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Improvement of cow manure anaerobic digestion performance by three different crop straw biochars

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ABSTRACT

Anaerobic digestion (AD) is an effective bioconversion technology widely used to treat agricultural waste and reduce environmental pollution. Although AD has been commonly applied to practical social needs, low yield and reactor instability limit its performance. In this study, potato, rape, and wheat straw were pyrolyzed to biochar at 600 °C and added to a batch AD system to improve biogas yield. Biochemical methane potential test was used to assess the methane production. The results showed that the three straw biochar had similar efficiency that increased cumulative methane production (35.45%–52.66%) compared with that in the control group (818.5 mL). Potato straw biochar presented the highest cumulative methane production (1249.5 mL). Significant differences in microbial communities between biochar and control groups were noticed. The microbial community structure in the AD system was significantly related to the biochar properties. The addition of biochar increased the abundance of *Methanobrevibacter* in the biochar group by 2–147 times. This study provides reference for the application of straw biochar to promote methane production.

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1. Introduction

The difficulty of disposing of organic waste and the energy crisis are prominent global issues. The main components of agricultural waste are agricultural straw and animal manure. If such a waste is not handled properly, it would result in a major failure to exploit available resources and environmental pollution. Incineration of agricultural residues and livestock manure generates greenhouse gas emissions globally. Anaerobic digestion (AD) is crucial in developing renewable energy and agricultural waste treatment (Zhao et al., 2021). When AD technology is extensively used, it can treat organic waste and produce clean energy by converting agricultural waste into valuable biogas. Methane from biogas can replace traditional fossil fuels (Arif et al., 2018). However, the widespread popularity of AD technology is limited by several challenges, such as operational instability, rapid acidification, low methane productivity, and hard-to-degrade byproducts (Rasapoor et al., 2020; Wang et al., 2018; Zabranska and Pokorna, 2018). Thus, it is urgently needed to improve the operational efficiency and enhance biogas quality.

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In recent years, several additives have been applied to AD systems to enhance digestion efficiency. Supplementation with additives such as graphene, carbon felt, and biological additives can significantly improve AD performance (Liu et al., 2021). Research has shown that carbon-based accelerant (e.g., activated carbon, carbon cloth, and graphene) and enzyme additives used for AD systems can increase biogas productivity. However, their use is limited by their high cost (Mao et al., 2015). Meanwhile, porous biochar produced from the thermochemical pyrolysis of biomass in an anaerobic environment is a promising additive in AD systems for enhancing operational stability and increasing methane yield (Chiappero et al., 2020; Zhang et al., 2019; Zhao et al., 2021).

Biochar has been widely used in waste treatment due to its unique characteristics. The key advantages of biochar are its porous structure, high specific surface area (SSA), special functional groups, and cation exchange capacity (CEC) (Cheng et al., 2020; Kumar et al., 2021). Furthermore, biochar in AD systems can change the composition of microbial communities, improve microorganism metabolism, maintain the stability of AD processes, and reduce the inhibitory effects of toxins (Zhang et al., 2019). It was reported that biochar addition to an AD system could immobilize microorganisms, shorten the lag phase, and promote electron transfer of methanogens (Liu et al., 2021; Pan et al., 2021). A previous report also showed that the biochar addition could increase biogas yield (22%–40%) in an AD system; accordingly, the relative abundances of methanogens and electroactive microorganisms rose by 43.8% and 24.61%, respectively (Zhao et al., 2021). Biochar provides a microbial habitat and excellent buffer capacity in AD systems.

Biochar produced from different feedstocks might vary considerably in its ability to improve AD performance. Although crop straw is widely used for the preparation of biochar, straw from various crops differs in its composition and texture. Therefore, biochar produced by different raw materials may have different effects on the AD process (Chen et al., 2019). Although many studies have been performed on the AD efficiency of biochar from straw of common crops (including corn straw, rice straw, and rice husk) (Liu et al., 2019b; Zhao et al., 2021), some types of straw biochar (including rape and potato straw biochar) have not been systematically studied for the enhancement of AD systems' digestion efficiency. There have also been few horizontal comparisons of the effects of various straw types in AD systems (Chen et al., 2019). The comparison of straw biochars from different raw materials is helpful to screen more superior biochar for improving the performance of AD. Additionally, microorganisms are the executors of methane production by anaerobic fermentation, and the microbial community structure plays a critical role in the AD process (Zhao et al., 2021). However, to date, there has been limited research on the interaction between biochar and microorganisms in AD. A deeper understanding of the interaction between the various properties of biochar and the structure composition in microbial community is very necessary. Studying the relationship between microbial community structure and biochar characteristics would facilitate targeted screening or transformation of biochar. Additionally, the biochemical methane potential (BMP) test is the most reliable method to determine methane production (Da Silva et al., 2018; Hafner et al., 2020; Holliger et al., 2021). However, few reports have been published on the BMP of livestock manure with biochar.

The transformation of agricultural waste into clean energy by AD has enormous application potential. There is thus a need to develop and improve AD as a renewable resource. In this study, potato, rape, and wheat straw were converted to biochar as additives for improving the efficiency of AD; the substrate of AD was cow manure. The effects of agricultural straw biochar produced from different raw materials on AD were investigated. The BMP test was used to assess the methane production in this context. The microbial diversity and community structure of AD were also analyzed. Moreover, the influences of biochar properties on microbial community composition and diversity in AD were investigated.

