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ABSTRACT

Biochar addition to anaerobic digestion systems generally improves the conversion of organic waste into methane, yet biogas residues are still unwanted byproducts that may pollute the environment. Therefore, we hypothesized that biogas residues could be recycled into new biochar. We produced biochar by heating at 500 °C the residue of anaerobic digestion of food waste. We measured methane production by adding either this residue biochar, or coconut shell biochar, or corn stalk biochar, in the anaerobic co-digestion of sewage sludge and food waste. Results show that the residue biochar produces the highest daily methane amount, of 432.2 mL per g of volatile solids, versus 377.7–386.3 mL for coconut and corn biochars. This finding is tentatively explained by the fact that the residue biochar has more basic groups, which neutralize fatty acids and, in turn, alleviates acidification. Another explanation is the higher abundance in sludge of electroactive *Clostridia*, *Methanobacterium* and *Methanobrevibacter*, which are known to accelerate methanation. Recyling biogas residues as biochar onsite would both decrease the amount of digestion byproducts and avoid long-distance transportation of biochar from remote biomass.

1. Introduction

Anaerobic digestion is a classical process allowing to produce biomethane by biodegradation of organic materials [1]. Strategies to enhance anaerobic digestion performance and achieve more energy production include the optimization of operating parameters, pre-treatment of feedstock, co-digestion and supplementation [2]. Anaerobic co-digestion of sewage sludge and food waste provides a more balanced nutritional diet, which alleviates the negative effects of mono-digestion [3]. However, the determination of optimal feedstocks ratios remains challenging since performance is controlled by various factors such as feedstock type, composition, trace element content, and biodegradability [4]. For instance, improper mixing may cause problems such as the lack of volatile fatty acids (VFAs) as essential nutrients. The use of additives is also promising [5,6]. Additives are generally carbon-based materials with conductive properties such as biochar, powdered activated carbon, granular activated carbon, graphene, graphite, carbon cloth, and multiwall carbon nanotubes or single-wall carbon nanotube [7,8].

Biochar refers to granular solids formed by pyrolysis and carbonization of biomass [9]. Biochar is stable and has a microporous structure and a large surface area. Biochar is relatively cheap and can be produced in high quantities [10]. Biochar is used for carbon sequestration and fertilization of soils in agriculture [11]. Biochar also enhances the performance of anaerobic digestion, and produce more biogas in general [12,13]. This improved performance is due to the fact that biochar enhances the buffering capacity, alleviates ammonia-acid inhibition, and improves microbial enrichment and interspecific electron transfer (IET, [14]. Other explanations are the following:

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Abbreviations

IET	interspecies electron transfer
DIET	direct interspecies electron Transfer
TS	total solids
VS	volatile solids
OLR	organic loading rate
HRT	hydraulic retention time
SCOD	soluble chemical oxygen demand
VFAs	volatile fatty acids
FID	flame ionization detector
TCD	thermal conductivity detector
SEM	scanning electron microscope
XRD	X-ray diffraction
FTIR	Fourier-transform infrared spectroscopy
DOM	dissolved organic matter

- the biochar porous structure favors the enrichment and growth of anaerobic microorganisms on biochar surface;
- biochar has strong adsorption ability, thus removing toxic compounds for microbes;
- biochar is electrically conductive and, in turn, acts as an electron conductor to promote direct interspecies electron transfer (DIET) between syntrophic bacteria and methanogen archaea, thereby improving the methane production [2,15,16].

The mechanisms by which carbon solids improve anaerobic digestion are debated. For example, granular activated carbon promotes the DIET between *Geobacter* and *Methanosaeta* [17]. On the other hand, biochar directly promoted the production of methane from propionic acid and butyric acid through DIET, and accelerated the decomposition of VFAs [18]. Since *Geobacter* is minor in sewage sludge, further research is needed to confirm whether biochar promotes DIET directly or other bacteria also participate in DIET. Biochar alleviates ammonia and acid inhibition, thereby shortening the hysteretic period and accelerating methane production [15]. Here, a meta-analysis shows that biochar promotes methane production and the overall performance of anaerobic digestion [13]. Results show that methane production is mainly controlled by feedstock type, pyrolysis temperature and biochar concentration, but not by pH, size, surface area and methanogen species.

Raw materials used to make biochar include wood, agricultural and forest residues, food waste, sugar, industrial organic waste, sewage sludge and stool [12]. Coconut husk and corn stalk are two common agricultural wastes. Adding coconut shell biochar and corn stover biochar to the anaerobic digestion promotes methane production [19,20]. Although these biochars are known to improve the performance of anaerobic digestion, the cost for collecting and transporting of carbon sources is high. On the other hand, huge amounts of unwanted biogas residues are produced after anaerobic digestion, which unfortunately become one of the reasons that restrict the development of anaerobic digestion facilities [21]. Theoretically, biogas residue may be applied to soil as fertilizer, because this residue is rich in nitrogen, phosphorus, potassium and humus [22]. However, direct land application is not feasible due to the presence of pathogenic microorganisms, odor, and high moisture content [23,24]. Therefore, other ways of recycling biogas residues are needed. Few studies have reported the preparation of biochar from biogas residue and further use of the produced residue biochar to improve anaerobic digestion. Generally, residue biochar is better in properties than biochar from other materials, in terms of pH, cation exchange capacity (CEC), hydrophobicity, carbon yield, and adsorption performance [25,26]. Recycling biogas residues into biochar that can be further used to improve anaerobic digestion would increase the circularity of the system, avoid transport of external biochar from remote biomass sources, and reduce greenhouse gas emission [27].

This study compared the performance and methane production of anaerobic digestion treating sewage sludge and food waste supplemented with biochars prepared from biogas residue, coconut shell and corn stover, respectively; the structure of biochar and microbial community were also analyzed to get insights on digestion mechanisms. This work evaluated the comprehensive performance of biogas residue biochar applied to anaerobic digestion and was expected to provide a strategy to improve the performance of anaerobic digestion while solving the problem of biogas residue handling from the source. In addition, this work further provided data support for the promotion and application of co-digesting food waste and sludge in large-scale projects.

