other less prevalent VFAs (formic acid, valerate, isohexanoate, hexanoate and heptanoate) are shown as stacked bars in Fig. 5 for the four cases considered. Data showing the mean and range of VFA concentrations observed for biological replicates is also provided in the Supplementary Material (Figures SM3 to SM5). In agreement with observations of higher total VFAs concentration, elevated concentrations of individual VFAs were observed in leachate from the control throughout anaerobic digestion. Acetate, butyrate and propionate were the most abundant VFAs in leachate extracted from LBRs for all cases at Day 6. By Day 13, the concentrations of acetate had declined by 39, 79, 89 and 92%, and butyrate by 16, 78, 98 and 99%, for the control, and cases with

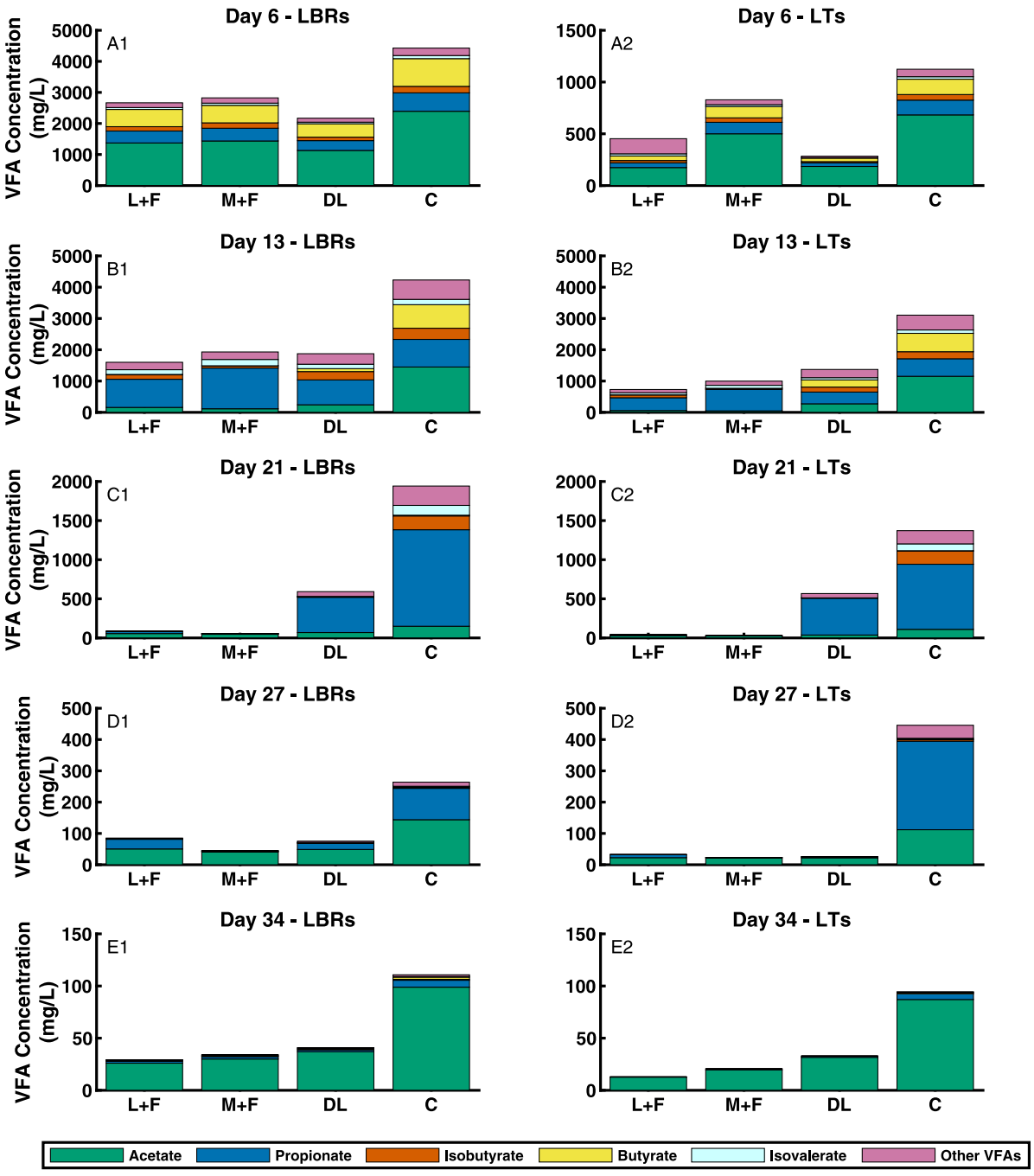

Fig. 5. The composition of VFAs in leachate samples extracted weekly from LBRs (A1 to E1) and LTs (A2 to E2) at days 7 to 35. The stacked bars show the mean concentrations of VFAs of biological replicates for the four cases considered. Specifically, the mean concentrations of acetate, propionate, isobutyrate, butyrate, isovalerate, and the sum of less prevalent VFAs (formic acid, valerate, isohexanoate, hexanoate and heptanoate), are shown. Data is presented for a control system without biochar (C), a system with a biochar layer in the LBR only (DL), and systems with an external biochar filter in addition to biochar mixed with feedstock (M + F), or a biochar layer (L + F), in the LBR. Note the difference in *y*-axis scale between subfigures.

biochar applied as a layer in the LBR only, biochar applied as a layer in the LBR with an LT filter, and biochar mixed in feedstock with an LT filter, respectively. This indicates that biochar, regardless of position in the system, promoted degradation of butyrate and acetate conversion. Furthermore, the application of biochar in both reactors resulted in the highest reductions of acetate and butyrate by Day 13. The enhanced rate of acetate and butyrate conversion supports observations of accelerated methane production for systems applying biochar in both reactors.

Propionate accumulation was observed in leachate for all cases from Day 6 to 13. Delayed propionate degradation is common in anaerobic digesters as thermodynamic barriers (i.e. higher Gibbs free energy) make syntrophic degradation of propionate unfavourable compared to acetate and butyrate conversion (de Bok et al., 2004; Xu et al., 2020). For cases applying biochar in both reactors, majority of propionate was degraded (98% reduction) between Days 13 and 21. Propionate degradation was slower when applying a biochar layer in the LBR only, with a 43% reduction between Days 13 and 21, then a 96% reduction between Days 21 and 27. In the control, propionate continued to accumulate until Day 21, followed by a 92% reduction in concentration between Days 21 and 27. This indicates that biochar application accelerated propionate degradation and that the use of biochar in