The aims of this work were as follows: (i) to determine the impact of potato, rape, and wheat straw biochar on an AD system, and to evaluate the methane yield by BMP test; (ii) to analyze the relationship between the promotion of methane production and the characteristics of biochar; (iii) to analyze the effect of biochar on the community structure of bacteria and archaea in the AD system; and (iv) to reveal the correlation between microbial community structure and biochar characteristics. This study analyzed the effect of biochar characteristics on the performance of AD systems and the structure of microbial communities. This work should help to optimize biochar or screen better straw biochar for promoting AD.

2. Materials and methods

2.1. Preparation of biochar

Rape, potato, and wheat straw were obtained from a local farm in Tianshui City, China. These three straw types were dried, ground with a multifunctional grinder, and sieved into pieces with particle diameters smaller than 0.45 mm. The straw was pyrolyzed in a temperature-controlled muffle furnace (VBF-1200X-H8, China) at a heating rate of 10 °C/min and kept constant for 2 h at 600 °C. The obtained three biochar samples were designated as wheat straw biochar (WSB), rape straw biochar (RSB), and potato straw biochar (PSB).

2.2. Determination of biochar properties

The biochar SSA was tested by the Brunauer–Emmett–Teller (BET) method. The pH values of the three straw biochar types were determined in a 5% (w/v) biochar/deionized water suspension stirred at 160 rpm for 24 h using a pH meter (PHS-3C, China). The carbon (C), nitrogen (N), hydrogen (H), and sulfur (S) contents of the three different biochar were

Table 1
Basic characteristics of three biochars used in AD.

Raw material	Biochar	Total C (%)	Total N (%)	Total H (%)	Total S (%)	CEC (cmol kg ⁻¹)	SSA (m ² g ⁻¹)	pH
Potato straw	PSB	60.44 ± 0.02	1.45 ± 0.004	2.32 ± 0.01	0.30 ± 0.007	18.8 ± 0.81	176.45 ± 12.05	11.66 ± 0.21
Rape straw	RSB	63.40 ± 0.29	0.90 ± 0.008	1.74 ± 0.02	0.77 ± 0.02	7.23 ± 0.76	13.48 ± 6.12	10.54 ± 0.23
Wheat straw	WSB	75.27 ± 0.42	0.69 ± 0.024	2.07 ± 0.01	0.45 ± 0.03	3.45 ± 0.29	290.10 ± 14.13	9.43 ± 0.19

determined using an isotope ratio mass spectrometer (IsoPrime 100, Elementar, Germany). The functional groups of the three biochar types were analyzed using Fourier transform infrared spectroscopy (FTIR) (Nicolet iS20, Thermo Scientific, USA). The FTIR spectra of the biochar samples were recorded at 400–4000 cm⁻¹. The method for determining the CEC was performed with reference to a previous study (Batista et al., 2018).

2.3. Anaerobic digestion

The cow manure was collected from a local dairy farm in Lanzhou, China. The total solid (TS) and volatile solid (VS) of cow manure was measured. Cow manure samples were used as AD substrates. The batch reactors were 250 mL glass bottles with a working volume of 200 mL. The cow manure substrate itself was used as the inoculum in the AD system. Bottles without biochar addition were set up as blanks, which are referred to here after as the control group. There were three groups of biochar-modified bottles (WSB, RSB, and PSB). In the three biochar groups, 10 g/L of WSB, RSB, or PSB was added. The mixture had a VS content of 4 g/L in each digestion bottle. The pH of all bulk batches was tested without adjustment. All the bottles were tightly sealed with rubber plugs. The anaerobic reactor was placed in an incubator at 38 °C for 22 days. The plunger displacement method was used to record the daily biogas production (Liu et al., 2019a). Biogas was taken from each bottle at predetermined times each day and liquid was taken every 4 days. A gas chromatograph (7890 A, Agilent, USA) was used to determine the concentrations of volatile fatty acids (VFAs) and the methane content was also measured using this device. The BMP of the AD system was assessed in accordance with the guidelines defined by Holliger et al. (2016). All experiments were performed in triplicate.

2.4. Microbial composition analysis

Total genomic DNA of the slurry in AD system was extracted using the TIANamp stool DNA Kit (Tiangen, China). The primers 338F and 806R were used for amplification of the V3–V4 regions of the bacterial 16S rRNA gene; meanwhile, the primers 524F and 958R were used to amplify the V4–V5 regions of the archaeal 16S rRNA gene. Sequencing was performed on Illumina MiSeq platform. The obtained low-quality reads and adapter sequences were removed for the subsequent assembly. All sequences were organized into operational taxonomic units (OTUs) based on 97% similarity. The predominant components of the microbial community at the phylum and genus levels were identified to determine differences and similarities among the different AD systems. The diversity and similarity of microbial communities were further analyzed, here, principal component analysis (PCA) was used to analyze the data at the phylum and genus levels using R (version 3.6.3). Correlations between the microbial community and biochar were analyzed by redundancy analysis (RDA). The Vegan package in R (version 3.6.3) was used for RDA. On the basis of 9999 permutations, only environmental variables that were significantly associated ($p < 0.05$) with the RDA model were selected for significance (He et al., 2021).