2. Materials and methods

2.1. Substrates and inoculum

The sewage sludge was taken from the non-dehydrated, secondary settling tank of the second Suzhou wastewater treatment plant. Degreased food waste was taken from a food waste plant in Suzhou, China. The inoculum for co-digestion was collected from a food waste anaerobic fermenter. The fresh sewage sludge and food waste were taken to the laboratory and stored at 4 °C before experimental use. The inoculated sludge was pre-cultured under anaerobic conditions for 14 days to remove organic pollutants. Table 1 lists the characteristics of sewage sludge, food waste and inoculum.

2.2. Biochar preparation

Biochars were prepared from three raw materials: 1) biogas residue was taken from an anaerobic fermentation tank of a food waste treatment plant in Suzhou, with 80.0% moisture content and 8.9% volatile solids (VS) after dewatering. 2) Coconut shells and 3) corn stalks were collected locally and washed. Raw materials were dried at 60 °C overnight, ground thoroughly, 100-mesh sieved, then heated in a Jingke OTF-1200X—S (Hefei) tubular resistance furnace at 500 °C for 2 h under N_2, at a heating rate of 10 °C/min. After cooling, molded biochars were stored in a dry dish filled with water-absorbing silica gel.

2.3. Experiments

The feed stock ratio used was sewage sludge/food waste 1/1, v/v (without dehydration). This mixture had 67.8 g/L total solids (TS) and 49.6 g/L VS. Anaerobic digestion was operated in a semi-continuous mode. The experiment was carried out in four identical 1 L conical flasks, equipped with a rubber plug with a feed port, a sampling port and a gas outlet. The gas sampling bag was connected to the gas outlet, and the daily biogas production was measured through a graduated 500 mL gas-tight syringe (Tongji 5 × 4U5, Ningbo, China).

In the domestication stage, 300 mL of inoculum were added to the bottle reactors in advance, then bubbled with dinitrogen for 5 min to ensure an anaerobic environment. The bottle reactors were placed in a shaker with high temperature of 55 ± 1 °C at 100 rpm. After that, 30 mL mixed stock was fed every day without discharging, and continuously operated for 10 days. The actual capacity of the system was 600 mL, which is suitable for stable

Table 1
Properties of feedstocks and inoculum[a].

Parameters	Unit	Sewage sludge	Food waste	Inoculum	Sewage sludge-food waste mixture (1/1, v/v)
pH	/	6.84	4.12	7.82	5.78
Total solids (TS)	%w	2.41	11.20	3.34	6.78
Volatile solids (VS)	%w	1.62	8.43	2.35	4.96
VS/TS	%w	67.22	75.27	70.36	73.16
C/N	/	5.86	11.08	14.31	9.22

[a] The inoculum was collected in the thermophilic (55 °C) tank of anaerobic digestion of food waste.

operation [28].

In the experimental phase, the organic loading rate (OLR) was set as 1.49 g VS/L/d, and the hydraulic retention time (HRT) was set as 20 d. 30 mL mixed substrate was fed in and 30 mL was discharged every day. The biogas production was measured every day, and the fermentation broth and biogas composition were analyzed every three days during the first 15 days, and every 5 days thereafter. All samples were analyzed in triplicate. On the 15th day, the fermentation broth in the bottle was taken for microbial community analysis. The pH was not adjusted during the whole experiment, and the pH value stabilized at 7.2–8.2 by internal adjustment of the anaerobic digestion system.

The optimal dosage of biochar is 7.5–15.0 g per L of working volume to improve the performance of anaerobic digestion [29]. In this study, the biochar dosage was set at 8.0 g/L. The experiment includes four type of runs: 1) a control where inert glass was added, 2) addition of coconut shell biochar, 3) addition of corn stover biochar and 4) addition of biogas residue biochar. The lost weight, about 0.24 g of biochar due to digestate discharge was supplemented into the corresponding digester every day, according to Ref. [30].

2.4. Analytical methods

2.4.1. Characterization of biochar

The elemental composition of biochar was measured using the element analyzer Vario EL cube, Elementar, Germany. Biochar pH was measured with a glass electrode using a biochar/deionized water ratio of 1/10, w/w. Before pH measurement, the mixed sample was shaken at room temperature at 120 r/min for 12 h. The quantity of acidic and basic functional groups was measured by the Boehm method [31]. The surface morphology of biochars was observed using a scanning electron microscope (SEM) from Hitachi, Ltd. S4800, Japan. Samples were covered with gold before SEM. The crystal structure of biochar was measured by X-ray diffraction (XRD, Rigaku Ultima IV, Japan). The scanning range was 5°–60° with a scan speed of 0.5°/min. Functional groups on the surface of biochar were qualitatively measured using a Fourier transform infrared spectrometer (FT-IR) from Bruker/Tensor 27, Germany, at 500-4000 cm^{-1}.

2.4.2. Biogas slurry analysis

The collected digestate liquid of the reactor was centrifuged at 4000 rpm for 10 min, and the extracted supernatant was immediately placed in the refrigerator and stored at 4 °C. The chemical determinations were performed within 24 h. For specific parameters, the supernatant was analyzed after being filtered with an organic filter column (0.45 μm). Concentrations of TS, VS, soluble chemical oxygen demand (SCOD) and ammonia nitrogen were determined according to standard methods [32]. The filtrate was digested with potassium dichromate reagent at 150 °C for 2 h to analyze SCOD concentration, then the colorimetry was tested in a DR2800 spectrophotometer (HACH Co., Loveland, CO, USA). Nessler's reagent was used to analyze concentration of ammonia nitrogen with the spectrophotometer Jingke 721G, Shanghai, at a wavelength of 420 nm. The pH of the slurry was measured by a pH meter (Leici PHBJ-260, Shanghai). The absorbance of coenzyme 420 was measured by ultraviolet spectrophotometry, according to Ref. [33]. Fatty acid concentrations were determined using an Agilent GC7890 gas chromatograph (USA) equiped with a flame ionization detector (FID) with nitrogen as the carrier gas and a 30 m × 0.32 mm × 0.5 μm DB-FFAP capillary column.

2.4.3. Biogas analysis

The biogas production was measured by a 500 mL graduated syringe Tongji 5 × 4U5, Ningbo China. Methane production was calculated by multiplying the biogas production by the methane content. Then, the conversion was performed according to the volatile solids of the daily feed to obtain the average daily methane production.

Biogas compounds were analyzed by a gas chromatograph equipped with a packed chromatographic column (30 m × 0.32 mm × 0.5 μm, Agilent Technologies, USA) and a thermal conductivity detector (TCD). Argon was used as carrier gas. The flow rate of the carrier gas was 25 mL/min, and the sample injection volume was 1 mL. The temperature of the injector and detector were set to 80 °C and 120 °C, respectively.

