Magnetic biochar affects the metabolic pathway in methanogenesis of anaerobic digestion of food waste

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Abstract
To alleviate pollution and promote clean energy production, food waste can be used to produce methane through anaerobic digestion (AD). In this study, wheat straw was used to produce biochar (BC) and magnetic biochar (MB) as additives for AD of food waste. MB2.5% and BC2.5% led to the highest methane production. The main action mechanism of the additives in AD was the change in environmental factors upon stimulating microorganisms to rapidly metabolize volatile fatty acids. The stimulation of microbial function increased the abundance of the methanogenesis pathways. Methanosarcina was the most dominant archaea in the four methanogenesis modules, and the microbial community changed more clearly with time than with treatment. This study is innovative in finding the effectiveness of BC and MB treatments attributed to the abundance of methanogenesis modules, especially MB2.5% treatment retained its advantage until the later stage of digestion in AD process.

KEYWORDS
anaerobic digestion, food waste, functional contribution, magnetic biochar, metabolism pathway, metagenome

Abbreviations: AD, anaerobic digestion; AN, ammonia nitrogen; BC, biochar; BET, Brunauer–Emmett–Teller; CD-HIT, Cluster Database at High Identity with Tolerance; FTIR, Fourier transform infrared; KEGG, Kyoto Encyclopedia of Genes and Genomes; MB, magnetic biochar; NCBI, National Center for Biotechnology Information; RPKM, reads per kilobase per million mapped reads; TS, total solid; VFs, volatile fatty acids.
1 | INTRODUCTION

Food is a basic human need, which is typically associated with high waste production. Studies have shown that more than 60 million tons of food waste are generated in Chinese cities every year, and each household also generates 129 kg of food waste every year (Jin et al., 2011). This food waste represents a wastage of resources and creates environmental impacts. In this context, anaerobic digestion (AD) is typically used for the utilization of food waste in the production of biogas. However, because of the complex composition of food waste, AD typically has a low initial pH, which can cause acidification; high nutrient to ammonia ratio, which can lead to poisoning; and slow decomposition, which hinders the process start-up (Xue et al., 2020). These disadvantages can lead to a low methane production. To overcome these obstacles and increase methane production, manual interventions can be conducted, including the use of additives. Biochar (BC) is one such additive which is increasingly attracting research attention in AD process. From an environmental and economic point of view, the addition of biochar with the same amount of input as in the conventional AD process can increase the biogas production, resulting in significant economic benefits (Luz, Cordiner, Manni, Mulone, & Rocco, 2018). Song et al. (2021) through cost analysis found that BC and modified BC could provide benefits to gas production, but the high cost of BC modification is a challenge in actual production.

BC can be prepared from agricultural waste, industrial organic waste, and municipal sludge (Tripathi et al., 2016). The modified biochar loaded with Fe₃O₄ is called magnetic biochar (MB) and it has different physical and chemical properties from BC. However, the differences between the mechanisms of BC and MB on food waste AD remain unclear. The current theory that explains the action of BC and MB in enhancing AD methane production can be divided into three parts as follows: providing microelements for microorganism growth (Sugiarto et al., 2021); conductivity affects the direct electron transfer between microorganisms (Yin et al., 2017); and the surface structure and function of BC or MB can increase the reaction contact area (Quintana-Najera et al., 2021). However, research on the influence of BC or MB on the four metabolic pathways of methane production and studies on the contribution of microbial methanogenesis to AD are lacking. For instance, there are no published reports on the microorganism characteristics at different digestion stages. In the digestion liquid, BC additives can adjust the pH (Paritosh & Vivekanand, 2019), increase the buffering capacity of the system, and stabilize the system dynamics (Li et al., 2018). The correlations between BC or MB and characteristics such as composition and function of microorganisms, environmental factors, and AD methane production have not been fully clarified.

In this study, wheat straw was used as raw material to prepare BC and MB additives, and food waste was used for the AD. The effect of additive type on the methane production by food waste AD was investigated considering indicators such as pH, concentration of volatile fatty acids (VFAs), and total ammonia nitrogen (AN). Furthermore, we analyzed variations in structure of archaic and bacterial community in the four modules of the methanogenesis pathway to reveal the novel regulators of methanogenesis. Additionally, metagenomic sequencing was conducted in the early, middle, and late stages of digestion. The differences between the time and treatment effectiveness of BC and MB on food waste AD were used to identify the main influencing factors. Subsequently, speculated a mechanism map of the effect of additives on AD. The findings of this study are expected to find the key steps and mechanisms by which MB affects AD, and provide a robust theoretical basis on the mechanism and use of additives to enhance methane production in subsequent AD practices.