3. Results and discussion

3.1. Properties of biochar

Table 1 shows the basic characteristics of three different biochars. The biochar produced from different raw materials showed various characteristics. PSB, RSB, and WSB showed high SSA at 600 °C, which is higher than that reported for corn straw and coconut shell biochar (Zhang et al., 2019). WSB showed the highest SSA (290.1 m²/g) and lowest pH (9.43). The pH of the three biochar ranged from 9.43 to 11.66, which was close to the reported biochars (Windeatt et al., 2014). However, the total N of WSB was the lowest (0.69), which was lower than that of RSB, PSB, and previously reported biochars (Zhao et al., 2021). PSB showed higher CEC (18.8 cmol kg⁻¹) than RSB and WSB. It has been reported that variation in the materials has a significant effect on the SSA, internal structure, and pore size distribution of biochar (Cantrell et al., 2012).

The main surface functional groups of each biochar were analyzed (Fig. S1). The three biochar contained C–O, CH, CH₂, or –CH₃ aliphatic CH_n (2945–2916 cm⁻¹) groups. Besides, PSB and RSB contained large numbers of hydroxyl (3727.96–3415.56 cm⁻¹) and amino groups compared with WSB. Moreover, RSB contains vinyl, in contrast to PSB and WSB. There is a certain correlation between these functional groups and methanogenesis during AD.

One report has shown that the pyrolysis parameters and changes in raw materials significantly affect the SSA value of biochar and its internal structure (Cantrell et al., 2012). High pyrolysis temperature and heating rate increase the SSA of biochar (Pandey et al., 2020). It has been reported that the SSA of biochar ranges from several to hundreds of square

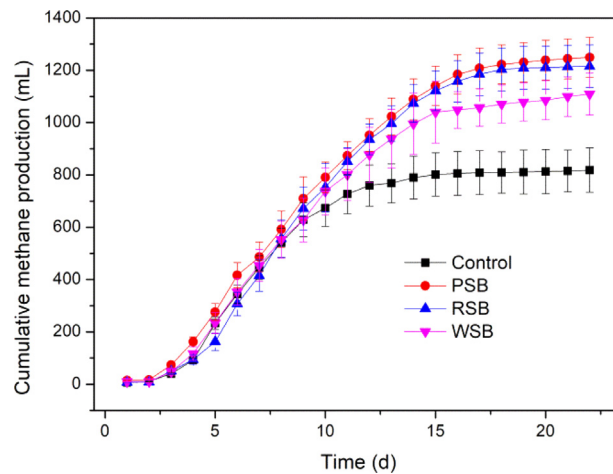


Fig. 1. Cumulative methane yield of control and biochar groups.

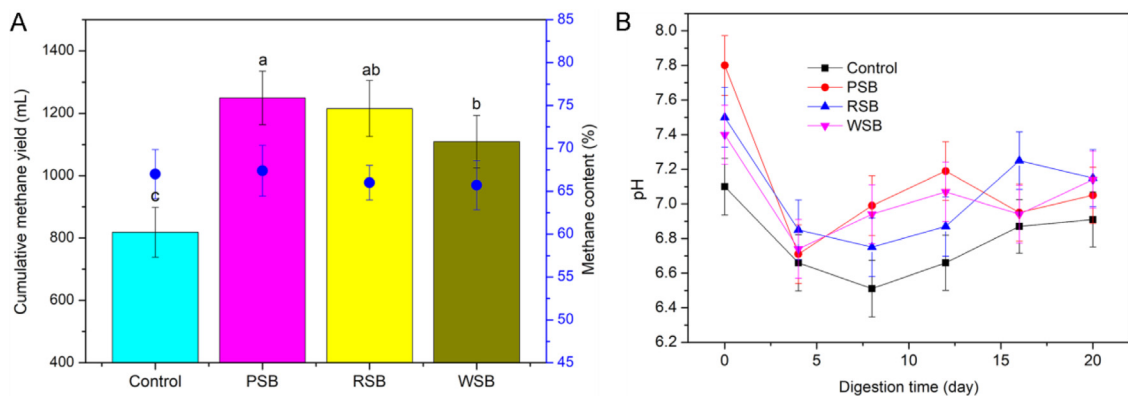


Fig. 2. (A) Total methane yield and biogas analysis of methane content. (B) Variations of pH in different groups of AD.

meters per gram (Zhao et al., 2021). WSB and PSB were produced at 600 °C and have higher SSA than RSB and other reported biochars (Xu et al., 2014). The rapid heating rates of biochar produced high alkalinities of biochar (Nzediegwu et al., 2021). Therefore, the straw was pyrolyzed at a fast heating rate of 10 °C/min in this study and the obtained straw biochar had high pH (9.43–11.66). Biochar has strong adsorption and immobilization abilities for the removal of ammonia, heavy metals, and other toxins due to its high SSA and abundant porosity (Zhao et al., 2021). These toxins seriously inhibit methanogenesis during the AD process. The additive biochar could adsorb those toxins on its surface by ion-exchange and electrostatic attraction (Zhao et al., 2021). Another study found that biochar can release the above inhibitory effect, and the adsorption capacity increased with increasing SSA of the additive biochar in AD systems, resulting in a high methane yield (Pan et al., 2019). Therefore, adding biochar in the form of WSB, RSB, and PSB can improve AD performance.

3.2. Effects of biochar on methane yield

The cumulative methane production of biochar and control groups is presented in Fig. 1. The results indicated that the three different straw biochars increased methane production to different degrees. The cumulative methane production of the control group was lower than that of the biochar groups, which can be attributed to the effects of crop straw biochars. The results of cumulative methane production indicate that biochar as an additive can promote the biogas production. The cumulative methane yield can illustrate the hydrolytic efficiency of substrates in AD systems (Zhang et al., 2019). Generally, a high cumulative methane yield implies good hydrolysis of substrates (Li et al., 2018). The cumulative methane yield obtained in the biochar group was markedly higher than that in the control group, implying that the addition of crop straw biochar can improve the hydrolysis of substrates, which is related to the biochar properties.