2.4.4. Microbial community analysis

The microbial communities in the sludge collected from the digesters during the relatively stable phase, on the 15th day of anaerobic digestion, were analyzed by high-throughput sequencing. Sludge samples, ca. 1.0 g, were collected in each bottle and stored at −20 °C. Subsequently, samples were sent to Majorbio, Shanghai for PCR amplification (PCR: polymerase chain reaction). For pyrosequencing, the V4 region of the 16S rRNA gene was amplified by PCR using the bacterial primer set 515F (5′-GTGCCAGCMGCCGCGG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′), while the archaea primer set, 524F10extF (5′-TGYCAGCCGCCGCGGTAA)-3′) and Arch958RmodR (5′-YCCGGCGTTGAVTCCAATT-3′) expanded the V4–V5 region of the 16S rRNA gene. PCR and sequencing were carried out according to Ref. [34]. The sequences obtained in different samples were then classified into phylum and genus level, and the pyrosequencing analysis was done on an Illumina platform (Illumina Miseq PE300).

3. Results and discussion

We studied the effect of adding biogas residue biochar, coconut shell biochar, corn stover biochar and inert glass, as control, in anaerobic co-digestion of sewage sludge and food waste respectively. We analyzed biochar structure, methane production, digestion performance and microbial communities.

3.1. Characterization of biochars

Table 2 shows the elemental composition of the three biochars. Results show that the C/N ratio of coconut shell biochar, of 48.4, and

corn stover biochar, of 30.4, is much higher than that in biogas residue biochar, of 10.6. The optimal C/N ratio for conventional anaerobic digestion processes is between 15.0 and 30.0, whereas for *methanogenic archaea,* the optimal C/N is between 16.0 and 19.0 [35]. Table 2 also shows that the pH of the three biochars prepared under the same conditions is very close, of 9.9–10.3. By contrast, the number of basic groups on the surface of the biogas residue biochar is 3.88 mmol/g biochar, much higher than that for the coconut shell biochar, of 2.42 mmol/g and corn stover biochar, of 2.96 mmol/g. This suggests that basic groups in the biogas residue biochar could buffer the build-up of excessive fatty acids during the anaerobic digestion.

Fig. 1a shows the SEM images of coconut shell biochar, corn stover biochar, and biogas residue biochar. Results show lumpy structures for the coconut shell biochar, lamellar shapes for the corn stover biochar, and flocculent morphology for the biogas residue biochar. The more porous structure of the biogas residue biochar suggests the presence of more accessible sites for the reaction of digestive materials, according to Ref. [36].

Fig. 1b shows the XRD analysis of biochars. Broad peaks at 25.0°–28.0° with different widths are attributed to the formation of the turbostratic domains in the biochar structure. The peaks of biogas residue biochar and corn stover biochar are narrower than that of the coconut shell biochar, indicating that the crystallinity of the biogas residue and corn stover biochars are higher, according to Ref. [37]. The peak of biogas residue biochar is shifted to the right compared to other biochars. This is due to the large sample volume and high sample layer thickness during sample preparation and tableting that caused the overall diffraction peak shift to a larger diffraction angle. The typical reflections at 29.4°, 36.0°, 39.4°, and 48.5° indicate the presence of calcite ($CaCO_3$) crystals in the biogas residue biochar, and the sharp peak at 29.4° suggests a good crystal structure [3]. The patterns reveal that corn stover biochar has strong diffraction peak at 2θ values of 26.0°, and the peak is the (0 0 2) characteristic diffraction peak of graphite crystals GP002 [38].

Fig. 1c shows the FTIR analysis of biochars. Biochars have acquired various surface functional groups after pyrolysis at 500 °C. The band at 1400 cm^{-1} may be caused by O–H in-plane bending and C–H bending, while the band at 3000 cm^{-1} represents the symmetric CH_3 stretching of the methoxy group, partially caused by O–H stretching [39]. The aromatic CH stretching region (900-700 cm^{-1}), the oxygen-containing functional group region (1800-1000 cm^{-1}), and the aliphatic CH stretching region between (3000-2700 cm^{-1}) are the three main regions influencing anaerobic digestion performance [40]. Our data shows that the vibrations of the biogas residue biochar in these areas are higher than that of corn stover biochar and coconut shell biochar, implying that the corresponding groups are more abundant on the surface of biogas residue biochar.

The spectrum above 3500 cm^{-1} represents the stretching vibration zone of N–H and O–H. Alkaline functional groups such as –OH, C≡C, –NH, C═O(CO) and CO_3^{2-} should increase the biochar alkalinity, and thus could buffer organic acids produced by anaerobic digestion [41]. Compared with coconut shell biochar, the peaks of the biogas residue biochar and the corn stover biochar at the corresponding positions are much more prominent, indicating that there are more redox active groups presented [39]. In addition, Table 2 shows that biogas residue biochar has more basic groups on the surface. These data indicate that biogas residue biochar has the potential to buffer the pH of anaerobic digestion, adjust formation of VFAs, and alleviate the inhibition of high rates of organic loading shocks.

Overall, the more porous and fragmented structure, and the presence of more functional groups, in particular basic groups in the biogas residue biochar suggest that this residue biochar would be more efficient in enhancing anaerobic digestion.

3.2. Methane production

Methane production results during anaerobic digestion of sewage sludge and food waste supplemented with biogas residue biochar, coconut biochar, and corn biochar, and a control group without biochar addition were demonstrated in Fig. 2. The addition of biochars increased the production of biogas on the first day significantly, with values of 1530.0 mL for biogas residue biochar addition, 1330.0 mL for addition of coconut shell biochar, 1220.0 mL for corn stover biochar addition and 1100.0 mL for control addition (Fig. 2a). The higher methane production for the biogas residue biochar addition group is obvious during the first 15 days (Fig. 2b) and continuously higher in average values over 35 days, with 432.2 mL/g VS/d for the biogas residue biochar addition group, 386.3 mL/g VS/d for the corn stover biochar addition group, 377.7 mL/g VS/d for the coconut shell biochar addition group and 295.7 mL/g VS/d for the control (Fig. 2c). These results confirm that biochar improves methane production of anaerobic digestion [19,42]. And the biogas residue biochar addition group presents the best methane yield, which was previously less known.