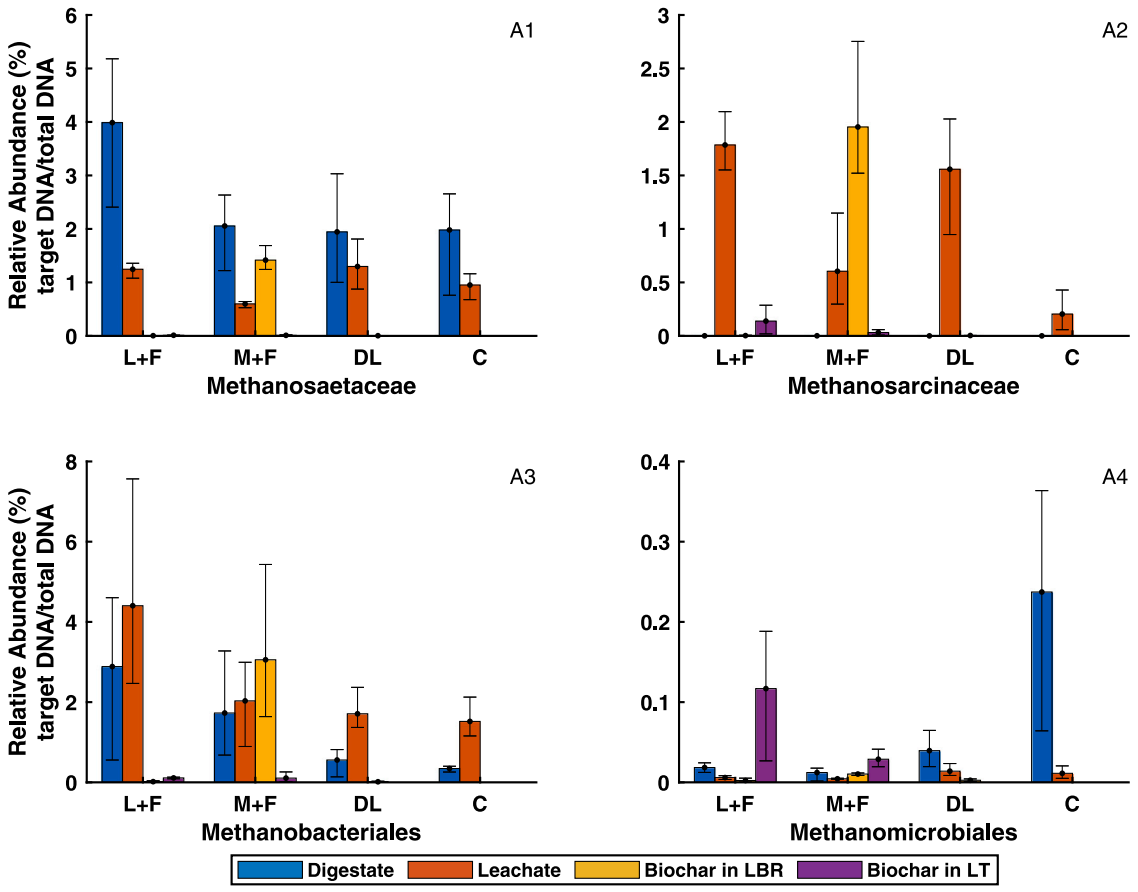

Fig. 6. The abundance of target methanogen relative to total DNA extracted from digestate, leachate (from the LT) and biochar samples after 51 days. Data is shown for a control system without biochar (C), a system with a biochar layer in the LBR only (DL), and systems with an external biochar filter in addition to biochar mixed with feedstock (M + F), or a biochar layer (L + F), in the LBR. The targeted methanogens are Methanosaetaceae (A1), Methanosarcinaceae (A2), Methanobacteriales (A3) and Methanomicrobiales (A4). The bars and range markers show the mean and variation between replicates, respectively. Note the difference in *y*-axis scale between subfigures.

both reactors enhances this effect. Although less abundant, isobutyrate, isovalerate, valerate and hexanoate (refer to Supplementary Material for the latter two VFAs) also accumulated in the control up to Day 21. The enhanced rate of methane production when applying biochar may be attributed to the accelerated degradation of propionate and these other less abundant VFAs.

The VFA composition of leachate samples extracted from the outlet of the LT were also analysed (Fig. 5A2 to E2). Note that as leachate had a hydraulic retention time of 5.8 d, the LBR samples from the previous week (Fig. 5A1 to D1) can be used to approximately indicate changes in the VFA composition of leachate across the LT. The application of biochar, regardless of position, enhanced the rate of VFA degradation in the LT compared to the control. However, direct application of a biochar filter in the LT promoted much faster degradation of all VFAs compared to the control and system with biochar in the LBR only. This faster consumption of VFAs in leachate when applying a filter in the LT likely promoted buffering of the LBR via leachate recirculation. This corresponds with the more rapid rise in pH and earlier methane production observed for systems applying biochar in both reactors.