Cumulative methane yield and average methane content were analyzed (Fig. 2 A). There were significant differences in cumulative methane yield of the three different biochars. The cumulative methane yields of the control group, PSB, RSB, and WSB were 733.8–903.3, 1172.0–1327.0, 1133.4–1297.4, and 1028.7–1188.7 mL, respectively. The cumulative methane

Table 2

Comparison of the effects of biochar prepared from different materials on AD performance.

Biochar material	Temp. (°C)	Impact on CH ₄	Reference
Potato straw	38	Cumulative CH ₄ increased by 35.45%	This study
Rape straw	38	Cumulative CH ₄ increased by 48.49%	This study
Wheat straw	38	Cumulative CH ₄ increased by 52.66%	This study
Dry <i>Spirulina</i>	35	CH ₄ yield increased by 12%	Zhang et al. (2020)
Waste apple tree branche	37	Cumulative CH ₄ increased by 50%–74.5%	Yan et al. (2020)
Sawdust	35	Average CH ₄ increased by 60.4%	Wang et al. (2020b)
Wood chips	36	Cumulative CH ₄ increased by 26%	Tsui et al. (2021)
Wood chips	51–53	CH ₄ increased from 50% to 60%	Bona et al. (2020)
Cow manure	55	Cumulative CH ₄ increased by 203%	Sun et al. (2019)
Corn stover	55	CH ₄ increased by 8.6%–17.8%	Wei et al. (2020)
Pine sawdust	37	CH ₄ increased by 46.9%	Sugiarto et al. (2021)
Orchard waste wood	35	CH ₄ increased by 32%–36%	Pan et al. (2019)

yield significantly increased by 52.66%, 48.49%, and 35.45% ($p < 0.05$) when the biochar PSB, RSB, and WSB were added, respectively, compared with the level in the control group. The results illustrated that the addition of 10 g/L biochar (PSB, RSB, and WSB) could promote AD performance and increase the methane production. Nevertheless, the average methane content of the control group, PSB, RSB, and WSB were 67.00%, 67.39%, 66.00%, and 65.7%, respectively. There was no difference in mean methane contents between different biochars.

The BMP test is the most reliable method used by academics to determine the maximum methane production (Da Silva et al., 2018). The byproducts from beer production used for AD generate the highest BMP at up to 515 mL g⁻¹ VS (Oliveira et al., 2018). A methane yield of 182.94 to 242.69 mL g⁻¹ VS was achieved in different ratios of vinegar residue feedstock to the BMP assay (Feng et al., 2013). The final methane yield of simulated food waste was 430 mL g⁻¹ VS (Heo et al., 2003). However, few reports have been published on the BMP of cow manure. In this study, the methane yield was up to 236.7–273.7 mL g⁻¹ VS for cow manure with biochar addition, a range that is close to that reported in previous reports.

Different types of biochar materials can affect the biochar properties. Previous reports have shown that the properties of biochar could affect its effectiveness in the anaerobic fermentation process (Zhang et al., 2019). The effect of biochar prepared with different materials on methane production performance is summarized in Table 2. The addition of biochar clearly increased the methane yield during AD, which may have been due to the enhanced substrate hydrolysis and the acceleration of direct interspecies electron transfer (DIET) between methanogenic archaea and acidogenic bacteria by the biochar (Sugiarto et al., 2021). Similarly, biochar addition could also accelerate rapid methanogenesis by promoting electron transfer (Struckmann Poulsen et al., 2022; Wang et al., 2021).

The electrochemical functional groups are distributed on the surface of biochar (Wang et al., 2018). Biochar is not only an electron conduit for enhancing DIET, but also its functional groups accelerate the electron exchange process between electron donor bacteria and electron acceptor methanogens (Zhao et al., 2021). Thus, biochar accelerates highly efficient methane production. Additionally, the biological electron components, such as cytochromes and electron shuttles, could also promote the extracellular electron transfer between biochar and electroactive microorganisms (Pan et al., 2019). Thus, the PSB group showed the highest total methane yield due to its high CEC and SSA and functional groups. Furthermore, compared with WSB and RSB, PSB with the highest pH can effectively alleviate acidification in the AD system. The above-mentioned parameters SSA, CEC, functional groups, and pH can affect the composition of the microbial community. The process of methane production is influenced by the coordination and interaction between chemical properties of biochar and microbial community structure. There is thus a need for further research on the interplay between biochar's chemical properties, microbial communities, and AD performance. Three kinds of biochar from straw materials are used to AD in this study. In order to screen and identify superior biochar materials for promoting AD in subsequent research, it is necessary to expand the range of straw types used. Moreover, future studies should consider scaling up the reaction and conducting comprehensive experiments.

3.3. Effect of biochar on pH in AD system

Fig. 2B shows the changes in pH value during the AD process. The mean pH values of the control, PSB, RSB, and WSB groups in the AD system were 6.80, 7.16, 7.18, and 7.10, respectively. In the biochar group, the mean pH was higher (5.1%) than that in the control group, which was attributed to the buffering capacity maintained by the biochar (Nzediegwu et al., 2021). Biochar has a robust buffering capacity against acidic/alkaline shock due to its abundance of metal ions and functional groups (Zhao et al., 2021). It was found that the pH of each bottle significantly decreased after the start of digestion and gradually recovered after the 12th day of digestion. The lower pH in the AD system was mainly attributed to the hydrolysis of some organics and acidogenesis (Zou et al., 2018). The control group without biochar addition attained the lowest pH of 6.51 on day 8, which was lower than those in the WSB (6.76), RSB (6.76), and PSB (6.72) reactors. In general, the original pH of biochar was alkaline, and its addition could significantly increase alkalinity to keep the pH of the AD system close to neutral. This is mainly attributed to the abundance of alkali metals, such as Na, Ca, K, and Mg. In addition, the basic functional groups of biochar are essential to maintain the buffer capacity (Wang et al., 2017).