Table 3 presents the effects of adding different biochars on anaerobic digestion performances under different substrates. In general, biochar addition effectively neutralizes fatty acids and accelerate their decomposition while maintaining the system's ammonia nitrogen concentration at a low level and relieving the accumulation of free ammonium; the biochar addition could also selectively enrich bacteria and archaea that contribute to methanogenesis in a specific environment, increasing biogas production and methane production.

3.3. Evolution of pH, ammonia nitrogen, F420 absorbance and VFAs

Fig. 3a shows pH evolution during anaerobic co-digestion of sewage sludge and food waste supplemented by inert glass (control), coconut shell biochar, corn stover biochar and biogas residue

Table 2
Elemental composition and physicochemical properties of biochars.

Parameters	Unit	Coconut shell biochar	Corn stover biochar	Biogas residue biochar
N	%	1.52	2.00	2.17
C	%	73.75	60.78	22.92
H	%	2.62	2.12	1.09
S	%	5.76	0.54	0.44
C/H	%	20.18	28.72	20.99
C/N	%	48.44	30.36	10.56
pH	/	10.06	10.30	9.91
Surface basic groups	mmol/g biochar	2.42	2.96	3.88
Surface acidic groups	mmol/g biochar	0.68	0.45	0.32

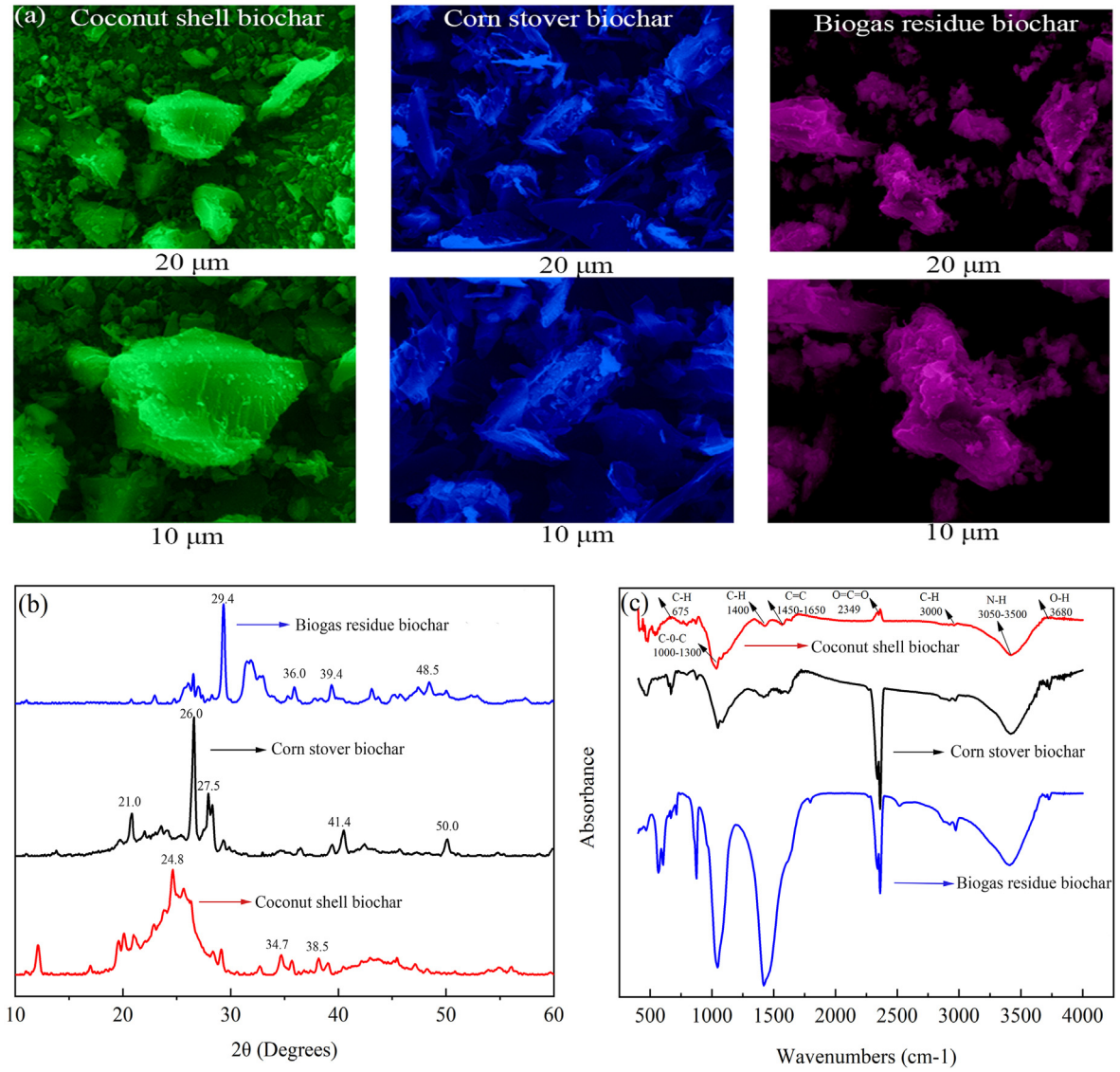

Fig. 1. Analysis of biochars by (a) scanning electron microscopy (SEM), (b) X-Ray diffraction (XRD) and (c) Fourier-transform infrared spectroscopy (FTIR).

biochar. These four runs show similar variations starting by a slight decrease from pH around 7.7 initially to pH 7.4 after 20 days, then an increase to pH 7.8 after 35 days. These pH values are within the optimal pH range for methane production [48,49]. Our results also show that the pH of the biogas residue biochar run is generally slightly higher than for other biochars. This suggests that the addition of biogas residue biochar maintains the acid-base stability better than other biochars.

Fig. 3b shows an overall decrease of ammonia nitrogen from about 2050 mg/L to 1750 mg/L after 35 days, with no clear difference between added solids. These ammonia nitrogen levels are too low to inhibit anaerobic digestion. Indeed, ammonia nitrogen levels higher than 3500 mg/L are leaded to inhibit anaerobic digestion [5,6,50]. This means that anaerobic co-digestion sewage sludge and food waste effectively alleviates inhibition by ammonia nitrogen [1,51]. To conclude, we did not find any clear effect of adding biochar on ammonia nitrogen levels.