2 | MATERIALS AND METHODS

2.1 | Biochar production

2.1.1 | Biochar preparation

The biochar preparation method was adopted from a previous study (Altamirano-Corona et al., 2021; Trakal et al., 2016). The BC used in this experiment was prepared from wheat straw, which was washed, dried, chopped, and pyrolyzed at 400°C. The BC was then sieved through a 60-mesh sieve.

The BC was modified using a co-precipitation method (Trakal et al., 2016). First, two solutions were prepared. The first solution was prepared by mixing BC with deionized water. The second solution was prepared by co-dissolution of FeCl₃·6H₂O and FeSO₄·7H₂O in deionized water. These solutions were mixed and stirred at 150 rpm for 30 min. Subsequently, 1 mol/L NaOH was added dropwise to the mixed solution to attain the pH of 11. The solution was then stirred for 2 h, and then heated to a slight boil, which was maintained for 1 h. The BC was then loaded with Fe²⁺ and Fe³⁺ to become MB. After MB was cooled, they were washed with deionized water and absolute ethanol, and then filtered to a pH of 7.

2.1.2 | Biochar property analysis

To calculate the pH, 0.5 g of each sample was weighed and placed in a flask, and then mixed with 10 ml of ultrapure water. The flask was placed in a constant temperature
At 35°C and continuously shaken at 0.56 g for 48 h, and the pH of the mixed solution was measured using a handheld pH meter (PHB-3, Ruipin Precision Instrument). Both BC and MB experiments were conducted in triplicate, and the average values were used.

To determine ash content, an empty porcelain crucible was weighed, and then, 0.5 g of BC was evenly placed on the bottom of the crucible. A muffle furnace was used to heat the sample to 500°C for 4 h, and after cooling to a constant temperature, the porcelain crucible was weighed again. The ash content was calculated following Equation (1):

$$\text{Ash} = \frac{m_2 - m_1}{0.5} \times 100\%$$  \hspace{1cm} (1)

Scanning electron microscopy (SEM; Zeiss MERLIN Compact) was used to detect the surface structure and composition of the samples. A full-automatic specific surface area analyzer (TriStar II 3flex, Micromeritics) was used to measure the Brunauer–Emmett–Teller (BET) surface area of the samples, and Fourier transform infrared (FTIR) spectroscopy (Nicolet iS10, Thermo Fisher) was used to identify the surface functional groups.

### 2.2 Anaerobic digestion batch test

The food waste for the AD substrate was obtained from the canteen of the Northwest A&F University (Yangling, Shaanxi), and it contained rice, vegetables, meat, oil, soy products, and other food products. The total solid (TS) content of the food waste was 19.42% based on a wet basis, and the C/N ratio was 29.38. The inoculum used was a biogas slurry from a well-functioning dairy farm biogas project (Baoji, Shaanxi), and the TS of inoculum was 4.39% (Table 1).

For the AD batch test, we set the digestion volume to 700 ml, TS to 8%, and food waste to inoculum ratio to 1:1, which was set based on the existing research on the digestion conditions of food waste (Leung & Wang, 2016). The different treatments based on the additive material and its concentrations were designated as follows: CK for 0% additives, BC2.5%, BC5%, BC7.5%, MB2.5%, MB5%, and MB7.5%. These concentrations selected in this study were within the appropriate dosage range of biochar added to food waste that has been previously researched (Altamirano-Corona et al., 2021; Cai et al., 2016). The experiments were performed in triplicate. Using the drainage gas collection method, each batch was monitored at 35 ± 1°C for 35 days.

A biogas analyzer (Gasboard-3200Plus, Ruiyizikong) was used to determine the daily methane content $c$ (%) and biogas product yield $Y$ (ml). The methane product yield $M$ (ml) was calculated by Equation (2):

$$M = Y \cdot c$$  \hspace{1cm} (2)

### 2.3 Indicators of anaerobic digestion

Since anaerobic digestion has the characteristics of rapid kinetic change rate in the early stage (Mao et al., 2017), 10 ml of digestive fluid was sampled on days 3, 7, 15, 25, and 35, respectively. In this study, the nonexperimental group was set with the same conditions as that of the experimental group. This nonexperimental group was used to supplement the experimental group with evenly stirred digestion liquid under the corresponding conditions after each sampling. The purpose of this was to eliminate the influence of sampling on the test results. The experiment was discontinued when the daily methane production from all digesters declined to less than 1% of the total methane production. The duration of this experiment was 35 days.