The functional groups generated during pyrolysis, such as (X-OH) and amine ($-\text{NH}_2$) groups, can maintain the pH of the AD system in a neutral condition by resisting acidic/alkaline shock (Ma et al., 2020). Consequently, biochar in the AD system can increase alkalinity and alleviate VFA inhibition (Lim et al., 2020; Meng et al., 2020). PSB and RSB have a higher pH than WSB, which may be attributable to their hydroxyl and amino groups. Phenolic hydroxyl species were considered as the essential functional groups accountable for their electron-donating capacity (Kumar et al., 2021). They determined the biochar's overall electron exchange capacity (Hoang et al., 2022). The pyrolysis temperature of potato, rape, and wheat straw is the same, and the functional groups generated are different, mainly due to different material sources. The surface functional group of straw biochar can promote the DIET of microorganisms and strengthen the metabolism of methanogens (Huang et al., 2023).

3.4. Effect of biochar on VFAs in AD system

Acetic acid, propionic acid, butyric acid, and valeric acid are the main components of VFAs, which are essential intermediates in the AD system. The concentrations of acetic acid were 0.61–2.94, 1.11–4.08, 1.59–4.22, and 1.96–4.66 g/L in control, PSB, RSB, and WSB groups, respectively. Compared with the level in the control group, the average concentrations of acetic acid in PSB, RSB, and WSB groups increased by 13.63%, 63.67%, and 86.53%, respectively. Propionic acid also showed a similar trend, with the level in the biochar groups being higher than that in the control group. The concentrations of propionic acid were 0.17–0.63, 0.33–0.91, 0.33–0.65, and 0.24–1.17 g/L in control, PSB, RSB, and WSB groups, respectively. The biochar groups showed higher levels (by 22.22%–76.11%) than the control group. Meanwhile, the concentrations of valeric acid and butyric acid in each reactor were below 0.2 g/L. The concentration of each VFA component in a reactor with biochar addition was higher than that of the control. The high VFA concentrations in biochar reactors were mainly attributed to the rapid decomposition of organic matter (Zou et al., 2018). The main components of VFAs were propionic acid and acetic acid during the AD process, while small amounts of butyric acid and valeric acid were also produced. This resembles the findings reported in previous studies (Li et al., 2022). The concentration of acetic acid during the AD process could represent the efficiency of the methanogenesis stage. The concentration of acetic acid often varies with the progress of digestion in the fermentation system. In the early stage of AD, the methanogens utilized acetic acid very slowly. Subsequently, the methanogens continued to grow with the progress of AD, and the decomposition rate of acetic acid increased (Li et al., 2022).

VFA generated during AD can reduce the pH (Wainaina et al., 2019). The excessive accumulation of VFA makes it easy for the AD system to enter an unstable state. The PSB, RSB, and WSB have high pH value that can alleviate acidification. The presence of a large number of alkaline functional groups in biochar is crucial for maintaining buffering capacity, leading to a significant increase in system alkalinity (Altamirano-Corona et al., 2021). Biochar can effectively promote the degradation of VFA during the AD process and alleviate the problem of VFA inhibition (Zhao et al., 2021).

3.5. Microbial community structure

3.5.1. Bacterial community composition

The bacterial communities of the AD system were analyzed using amplicon sequencing of 16S rDNA. The results of analysis of bacterial communities at the phylum level are shown in Fig. S2. The most abundant bacterial phylum in the biochar group was *Firmicutes*, followed by *Bacteroidota*, *Cloacimonadota*, and *Spirochaetota*. In the control group, the dominant phyla included *Firmicutes*, *Bacteroidota*, and *Actinobacteriota*. Compared with the findings in the control group, there were significant differences in the relative abundances of *Cloacimonadota* and *Spirochaetota* in the biochar groups. In particular, the abundance of *Cloacimonadota* in the PSB group was higher than in the other groups. Previous studies reported that the dominant phylum in AD was *Firmicutes* (Zhang et al., 2020). *Firmicutes* is a type of hydrolytic bacteria that degrade some proteins and carbohydrates (Lim et al., 2014); these bacteria can also produce some key enzymes (such as protease, cellulose, hemicellulose, and lipase) that facilitate the decomposition of organic matter (Chen et al., 2019). This result demonstrated that the addition of biochar clearly altered the microbial community composition of AD. Biochar can increase buffering abilities, provide microbial habitats, and promote electronic transmission between methanogens and digestive bacteria, thereby improving AD performance (Zhang et al., 2022).

The composition of bacterial communities was also analyzed at the genus level (Fig. S3). The dominant genus was *Hydrogenispora* in all samples. Meanwhile, *Turicibacter* in the biochar groups was higher than in the control group. This taxon participates in fermentation metabolism and its main product is lactic acid, which plays an important role in methanogenesis. *Romboutsia* also exhibited a similar trend to *Turicibacter*. Moreover, the abundance of *Bacillus* in the PBS group was higher than in the other three groups. *Bacillus* might be related to methane yield. *Caldicoprobacter* was also widespread in the AD system. It has been reported that *Caldicoprobacter* can degrade galactose, xylose, glucose, raffinose, cellobiose, and cellulosic metabolites, participating in the metabolism of acetate, monosaccharides, CO_2 , and H_2 (Zhao et al., 2021).