Fig. 3c shows the evolution of coenzyme F420 absorbance. Indeed, coenzyme F420 is a unique electron transport enzyme in the methane production pathway, and therefore coenzyme F420 is used to monitor methanogen activity to a certain extent [52]. Our results show that at the start of digestion, the coenzyme F420 activity of biochar-added runs are higher that the control. This suggests that biochar favors methanogen activity. Moreover, at that time, coenzyme F420 activity is the highest for the biogas residue biochar, and corresponds to the highest initial methane production rate (Fig. 2b). Afterwards, coenzyme F420 absorbance show an 'S' shaped trend and there is no clear difference between added solids.

Fig. 4 shows the profiles of fatty acid content during anaerobic digestion. Here we assume that levels of volatile fatty acids exceeding 3500 mg/L is an indicator of anaerobic digestion inhibition, according to Ref. [51]. We observed that during the first 15 days only mild inhibition occurred for the control (inert glass) and coconut shell biochar. On the 12th day, all runs switched to acetic acid-dominated fermentation. Indeed, propionic acid and butyric acid are first converted into acetate and CO_2/H_2 by acetogens, then utilized by aceticlastic- and hydrogenotrophic methanogens [53]. Also, the conductivity of biochar acts as a bridge between fermenting bacteria and methanogens, and thus promotes the production of methane by conversion of propionic acid and butyric

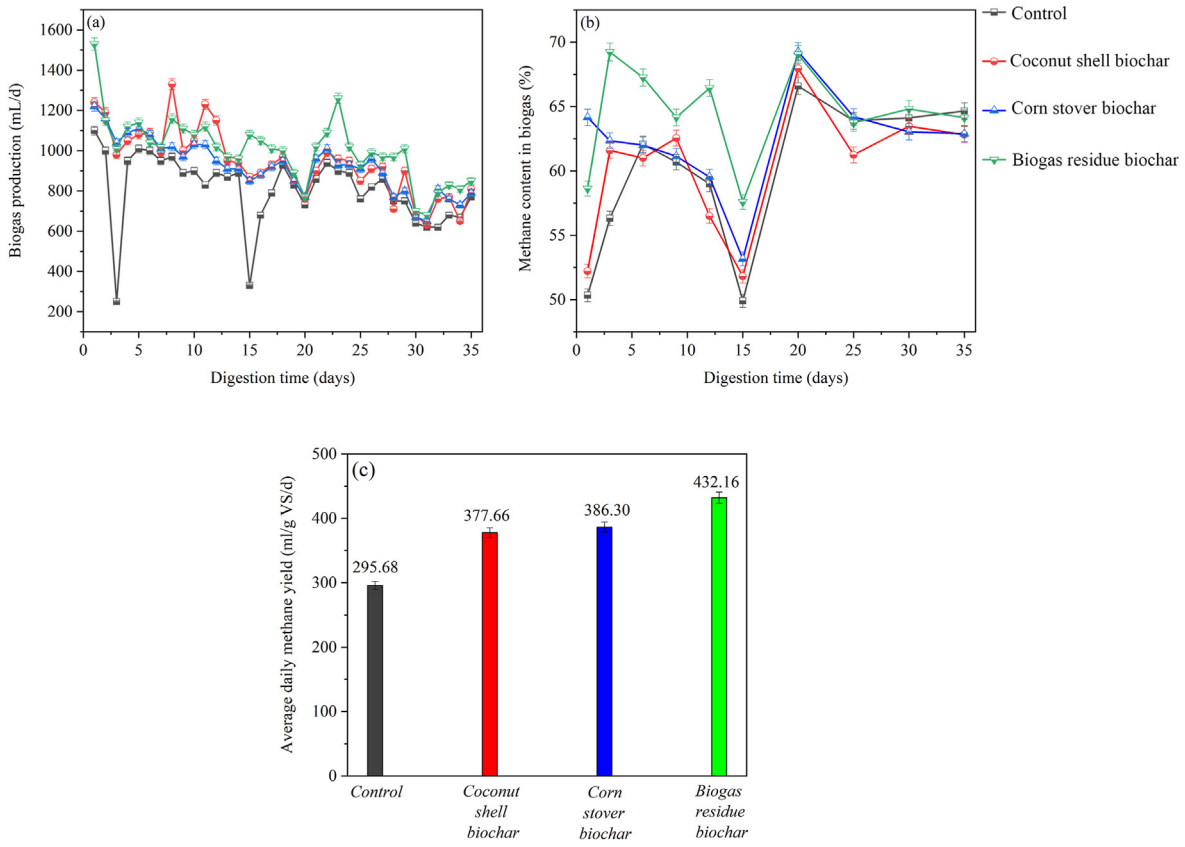

Fig. 2. Biogas produced during anaerobic digestion of sewage sludge and food waste supplemented with inert glass (control), coconut shell biochar, corn stover biochar and biogas residue biochar. (a) Daily biogas production, (b) methane content in biogas produced daily and (c) average daily methane yield during the entire digestion process duration of 35 days. Daily biogas production was calculated in a period of 24h.

Table 3
Effects of adding biochar in anaerobic digestion.

Digestion substrates	Biochar	Effects	References
Sewage sludge; food waste	Citrus peel	- Effectively neutralized fatty acids (FAs) - Alleviated systematic acidification - Increased methane yield	[3]
Poultry litter	Wood pellets	- Increased methane yield and maximum biogas production - Shortened lag phase time.	[43]
Algal biomass; food waste	Algal	- Improved microbial activities increased methane yield.	[44]
Food waste	Wood chips	- Accelerated and stabilized start-up of thermophilic semi-continuous anaerobic digestion - Enhanced process stability - Promoted interspecies electron transfer (IET)	[45]
Chicken manure	Fruitwood	- Reduced concentration of organic acids and total ammonia nitrogen - Boosted methane production	[46]
Sorghum	Rice husk	- Increased the sorghum maximum methane production rate - Shortened lag phase time - Increased alkalinity	[47]

acid into acetic acid [54]. On day 15, fatty acid levels were minimum, in agreement with the low methane content (Fig. 2b). The slight decrease of methane production after 20 days (Fig. 2b) is probably due to the rise of propionic acid (Fig. 4) in the digestive system, according to Ref. [3]. Overall, we did not observe systematic differences between added biochars.