We measured the pH, AN, VFA, and composition of the sample supernatant. The sample supernatant was obtained after centrifugation at 5595 g for 5 min. A handheld pH meter was used to measure sample pH. The sample AN was obtained using the Kjeldahl method (Wang et al., 2012). The VFA and sample composition were measured after the supernatant was mixed with phosphoric acid until the pH was <3. The sample was centrifuged again, and the liquid was passed through 0.45 and 0.22 μm filters. The VFA composition was determined by gas chromatography (GC-2010 PLUS) by setting the inlet temperature to 220°C, detector temperature to 250 and 100°C, retention time to 0 min, and heating to 180°C at 8°C/min (Xue et al., 2020).

### 2.4 DNA extraction and metagenomic sequencing

Total genomic DNA was extracted from samples using the EZNA® DNA Kit (Omega Bio-tek) according to the manufacturer’s instructions. The concentration and purity of the extracted DNA were determined using a...
TBS-380 fluorimeter and NanoDrop2000 UV-Vis spectrophotometer, respectively. The DNA extract quality was confirmed under 1% agarose gel. The DNA extract was fragmented to an average size of approximately 300 bp using Covaris M220 ultrasonicator (Gene Company Limited) to construct a paired-end library using a TruSeqTM DNA Sample Prep Kit (Illumina). Adapters containing the full complement of the sequencing primer hybridization sites were ligated to the blunt-end of the fragments. Paired-end sequencing was performed on an Illumina HiSeq4000 platform (Illumina Inc.) at Majorbio Bio-Pharm Technology Co., Ltd. using HiSeq 3000/4000 PE Cluster Kit and HiSeq 3000/4000 SBS Kit according to the manufacturer’s instructions (www.illumina.com).

2.5 | Gene taxonomy and functional annotation

All predicted genes with 95% sequence identity (90% coverage) were clustered using the Cluster Database at High Identity with Tolerance (CD-HIT) (Fu et al., 2012) (http://www.bioinformatics.org/cd-hit/). The longest sequences from each cluster were selected as representative to construct a nonredundant gene catalog. The reads after quality control were mapped in the representative sequences with 95% identity using the SOAPaligner software (Li et al., 2008) (http://soap.genomics.org.cn/), and the gene abundance in each sample was evaluated. Representative sequences for the nonredundant gene catalog were aligned to the National Center for Biotechnology Information (NCBI) NR database with an e-value cutoff of 1e−5 using the BLASTP programme (Version 2.2.28+, http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul et al., 1997) for taxonomic annotations. The annotation of the cluster of orthologous groups of proteins (COG) for the representative sequences was performed using BLASTP against the eggNOG database (Jensen et al., 2008; Tatusov et al., 2003) (Version 4.5) with an e-value cutoff of 1e−5. The annotation of the Kyoto Encyclopedia of Genes and Genomes (KEGG) was conducted using BLASTP (Version 2.2.28+) against the KEGG database (Xie et al., 2011) (http://www.genome.jp/keeg/), with an e-value cutoff of 1e−5. The accession number of raw pyrosequencing data submitted to NCBI database was SRA: SRP350113.

3 | RESULTS AND DISCUSSION

3.1 | Biochar characteristics

Although the pH values of BC samples prepared from different substances were typically different, they were commonly alkaline (Pan, Ma, Zhai, et al., 2019). In this study, there was no significant difference between the pH values of both additive types, which were approximately 8.2 (Table 2). As BCs are alkaline, they can increase the system initial pH and improve the buffering performance when added to the AD of food waste with low pH. However, the ash content of MB was higher than that of BC. This might be attributed to the MB higher load of inorganic salts that cannot be volatilized at high temperatures. The BET-specific surface area, pore volume, and pore size are the main indicators used to measure the performance of a BC; however, they are affected by the type of raw material, pyrolysis temperature, modification treatment, and other aspects (Li et al., 2018). The microstructures observed in SEM photomicrograph images at ×3500 magnification (Figure 1a,b) indicate that the MB surface after being corroded by the chemical solution was rougher than the BC surface, and nonsmooth foreign matter was attached to it. This might have added other stimulants (in addition to BC) containing Fe elements to the AD digester (Choudhury & Lansing, 2020). Figure 1c shows the detection status of the surface functional groups on BC and MB. The intensities of the absorption peaks obtained for BC and MB were different. MB presented stronger absorption peaks than BC, at wavelengths of 3365 and 2923 cm⁻¹, representing the -OH and C-H stretching vibrations, respectively (Chen et al., 2008, 2014). This occurred because the modification attracted a large number of hydroxyl groups. The stretching vibrations of lignin aromatic nucleus C=C, cellulose C–O–C, and lignin aliphatic were observed at wavelengths of 1558–1617, 1096, and 668 cm⁻¹ (Huang et al., 2020; Xiao et al., 2014), respectively, which slightly increased the MB absorption peaks.