Bacteria are responsible for the hydrolysis, acidogenesis, and acetogenesis stages during the AD process (Cai et al., 2023). The biochar can provide SSA for microbial colonization and enrich specific microbes. The large SSA of biochar is conducive to the enrichment of microorganisms (such as *Oxobacter* and *Caldicoprobacter*) and enhances the growth. *Bacteroidetes* responsible for the degradation and generation of VFAs in the AD system increased with PSB and RSB

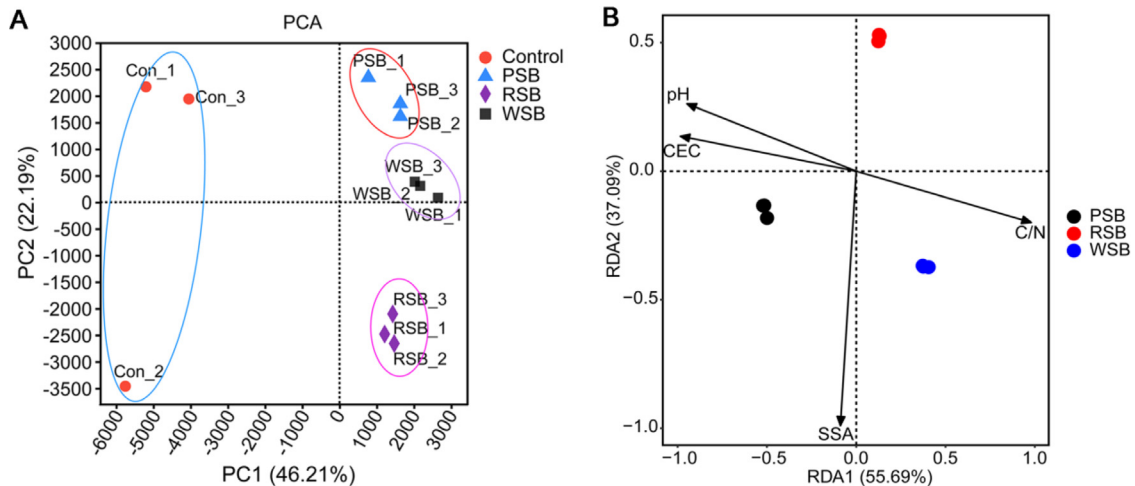


Fig. 3. (A) Scatter plot of PCA scores depicting variance of microflora in AD at phylum level. (B) RDA plot depicting the correlation between biochar properties and bacterial communities in AD system at the operational taxonomic units level in AD. Con: Control. C.N: biochar C:N ratio. Numbers (1, 2, 3) at the end of each group represent three replicates.

addition, which may be due to their high pH. CEC seems to be proportional to surface oxidized functional groups (Hoang et al., 2022). Biochar facilitates the exchange of cations, enhances the stability of biochar bacteria, and enhances electrical conduction. Therefore, owing to the high pH and CEC, and similar functional groups of PSB and RSB, the trends of change of specific microorganisms in PSB and RSB groups were similar.

PCA analysis results at the OTU level indicated a significant difference in bacterial communities between the control and biochar groups (Fig. 3 A). However, the differences among PSB, RSB, and WSB were also clear, indicating that the different biochars had different effects on the AD bacterial community. This may have been related to particular characteristics of the biochar, such as SSA, pH, and CEC. Microorganisms are key substrate decomposers and also play an essential role in anaerobic fermentation. The abundance and diversity of microorganisms directly affect substrate decomposition, acidogenesis, methanogenesis, and acetogenesis.

Fig. 3B shows the relationship between bacterial community and environmental factors. The effects of biochar properties (C:N ratio, SSA, pH, CEC) on bacterial community structure were analyzed. RDA was applied to illustrate the correlations between biochar characteristics and bacterial community composition. There were clearly high correlations between bacterial communities and biochar properties. Biochar properties including SSA ($p < 0.01$), pH ($p < 0.01$), C:N ratio ($p < 0.01$), and SSA ($p < 0.01$) had significant effects on bacterial community structure. The RDA results showed that biochar SSA had the highest correlation with the variation of microbial communities. The RSB group has lower SSA than the WSB and PSB groups. The C:N ratio is related to raw materials at the same preparation temperature. In this study, the C:N ratio of PSB was significantly lower than those of RSB and WSB, which may be one of the main factors affecting AD. The WSB group also had the lowest pH, while the PSB group had higher pH and CEC than the WSB and RSB groups. This might be the main reason why PSB had high total methane yield in AD.