3.4. Evolution of dissolved organic matter and removal of SCOD

Fig. 5 shows the evolution of dissolved organic matter (DOM) during anaerobic co-digestion of sewage sludge and food waste supplemented by inert glass (control), coconut shell biochar, corn stover biochar and biogas residue biochar, on days 6, 15 and 35. The two green fluorescent spots correspond to the protein-like region, of Ex/Em 280/300–350 nm, and the ligno-cellulosic region, of Ex/Em 220/300–350 nm [20].

On day 6, we observed that the fluorescence intensity of biochar runs is slightly higher than control. This may be explained by the precipitation of protein and cellulose [55]. This is consistent with the increase of SCOD on day 15 (Fig. 6). This is consistent with the decrease of methane content on day 15, followed by an increase (Fig. 2). Indeed, precipitation of protein and cellulose is likely to inhibit physically methanogen colonies, then nutrients freed by precipitation would rapidly activate methanogen growth.

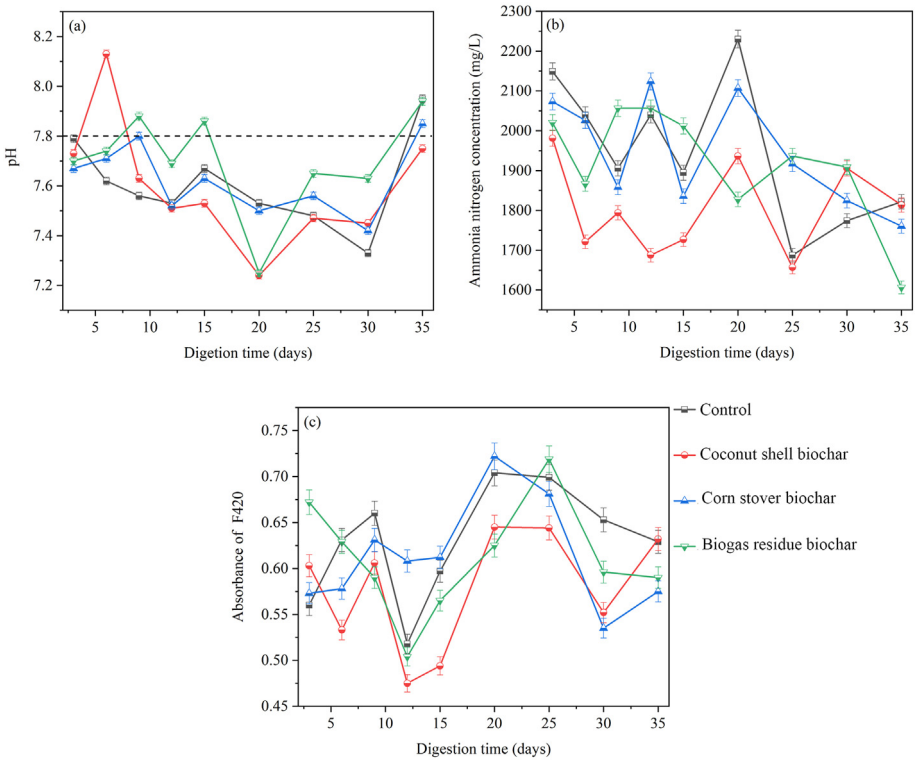

Fig. 3. Evolution of pH (a), ammonia nitrogen concentration (b) and coenzyme F420 absorbance (c) during anaerobic co-digestion of sewage sludge and food waste supplemented by inert glass (control), coconut shell biochar, corn stover biochar and biogas residue biochar.

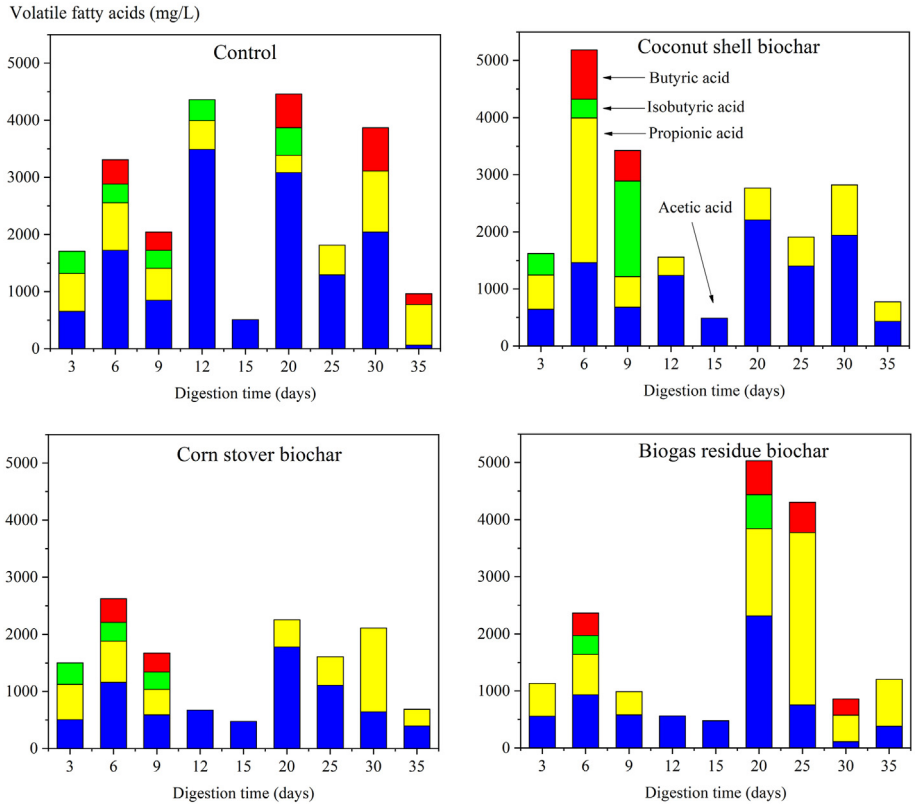

Fig. 4. Evolution fatty acid composition during the anaerobic co-digestion of sewage sludge and food waste supplemented with inert glass (control), coconut shell biochar, corn stover biochar and biogas residue biochar.