|                | pH    | Ash (%) | BET surface area (m²/g) | Pore volume (cm³/g·10⁻³) | Pore size (nm) |
|----------------|-------|---------|------------------------|--------------------------|---------------|
| BC             | 8.19  | 20.04   | 4.496                  | 0.789                    | 7.021         |
| MB             | 8.26  | 41.33   | 121.846                | 29.475                   | 9.676         |

Abbreviations: BC, biochar; BET, Brunauer–Emmett–Teller; MB, magnetic biochar.
3.2 | Anaerobic digestion system

3.2.1 | Methane production

Many studies have reported the effects of BC on methane production in anaerobic digestion. For instance, water hyacinth BC loaded with Fe₃O₄ can increase the removal of chemical oxygen demand and production of methane (Zhuang et al., 2020). BC can also reduce the initial lag period in a slow digestion process (Fagbohungbe et al., 2016), and promote a normal start-up for systems with high organic loads (Wang et al., 2017). The AD methane production obtained in this study is shown in Figure 2. The CK samples (without BC) presented the lowest cumulative methane output, and the methane output increased significantly upon the use of additives, particularly in BC2.5% and MB2.5%. From the perspective of daily behavior, all digesters presented a lag period, and the first peak of methane production occurred after this lag. CK presented the longest lag time, and its first methane peak occurred on day 18. In contrast, MB7.5% and MB5% presented the shortest lag times, and their first peaks occurred on day 9, followed by MB2.5% and BC7.5% on days 11 and 12, respectively, and BC2.5% and BC7.5% both on day 15. Therefore, although both additives shortened the lag time of AD, MB presented a better performance than BC. In addition, there was a gentle second peak of methane production from day 18 to 30. Overall, BC2.5% and MB2.5% were the most optimal additives for food waste AD.

3.2.2 | Volatile fatty acids, pH, and total ammonia nitrogen

VFA, pH, and AN are important environmental factors in an AD system, and they can reflect the progress and stability of the digestion process. Figure 3 shows that the pH was low in the early AD stage, and then gradually increased and stabilized at approximately 7.3. Among all samples, MB2.5%, MB5%, and MB7.5% presented the fastest pH increase. In contrast, AN fluctuated over time, and the final AN values of CK, BC2.5%, BC5%, and BC7.5% were lower than their respective initial values, which was opposite to the MB trend. Studies have shown that BC can improve alkalinity and alleviate ammonia inhibition by providing continuous process stability in AD (Shen et al., 2015). However, BC cannot alleviate severe ammonia removal (Mumme et al., 2014). For all treatments, AN initially increased and
subsequently decreased. However, the VFA concentration varied among treatments because BC can adjust the digestion power and accelerate the conversion from acid phase to solvent phase (Wu et al., 2019). The VFA of CK did not change significantly until day 15, and it decreased significantly on day 25. Although VFA presented a slight decrease in the BC treatments, it presented a sharp decrease in the MB experiments on day 15 as the pH increased. Acetic, propionic, and butyric acids accounted for a large proportion of the VFA composition, and their changes were consistent with the total VFA trend. This result was similar to the findings of a previous study, in which the addition of BC and Fe-containing substances was beneficial to the dynamics of the digestive system and reduced the content of acetic and propionic acids (Capson-Tojo et al., 2019).

3.3 | Microbial activity characteristics

We selected BC2.5% and MB2.5% for further investigation because they presented the best methane production, and CK was used to investigate the microbial properties. The analyses were performed for the early (day 3), middle (day 15), and late stages (day 35) of the AD process, and the samples were accordingly named as BC3, BC15, BC35, MB3, MB15, MB35, CK3, CK15, and CK35.