3.5.2. Archaeal community composition

To obtain more insights into microbial changes, the archaeal communities were investigated at the phylum level (Fig. 4 A). There were differences of archaeal communities in the different groups. In the AD system, the dominant archaeal phyla were *Halobacterota*, *Crenarchaeota*, and *Euryarchaeota*. Nevertheless, compared with the level in the control group, the abundance of *Euryarchaeota* in the biochar groups was higher. *Euryarchaeota* including many methanogenic archaea produces methane in the intestines of animals. The archaeal communities were investigated at the genus level (Fig. 4B). Among the methanogens, the most abundant genus was *Methanosarcina*, followed by *c_Bathyarchaeia*. In the PSB group, the abundance of *Methanocorpusculum* was significantly higher than in the other groups, which might have been associated with the levels of production of methane. *Methanobrevibacter* and *Methanosphaera* were abundant in the AD system. These genera belong to the methanogenic family *Methanobacteriaceae*, representing hydrogenotrophic and acetoclastic methanogens. *Methanobacteriaceae* could use H_2 or CO_2 as a substrate for methanogenesis. It is one of the most commonly methanogens in anaerobic reactors. The abundance of *Methanobrevibacter* in the biochar groups increased by 2–147 times. Meanwhile, the abundance of *Methanosphaera* was higher in the PSB group than in the others, and this trend was similar to the findings on total methane production. Thus, *Methanosphaera* is related to the methane yield.

Methanogenic archaea complete the methanogenesis stage. In this study, it was found that the abundance of the methanogen community was altered by the addition of biochar. The specific elevation of archaea in biochar-added reactors depended on biochar-related parameters (Pan et al., 2019). The major roles of biochar are biofilm formation, shifts of

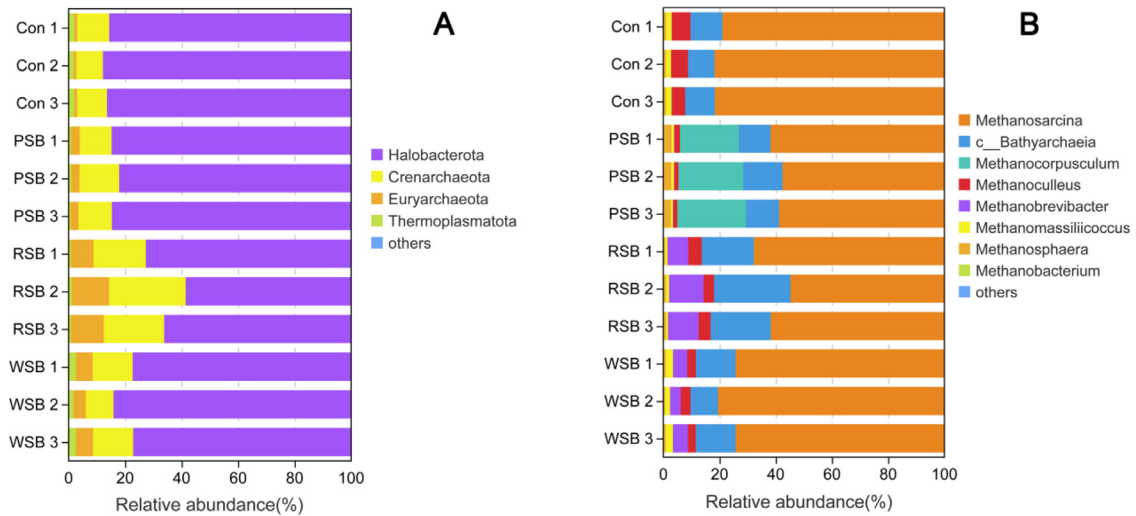


Fig. 4. The shifts of archaeal communities of AD system at the phylum level (A) and genus level (B). Con: Control. Numbers (1, 2, 3) at the end of each group represent three replicates.

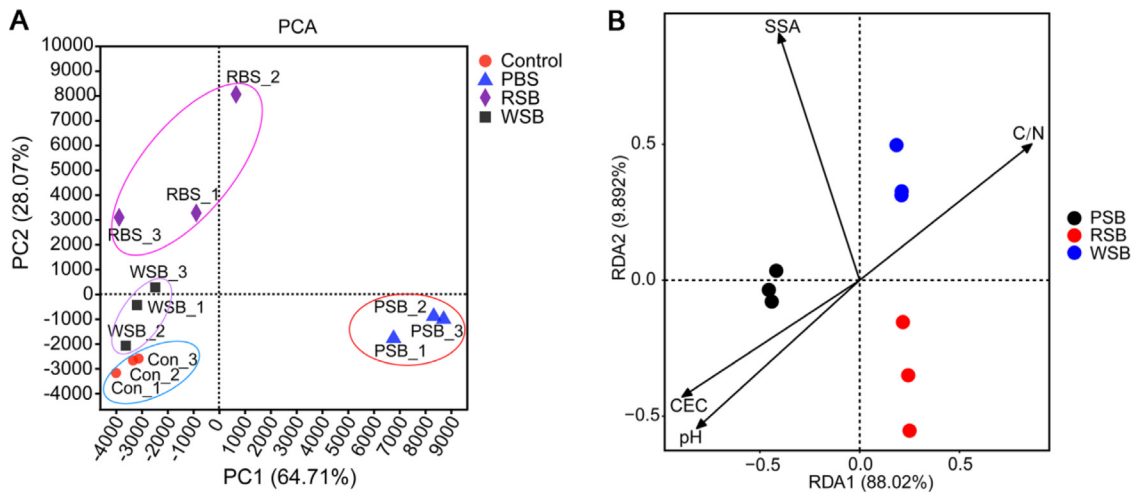


Fig. 5. (A) Scatter plot of PCA scores depicting variance in archaeal community. (B) RDA plot depicting the correlation between biochar properties and archaeal communities in AD system. C.N: biochar C:N ratio. Con: Control. Numbers (1, 2, 3) at the end of each group represent three replicates.

microbial populations, and promotion of DIET (Cruz Viggi et al., 2017). There is also a strong direct correlation between CEC and the stimulation of methane production, which also helps with the growth of microorganisms in the early stages of the AD process (Qin et al., 2020). Compared with the findings in the RSB and WSB groups, PSB exhibited significant differences in the abundance of archaea, which may have been mainly attributable to the high CEC, SSA and pH.