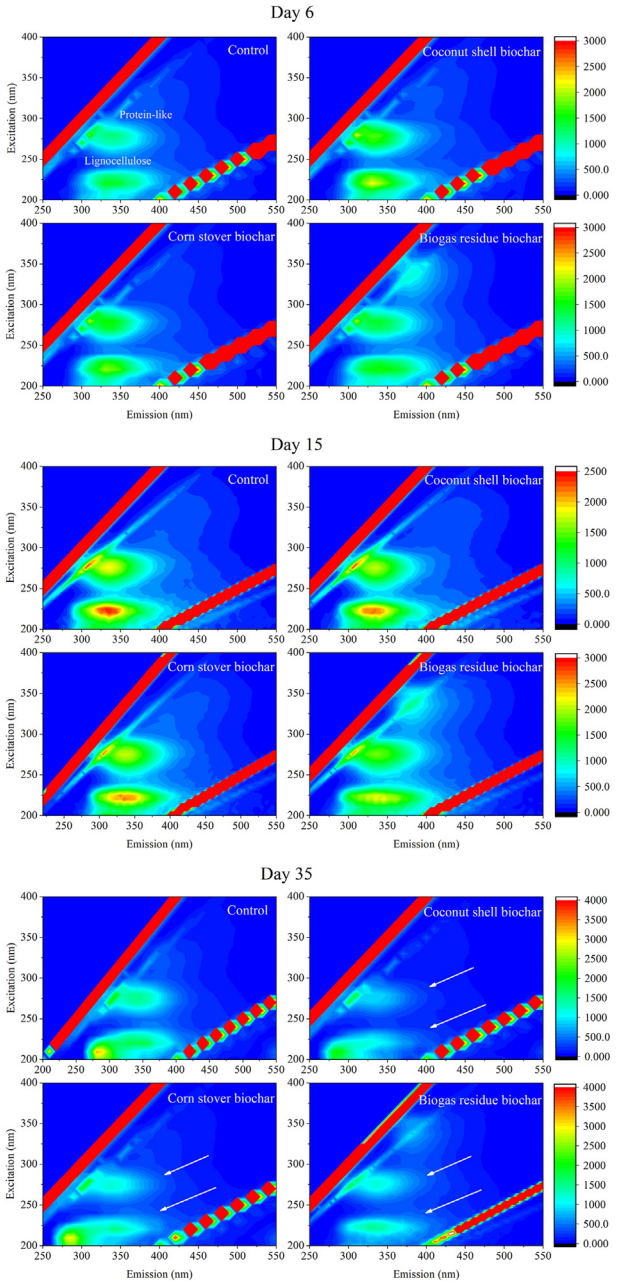

Fig. 5. Evolution of dissolved organic matter (DOM) during anaerobic co-digestion of sewage sludge and food waste supplemented by inert glass (control), coconut shell biochar, corn stover biochar and biogas residue biochar, on days 6, 15 and 35. White arrows shows the lower fluorescence of protein-like and ligno-cellulosic spots for biochar runs versus control.

On days 15 and 35, the fluorescent spots of the control run (inert glass) were more intense than for biochar runs (Fig. 5). This indicates that, after 15 days, biochar accelerates the degradation of protein and cellulose in the digesters. The fluorescence decline is particularly pronounced for the biogas residue biochar on day 35. This suggests that the biogas residue biochar is more efficient at improving digestion, and agrees with the lowest SCOD content, of 2480 mg/L (Fig. 6). Overall, fluorescence analysis reveals that adding biochars decreases protein and ligno-cellulosic matter, and that effect is more pronounced for the biogas residue biochar. Total carbohydrate and protein are the main components of DOM and two important indicators in the anaerobic digestion. Future study

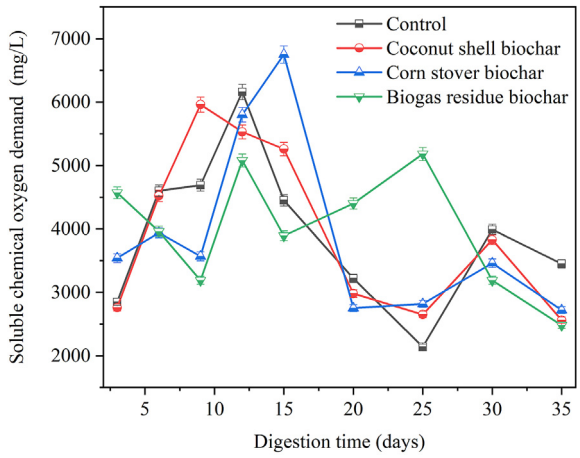

Fig. 6. Evolution of the soluble chemical oxygen demand (SCOD) during anaerobic co-digestion of sewage sludge and food waste supplemented by inert glass (control), coconut shell biochar, corn stover biochar and biogas residue biochar.

can be considered to measure the concentration of total carbohydrate and protein combined with three-dimensional fluorescence spectroscopy to monitor the changes of DOM composition during the anaerobic digestion process, so as to strengthen the evidence of the advantages of adding biogas residue biochar.

3.5. *Microbial community analysis*

We analyzed species and community structures of bacteria and archaea in slurry of anaerobic co-digestion of sewage sludge and food waste supplemented by inert glass (control), coconut shell biochar, corn stover biochar and biogas residue biochar at day 15 (Fig. 7). Under thermophilic conditions, *Thermotogota*, *Firmicutes* and *Bacteroidota* dominate the four groups of the anaerobic digestion reactors, accounting for 40.8%–48.6%, 24.1%–29.0% and 10.2%–11.3% of the total community, respectively. This observation is consistent with previous reports [29,56]. Compared with the control run (inert glass), the abundance of *Thermotogota* in the biochar runs is reduced, which could be attributed to the influence of temperature and feedstocks [57]. The content of *Firmicute* is higher for coconut shell biochar, of 29.0%, and for biogas residue biochar, of 25.7%, than for the corn stover biochar, of 24.1%, and for the control, of 24.9%. For thermophilic anaerobic digestion reactors, *Firmicutes* plays an important role in converting organic matters into metabolites such as acetate acid [58,59]. *Bacteroidota* in the biochar group increased by about 10% compared to the control group (Fig. 7a). *Bacteroidota* is known to favor hydrolysis and acetogenesis of organic compounds for anaerobic digestion [56]. Therefore, our finding supports the promotion of methanogenesis by biochar.

Clostridia is an important species in *Firmicutes*. The addition of biochar can promote the growth of electroactive *Clostridia* and other electroactive bacteria and, in turn, can promote direct interspecies electro transfer (DIET) using biochar as electronic conduits [45,58,60]. Our results indicate that the abundance of *Clostridia* in the four groups decreases in the order: biogas residue biochar, coconut shell biochar, corn stover biochar, then control (Fig. 7b).