3.3.1 | Microbial community composition

The composition of the bacterial community of the samples in this study at the phylum level is shown in Figure 4a (composition of bacterial community at the genus level is provided in Appendix). At the genus level, the Bacteroides relative abundance increased in BC and MB at the early stage, which was similar to the results of studies on supplementing activated carbon in digestion systems (Park et al., 2018). And unclassified_o_Bacteroidales was the dominant bacteria (Figure A1, in Appendix). At the corresponding phylum level, Bacteroidetes and Firmicutes were the dominant bacteria, and Bacteroidetes gradually decreased with AD time. Fat, protein, and carbohydrates, which were present in the food waste, can be degraded by Firmicutes (Turker et al., 2016). However, Firmicutes first increased in CK and BC and then decreased, and they gradually increased in MB. When the proportion of Proteobacteria increased, the proportion of Synergistetes decreased, and vice versa (Figure 4a).

From the perspective of archaeal genus level, Methanosarcina was the absolute dominant bacteria regardless of time or treatment, and it accounted for the highest proportion of the total archaea during the peak of methane production on day 15. The proportion of Methanobacterium, unclassified_o-Methanomicrobiales, Methanoculleus, and Methanothrix increased mildly in BC35 and MB35, which did not occur in CK35. The addition of BC and MB did not cause a large-scale change in the composition of the microbial community. However, the microbial community changed significantly over time. In addition, the results of the microbial community composition showed no significant difference between CK and treatments at the same sampling time. Since the composition of the microbial community was more affected by the inoculum than by the treatments provided during AD.

**Figure 3** VFA, pH, and AN of waste food upon different AD treatments on days 3, 7, 15, 25, and 35
FIGURE 4  Community composition of (a) bacterial community at the phylum level and (b) archaeal community at the genus level.

FIGURE 5  (a) Modules of main methanogenesis pathways. Note: Squares and colors represent relative abundance (%) of enzymes. (b) Abundance (RPKM) of modules. (c) Archaea genus level and functional contribution analysis.
3.3.2 | Methanogenesis pathway change

The four modules of metabolic pathways for methane production are M00567, M00357, M00356, and M00563, which correspond to the methanogenesis of CO₂, acetate, methanol, and methylamine/dimethylamine/trimethylamine, respectively (Ma et al., 2020). The differences between the abundance of the four modules was calculated by reads per kilobase per million mapped reads (RPKM). These differences were not clear in CK3, BC3, and MB3 (Figure 5b). By day 15, the abundance of the four modules increased in all samples, and the changes in MB15 were significantly higher than those in CK15. The acetate methanogenesis pathway continued to increase in CK35, whereas it decreased in MB35. The abundance of the four modules in these two treatments was nearly the same, and the main difference was that they were significantly reduced in BC35. Correspondingly, enzymes with the highest proportion in the methanogenesis of acetate and CO₂ are shown in Figure 5a. Furthermore, in the CO₂ methanogenesis, the enzymes with highest concentration on day 15 were EC1.2.7.12 (formylmethanofuran dehydrogenase), EC2.3.1.101 (formylmethanofuran-tetrahydrodismethanopterin N-formyltransferase), EC3.5.4.27 (methenyltetrahydrodismethanopterin cyclohydrolase), EC1.5.98.1 (methylene tetrahydrodismethanopterin dehydrogenase), and EC1.5.98.2 (5,10-methylene tetrahydro methanopterin reductase). Their high abundance was maintained in MB35, but decreased in CK35 and BC35. In addition, the abundance of enzymes for acetate methanogenesis was the highest on day 3 (CK3, BC3, and MB3), including EC2.7.2.1 (acetate kinase), EC6.2.1.1 (acetate-CoA ligase), and EC2.3.1.8 (phosphoryl acetyltransferase). The enzymes EC2.1.1.86 and EC2.8.4.1 were present in all four methanogenesis pathways, and the changes in their abundance were consistent with the trends of enzymes in the CO₂ methanogenesis.