At the OTU level, PCA analysis (Fig. 5 A) indicated the slight similarities in archaeal community structures between the control and WSB groups. This may be related to the methane yield because the total methane yield of these two groups was lower than in the other groups. In the PCA analysis, control and PSB samples were strongly separated, indicating that the dominant archaeal genera of the control and PSB were distinctly different. The above results indicated that the addition of biochar alters communities of methanogens. Moreover, the sample points of RSB and WSB in the PCA plot were much closer to each other than to PSB, indicating that the effects of RSB and WSB on archaeal community in AD are similar.

The RDA analysis (Fig. 5B) showed that the archaeal community at the OTU level was highly related to biochar pH ($p < 0.001$), SSA ($p < 0.01$), C:N ratio ($p < 0.01$), and CEC ($p < 0.01$). The pH of biochar had the most significant impact on archaea communities. A strong correlation was also found between the characteristics of biochar and AD, which was also proven in a previous report (Zhao et al., 2021). In this study, the PSB group showed high methane production, which may have been related to its low C:N ratio and high SSA, pH, and CEC. Meanwhile, the RSB group had low SSA, while the WSB

group had relatively low CEC and pH. Furthermore, the RSB and WSB groups showed low methane production. Thus, PSB showed a high total methane yield due to high CEC, SSA, and pH.

The addition of biochar provides a significant amount of SSA for biofilm formation, which reduces microbial loss during the AD process. A previous study demonstrated a positive correlation between maximum methane production and SSA of the biochar in AD systems (Qin et al., 2020). Biochar has a porous structure and acts as a carrier for microorganisms. Furthermore, the addition of biochar to AD systems could optimize microbial communities, improve microorganism metabolism, and improve the stability of AD (Zhang et al., 2019). Despite biochar playing a vital role in AD processes, the underlying mechanisms require in-depth clarification (Zhao et al., 2021).

Biochar with immobilization ability can result in the enrichment of certain functional microorganisms. In one study, it was concluded that every pore of biochar hold about 10–100 methanogenic cells (Zhao et al., 2021); thus, in the AD system, there is a positive correlation between maximum methane yield and SSA of the biochar (Qin et al., 2020). Additionally, some elements (such as N, P, Mg, and Na) in biochar are necessary for microbial growth and metabolism, which improve the composition of the microbial community (Zhao et al., 2021). Biochar with conductivity builds a DIET pathway between methanogens and electroactive bacteria (Wang et al., 2020a). Research has proven that biochar significantly enriches methanogenic archaea (Kumar et al., 2021). In this study, the biochar group exhibited the enrichment of methanogenic archaea, which is similar to a previously reported finding. The above functions of biochar supplementation may cause significant variation in microbial diversity, and methane production and AD performance are improved. The PSB group showed the highest total methane yield, with the following main influencing factors: low C:N ratio, high pH, SSA, and CEC. Moreover, these properties of biochar had an impact on the microbial community structure; for example, the abundances of *Cloacimonadota* and *Methanocorpusculum* in the PSB group were high. The above factors led to higher methane production in the PSB group. While the analysis of the relationship between biochar properties and microorganisms has been conducted, additional comprehensive research is required to incorporate a broader range of biochar properties for large-scale applications.

4. Practical applications and future research prospects

The current research shows the effects of three straw biochar types on AD of cow manure. The results showed that straw biochar from different crops can promote the performance of AD to different degrees. The PSB presented the best ability to promote methane production, which is related to the characteristics of biochar itself. Crop straw is cheap and difficult to use, and burning it can cause air pollution. However, the straw biochar can increase methane production in AD, which enables full use of the straw to be made and improves energy efficiency. Therefore, it has broad application prospects. The research in this work is based on a small-scale approach, and analyses of the digestive system will continue to be expanded in subsequent research. It still needs validation for both technical and economic feasibility in pilot-scale and large-scale experimental tests. Additionally, large scale applications require consideration of many limiting factors, such as operating equipment, fermentation temperature, and stability of bacteria and archaea. The specific mechanism for promoting methane production by straw biochar needs further in-depth research. Efforts to clarify the mechanism of the interaction between biochar and microorganisms should also be continued.

5. Conclusions

Three agricultural straw types were used to prepare biochar, which was added to an AD system to improve the efficiency of anaerobic fermentation. The addition of biochar enhanced methane yield as evaluated by the BMP test. Among the three different types of biochar, PSB showed the highest ability to promote methane production, compared with the control group without biochar addition. There is a high correlation between the structure of microbiota in AD and biochar characteristics (pH, SSA, CEC, and C:N ratio). Biochar combined with AD technology can improve the efficiency of converting agricultural waste into clean energy.

CRedit authorship contribution statement

Minrui Liu: Conceptualization, Data curation, Writing – review & editing, Funding acquisition. **Zhengning Li:** Resources. **Xing-e Qi:** Investigation, Data curation. **Zhengjun Chen:** Investigation, Formal analysis. **Hongyuhang Ni:** Investigation, Writing – review & editing. **Yuan Gao:** Investigation. **Xia Liu:** Investigation.

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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Appendix A. Supplementary data

Supplementary material related to this article can be found online at <https://doi.org/10.1016/j.eti.2023.103233>.

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