We studied the composition of *archaeal* community in the control group and the biogas residue biochar runs (Fig. 7c). We found that *Methanosarcina* and *Methanobacterium* were major species in the reactors, which suggests their role in methane production since *Methanosaeta* and *Methanosarcina* can accept electrons directly from conductive materials [61]. Surprisingly, their

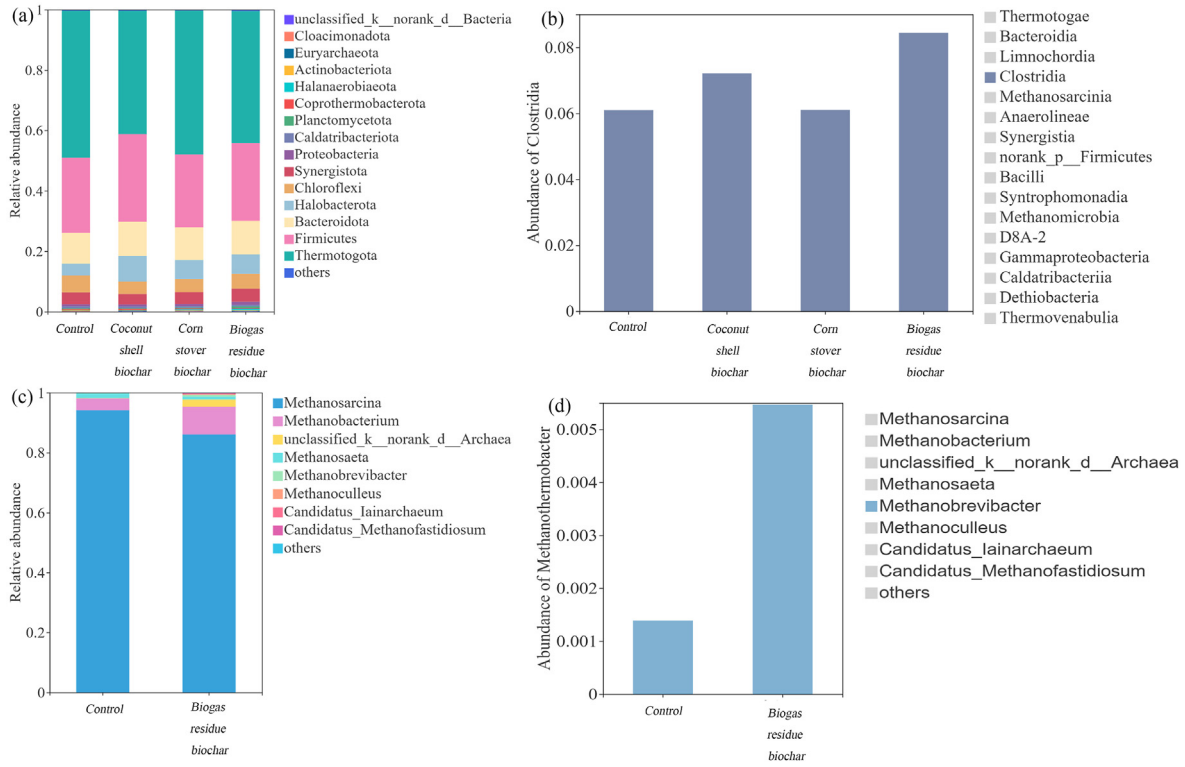

Fig. 7. Species and community structures of bacteria and archaea in slurry of anaerobic co-digestion of sewage sludge and food waste supplemented by inert glass (control), coconut shell biochar, corn stover biochar and biogas residue biochar on day 15. (a) Distribution of bacteria at phylum level, (b) classification of Archaea at genus level, (c) abundance of *Clostridia* in the four groups and (d) abundance of *Methanobrevibacter*. Relative abundance higher than 0.1%.

abundance is higher in the control run versus the biogas residue biochar run (Fig. 7c). But the methane production rate is still lower in the control run versus the biogas residue biochar run (Fig. 2). To explain this apparent discrepancy, we suggest that other species are potentially inducing DIET methanogenesis in the thermophilic anaerobic digestion. This is supported by our data showing that the number of *Methanobacterium* in the biogas residue biochar run is much higher, of 9.2%, than in the control group, of 4.0%. This was also observed by Ref. [62], who pointed out that *Methanobacterium*, *Methanosarcina* and *Methanothermobacter* can use hydrogen to reduce carbon dioxide, and *Methanobacterium* accounts for more than 60.0% of the methanogen.

In addition, Genus *Methanothermobacter*, a frequently observed thermophilic methanogen with an optimal metabolic temperature between 55 °C and 65 °C, is able to produce CH_4 using H_2 and CO_2 as substrates via hydrogenotrophic methanogenesis. Here we found that *Methanobrevibacter*, which has similar traits to *Methanothermobacter* is four times more abundant in the biogas residue biochar run versus the control run (Fig. 7d). Therefore, in agreement with [62], we suggest that under thermophilic conditions, *Methanobacterium* and *Methanobrevibacter* are involved in the DIET process.

4. Conclusions

The effect of biogas residue biochar on thermophilic anaerobic co-digestion of sewage sludge and food waste was studied. The total methane production is highly improved by adding biogas residue biochar, versus coconut shell biochar, corn stover biochar and inert glass control. For instance, at a biochar dosage of 8.0 g/L, the average daily methane yield of the biogas residue biochar run is 46.2% higher than the control group, e.g. 432.2 versus 295.7 mL/g of volatile solids per day. During the early stage of anaerobic digestion, the addition of biogas residue biochar significantly increases the activity of coenzyme F420. Furthermore, the unique structure and composition of biogas residue biochar effectively neutralize fatty acids produced during anaerobic digestion. Finally, the addition of biogas residue biochar enhances DIET of microbes and promotes the growth of bacteria and archaea, which improved the production of biogas from anaerobic digestion.

CRediT authorship contribution statement

Hongbo Liu: Funding acquisition, Conceptualization, Project administration, Supervision, draft manuscript. **Xingkang Wang:** Lab investigations and part of the pilot investigations, mechanism modelling. **Yueying Fang:** Investigation, Data curation. **Wenjia Lai:** Methodology, Data curation. **Suyun Xu:** Project administration, Supervision. **Eric Lichtfouse:** 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.

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