Figure 5c shows the contribution of archaea in the four modules of methanogenesis pathways. Although their contribution degree was similar to that of microbial community composition, there were differences between treatments and modules. Methanosarcina accounted for the highest proportion, regardless of modules, time, and treatments. Methanobacterium in M00357 (acetate methanogenesis) contributed more than the other three modules, but Methanosarcina exhibited the highest contribution in M00563. From a time perspective, the highest abundance of Methanobacterium occurred on days 3, 35, and 15. The highest contribution of Methanosarcina occurred on day 15. The unclassified_o-Methanomicrobiales contribution was higher in BC35 and MB35 than in CK35. In addition, the Methanothrix of BC35 and MB35 was lower than BC3 and MB3, respectively, in the four modules, whereas CK3 was more than CK35. The iron-containing enzymes required for methanogenesis and acidogenesis were activated by BC (Xu et al., 2020). In a word, the addition of both BC and MB increased the enzyme activity during the peak methane production. However, only MB maintained the advantage of enzyme abundance until the end of the AD process.

3.4 | Mechanism of biochar action on AD

The relationship between environmental factors and methane in the AD system is shown in Figure 6a. The most important components of VFA were acetic, propionic, and butyric acids, which presented a significant negative correlation with pH. Acetic and butyric acids were also negatively correlated with the methane production, whereas pH was positively correlated with it. There was no clear correlation between AN and methane production. Therefore, we investigated the main microorganisms affected by the changes in environmental factors related to methane production. Microorganisms were the main factors consuming the system substances, and they can both adapt to and change the environmental factors. Methanoseta can decompose acetic acid and accelerate the decomposition of acetate in the presence of BC with conductive affinity (Lu et al., 2016). Figure 6b shows that the dominant bacterial phylum Bacteroidetes presented a significant positive correlation with the concentration of acetic and butyric acids and a significant negative correlation with pH, whereas Chloroflexi exhibited an opposite trend. There was a positive correlation between pH and the dominant archaea Methanosarcina. In summary, the effects of BC and MB on the improvement of methane production in the AD of food waste can be potentially attributed to the stimulation of the four methanogenesis modules on day 15. The main difference was that, unlike BC, MB maintained the module enhancement until day 35. In addition, the environmental factors of the digestive liquid are associated with a series of changes, including pH increase and VFA degradation speed, through the action of archaebacteria. The schematic of the AD mechanism is shown in Figure 6c.

4 | DISCUSSION

Biochar properties are affected by many preparation factors. For instance, increasing the pyrolysis temperature during BC preparation can sharply decrease the amount of volatile solids (Luz, Cordiner, Manni, Mulone, Rocco, Braglia, et al., 2018), and the nature of raw materials such as animal manure and tree branches can directly affect
The modification of BC can eliminate elements in the alkyl, aromatic, ester, and hydroxyl groups, thereby forming vacancies (Ayaz et al., 2021), which significantly increase their BET-specific surface area. This increase in pore volume and size is conducive to adsorption, as they increase the MB contact with the digestion liquid (Leng et al., 2021). And many studies have reported the effects of BC on methane production in anaerobic digestion. For instance, water hyacinth BC loaded with Fe₃O₄ can increase the removal of chemical oxygen demand and production of methane (Zhuang et al., 2020). BC can also reduce the initial lag period in a slow digestion process (Fagbohungbe et al., 2016), promote a normal start-up for systems with high organic loads (Wang et al., 2017), and it has certain recovery ability to unstable anaerobic fermentation system (Capson-Tojo et al., 2019). This is similar to the results of this study. However, methane production was not positively correlated with BC dosage, and BC exceeding the appropriate range may have an inhibitory effect (Shen et al., 2016). The reason for the low methane yield may be due to the adsorption of carbon source by BC, and the consumption of methane and other organic substrates by *Norank_c_Bathyarchaeia* (Xie et al., 2022).

The influence of BC and MB on biogas production can be reflected in changes in environmental factors and microbial characteristics. The optimum pH range of most mesophilic methanogens was 6.8–7.2, which was consistent with the optimum pH of digestion (Fang & Liu, 2002), but there are differences in the optimal pH of different methanogens (ranging from 6.0 to 8.5). If pH exceeds the threshold, the activity of methanogens will be inhibited (Lee et al., 2009). However, metal elements and basic functional groups on the surface of biochar can improve the buffering capacity and stability of digestion system, and contribute to methane generation and intermediate acid degradation (Li et al., 2019). As for ammonia nitrogen content, Jindrich et al. (2012) indicated that the concentration of ammonia nitrogen not only affected the stability of the anaerobic digestion process but also affected the
methane production in the anaerobic digestion system. In this study, ammonia nitrogen content in anaerobic digestion system was still in a state of fluctuation but tends to be stable after adding BC and MB, but there was no significant correlation with methane production.