In summary, observed changes in VFA composition suggest that biochar application in this leachate recirculating system accelerated the degradation of three- to six-carbon VFAs to substrates (acetate, formate and hydrogen via acetogenesis) that can be consumed for methane production, as well as the conversion of acetate to methane. The degradation of these longer chain VFAs is only thermodynamically feasible if the concentrations of by-products (hydrogen and formate) are kept low through an obligate syntrophic relationship between acetogenic bacteria and by-product consuming bacteria and methanogenic archaea (de Bok et al., 2004; Batstone and Jensen, 2011). Therefore, improved performance due to biochar application may be due to enhancement of this syntrophic relationship. This effect has been proposed to explain improved anaerobic digestion of chicken litter (Indren et al., 2020a,b, 2021) and dairy and swine manures (Jang et al., 2018; Xu et al., 2020) due to biochar addition. Further discussion on potential enhancement via this mechanism is provided in Section 3.4.

3.4. Effect of biochar position on methanogen populations

Methanogen populations within the system were analysed at experiment termination to further understand the effect of biochar application in different positions on methane production. Fig. 6 shows the relative abundance of the four target methanogen orders and families present in samples of digestate and leachate, and if applicable biochar from the LBR and LT, for each case. For all cases, the methanogen population in digestate consisted predominantly of *Methanosaetaceae* and *Methanobacteriales*. An elevated abundance of *Methanosaetaceae* is expected based on the dominance of this family in the inoculum (refer to Figure SM8 in the Supplementary Material). For cases applying biochar in both reactors, higher mean relative abundances of *Methanobacteriales* ranging from 1.7 to 2.9% were observed compared to the control and case with biochar in the LBR only (0.3 to 0.6%). This suggests that applying biochar in both reactors promoted the activity of *Methanobacteriales* within feedstock. As the *Methanobacteriales* order is strictly hydrogen-consuming, increased methane production via the hydrogenotrophic pathway may contribute to the enhanced methane production rate observed in LBRs for these cases. Faster consumption of hydrogen (an acetogenesis by-product) could also promote the

degradation of longer chain VFAs, in turn further accelerating methane production.

A more diverse methanogen population was observed in leachate with significant relative abundances of *Methanosarcinaceae* in addition to *Methanosaetaceae* and *Methanobacteriales*. This has also been observed when applying biochar, polystyrene and glass as filter media in the LT for the same feedstock and system (Collins et al., 2023b). The *Methanosarcinaceae* family can use both the acetate-cleaving (acetoclastic methanogenesis) and hydrogen-consuming pathways for methane production, while the *Methanosaetaceae* family are obligate acetate cleavers. The mean relative abundances of *Methanosarcinaceae* in particular were higher in leachate for cases applying biochar (0.6 to 1.8%) than the control (0.2%). Increased methanogen diversity in leachate when applying biochar may promote degradation of longer chain VFAs and thus faster methane production compared to the control. The low relative abundance of methanogens on biochar sampled from filters compared to leachate may also suggest that microbial consortia suspended within leachate are primarily responsible for methane production in the LT. This is supported by similar methane yields from the LT for cases with and without biochar filters.

Despite the accelerated methane production rate when mixing biochar with feedstock in addition to a filter in the LT, comparison of the two cases applying biochar in both reactors reveals lower mean relative abundances of *Methanobacteriales*, *Methanosaetaceae* and *Methanosarcinaceae* in digestate and leachate for this case. However, the only biochar samples with relative abundances of methanogens comparable to digestate and leachate were those that were mixed in feedstock. Preferential growth and activity of methanogens on biochar within feedstock may explain the lower relative abundances observed in digestate and leachate for this case. Interestingly, although *Methanosarcinaceae* were not observed in digestate samples, a relative abundance similar to the highest for leachate was observed on biochar samples extracted from digestate. Enhanced methanogen diversity within feedstock (on biochar) due to the presence of biochar may contribute to explaining the enhanced rates of VFA degradation and methane production despite the lower abundances of methanogens detected in digestate samples.

Attachment of microorganisms to biochar was observed on samples from within the system using scanning electron microscopy (SEM). The key differences observed between biochar located in and outside feedstock are shown in the Supplementary Material (Figure SM9). Cocci and rod-shaped microorganisms were observed on the surface of samples from all positions, often located in close proximity. However, biochar located within feedstock showed larger groups of microorganisms on the surface coated in thicker layers of extracellular polymeric substance (indicating mature biofilm). Direct contact with feedstock and inoculum would be expected to promote microorganism attachment to biochar, as well as biofilm formation in response to elevated environmental stresses within feedstock. These larger communities of microorganisms in close proximity may enable more efficient interspecies electron transfer (via hydrogen and formate) promoting VFA degradation and methane production (de Bok et al., 2004). *Methanosaetaceae* and *Methanosarcinaceae* that were both significant on biochar within feedstock are also capable of participating in direct interspecies electron transfer (DIET) when attached to biochar (Rotaru et al., 2014). These mechanisms could both contribute to enhancing the syntrophic relationship between acetogenic bacteria and methanogens. It should also be noted that sulfate-reducing bacteria compete with methanogens for acetate and hydrogen (Wang et al., 2019; Tsui et al., 2022), and that biochar has been suggested to enrich the coexistence of these microorganisms to promote substrate degradation and methane production, while reducing hydrogen sulfide generation (Oliveira et al., 2020; Tsui et al., 2022). Further analysis considering bacterial diversity could provide insight into the influence of biochar position on relationships between different bacteria and methanogens, and thus VFA degradation, hydrogen sulfide generation and methane production. However, to accurately resolve the details of microbial structure, genus and the functionality of the bacterial and archaeal elements within the system, there would need to be a series and combination of experiments to provide a further depth of analysis that is outside the scope and aims of this study. Regardless, the results from this work indicate that mixing biochar in feedstock promoted microbial attachment and activity on biochar that positively influenced anaerobic digestion of chicken litter in this leachate recirculating system.

4. Conclusions

Chicken litter is becoming a problematic waste due to the rapid growth of the poultry sector. Anaerobic digestion is a strategy that can mitigate environmental issues while producing valuable by-products. Further research improving anaerobic digestion of chicken litter is needed to increase adoption of this strategy. This study extends knowledge regarding biochar application to enhance high-solids anaerobic digestion of chicken litter by considering the influence of biochar position in an LBR system. Application of biochar in both the LBR (mixed in feedstock or as a layer below feedstock) and LT increased methane yield (6 to 8% at 51 days) and accelerated VFA degradation and methane production compared to a system without biochar. For shorter solid retention times, biochar application in both reactors resulted in significantly higher methane yields. Mixing biochar with feedstock in addition to a biochar filter in the LT maximised both methane production rate and hydrogen sulfide reduction (77% compared to the control). Furthermore, this case improved biogas quality with hydrogen sulfide contents below 500 ppm observed in both reactors. These findings promote biochar addition to feedstock in the LBR and the use of a biochar filter in the LT to enhance anaerobic digestion of chicken litter in this system.

The enhanced rates of VFA degradation and methane production can likely be attributed to differences in microbial activity depending on the position of biochar application. Elevated abundances of *Methanobacteriales* in digestate, and *Methanosarcinaceae* in leachate, were observed compared to the control when applying biochar in both reactors. Furthermore, significant abundances of methanogens were observed on biochar that was mixed in feedstock. Interestingly, despite lacking in digestate samples, *Methanosarcinaceae* were present on biochar extracted from digestate. These observations highlight enhanced methanogen diversity due to biochar application that likely contributed to enhancing anaerobic digestion.

CRediT authorship contribution statement

Ben A. Collins: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Cristian H. Birzer:** Conceptualization, Supervision, Writing – review & editing. **Stephen P. Kidd:** Conceptualization, Supervision, Writing – review & editing. **Tony Hall:** Methodology, Investigation, Formal analysis. **Paul R. Medwell:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.jenvman.2023.118404.

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