From the perspective of microorganisms, the relatively high surface area of biochar additives is conducive to the adhesion and growth of microorganisms, while promoting the formation of electroactive bacteria, and improving the anabase of bacteria and methanogenesis (Judith Martinez et al., 2018). In addition, there was an electronic polymerization between methanogens and Geobacteria, and BC promoted methane production by facilitating direct interspecies electron transfer between microorganisms (Yuan et al., 2018). BC and MB can not only realize long-distance electron exchange between species (Kato et al., 2012) but also contribute to the stability of microbial adsorption and fixation, forming clusters or biofilms (Li et al., 2017), promoting microbial connection, and indirectly affecting the occurrence of direct interspecies electron transfer. Bacteroidetes, Firmicutes, and Proteobacteria are all anaerobic or facultative anaerobic bacteria that often exist in the digestion system (Bellucci et al., 2020). The microbrial community composition of a material varies with the environmental conditions. For instance, the presence of BC under ammonia stress can increase the abundance of Methanoregulaceae, Bacteroidales, Anaerolineales, and Syntrophobacterales (Su et al., 2019). Methanosarcina was easily attached to the surface of large particles (2–5 mm) in the BC, whereas Methanoseta preferred loosely bound fractions of all sizes in BC particles (Luo et al., 2015). Furthermore, the BC supplement increased the electron transfer between microorganisms, which was beneficial for the AD reaction (Wang et al., 2021). A higher expression of hydrolase gene was observed upon addition of BC (Yan et al., 2020). From the perspective of KEGG methanogenesis metabolic pathway, Methanosarcina can convert acetyl-phosphoric acid to acetyl-CoA by acetyl kinase EC2.7.2.1 and phosphotransacetylase (EC2.3.1.8), and Methanothrix was shown to directly convert acetate to acetyl-CoA using acetyl-CoA synthase (EC6.2.1.1) (Smith & Ingram-Smith, 2007). However, acetyl-CoA synthase exists in a variety of metabolic pathways (e.g., carbon metabolism, etc.). Moreover, the enzymes produced by the microorganisms were directly related to the methanogenesis pathways. Methanosarcina can use acetate kinase and phosphotransacetylase to participate in acetate methanation, and Methanosaeta can use acetate kinase to participate in acetate methanation (Ma et al., 2020). The most important electron carrier in this metabolic pathway is the coenzyme F420 (Mu et al., 2011). And this study showed that BC and MB supplementation had a positive effect on stimulating the enzyme activity of metabolic pathway during peak methane production.

Some studies suggest that the addition of biochar will first generate corresponding functional bacteria, which will significantly change the microbial community (Yin et al., 2018). Some scholars have also found that the main driving factors of the digestion system succession along with the process through the coupling relationship of the digestion system. This is because there is a significant correlation between archaea and digestion process factors, and different strains have different response mechanisms to digestion process factors due to different digestion conditions (Mao et al., 2019). This study showed that only MB maintained the advantage of enzyme abundance until the end of the AD process. This may have been caused by two aspects. First, the large surface area and roughness of MB were more suitable for the contact of microorganisms and enzymes with the digestate. Second, irritating magnetic substances were added upon the modification. Moreover, this result was different from the previous studies on the AD metabolic pathways with magnet powder addition (Gao et al., 2021). This further illustrated that the internal mechanisms of the different additives for increasing the AD methane production were different.

5 | CONCLUSIONS

In this study, the addition of BC and MB to food waste AD was investigated. The results showed the highest methane production in BC2.5% (85%) and MB2.5% (89%). The BC treatments increased the VFA degradation rate and pH. The main environmental factors affecting methane production were acetic acid, butyric acid, and pH, which also greatly affected bacteria and archaea communities. The influence of BC and MB in the mechanism of food waste AD was observed to be similar on the 15th day of the experiment. It was shown that both the abundance of the four methanogenesis modules and methane production were significantly increased at BC2.5% and MB2.5% in the digestion middle stage. A remarkable finding obtained in this study was that MB retained its positive effectiveness, even on the 35th day of digestion, whereas BC did not. However, the limitation of this research is that the study was conducted in a batch mode at the laboratory scale. In the future, research should explore whether MB has the characteristics of both magnet powder and BC, and if it can exceed the cumulative effect of the two. And further research is required to determine whether these laboratory scale experiments can be scaled-up and implemented on a factory scale.

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