

Effect of biochar addition on the anaerobic digestion of food waste: microbial community structure and methanogenic pathways

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ABSTRACT

This study assessed the effects of the addition of biochar prepared at 700 °C with different dosages on the anaerobic digestion of food waste. The biochar addition at a concentration of 10.0 g/L increased the cumulative methane yield by 128%, and daily methane production was also significantly promoted. The addition of biochar derived from poplar sawdust significantly increased the relative abundance of dominant bacteria for anaerobic digestion by 85.54–2530% and promoted the degradation of refractory organic matter and the transfer of materials between the hydrolysis and acid production stages. Further analysis has demonstrated that *Bathyarchaeia* and hydrogenotrophic methanogens were enriched by the biochar addition. Meanwhile, the relative abundances of functional genes, including C5-branched dibasic acid metabolism, and pyruvate metabolism, were increased by 11.38–26.27%. The relative abundances of genes related to major amino acid metabolism, including histidine metabolism, lysine biosynthesis, and phenylalanine, tyrosine, and tryptophan biosynthesis, were increased by 11.96–15.71%. Furthermore, the relative abundances of genes involved in major replication and repair were increased by 14.76–22.76%, and the major folding, sorting, degradation, and translation were increased by 14.47–19.95%, respectively. The relative abundances of genes related to major membrane transport and cell motility were increased by 10.02 and 83.09%, respectively.

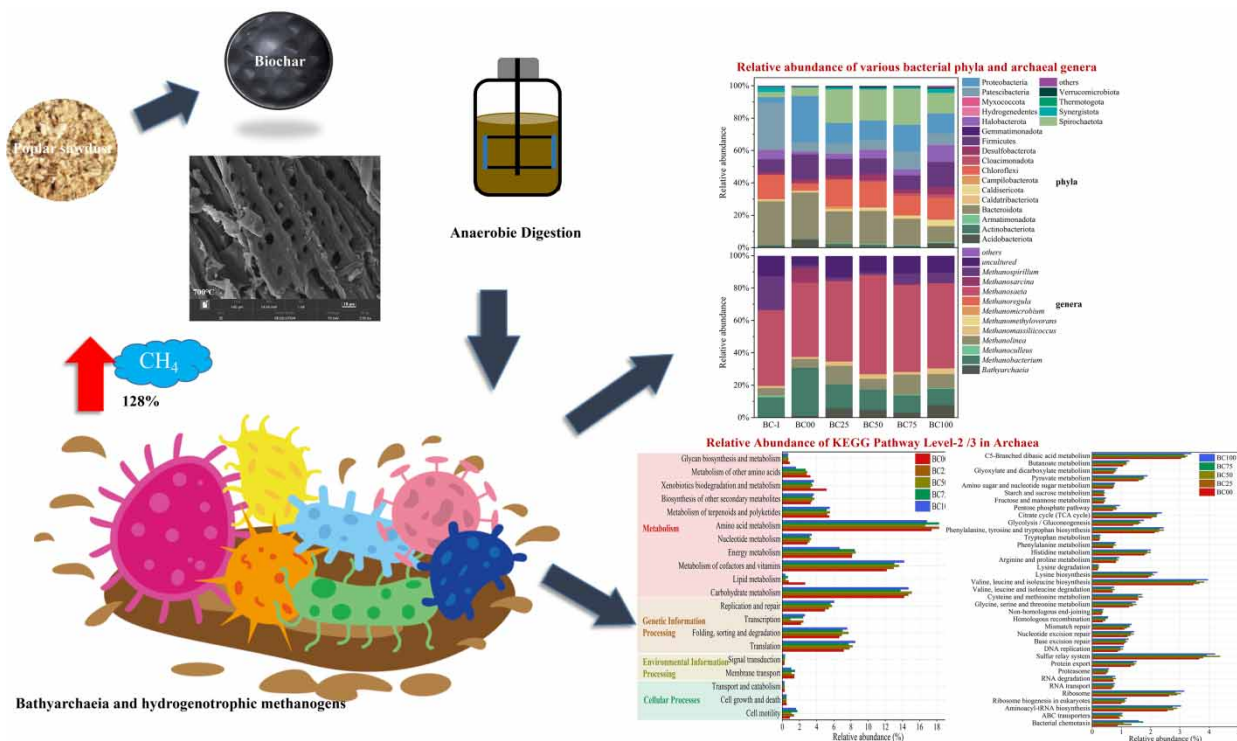
Key words: anaerobic digestion, carbohydrate and amino acid metabolism, hydrogenotrophic methanogens, poplar sawdust

HIGHLIGHTS

- Different dosages of biochar in the anaerobic digestion process of food waste were studied.
- 16S rRNA gene sequencing and PCR were used to analyze bacterial and archaeal domains.
- The addition of biochar has promoted the improvement of anaerobic digestion performance, e.g. the improvement of methane production capacity, the increase of anaerobic digestion-dominant bacterial communities, and the abundance of functional genes.

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GRAPHICAL ABSTRACT



1. INTRODUCTION

The growing production of food waste, containing food residues, grains, and vegetables, has become a major challenge for municipal solid waste treatment and disposal (Devi *et al.* 2023; Liu *et al.* 2023). Improper treatment and disposal of food waste may cause serious environmental burdens, such as land use, greenhouse gas emissions, and groundwater pollution risk (Gu *et al.* 2022; Liu *et al.* 2023). As a waste-to-energy technology, the anaerobic digestion of organic wastes has attracted widespread attention (Devi *et al.* 2023). However, as anaerobic digestion is a complex biological process, the high organic content of food waste could cause acidification, ammonia nitrogen inhibition, and consequently low process efficiency (Li *et al.* 2018a; Rasapoor *et al.* 2020).

Biochar is a highly aromatic carbon-rich product with a well-developed pore structure, a high specific surface area, and abundant oxygen-containing functional groups and can be prepared by the thermal conversion of biomass under oxygen-depleted conditions (Visiy *et al.* 2022; Valenzuela-Cantú *et al.* 2024). As an additive material, biochar has been widely used to improve anaerobic digestion performance, such as increasing methane yield, improving system stability, shortening hysteresis, and improving biogas quality through buffering effect, direct interspecific electron transfer, microbial immobilization, and disinhibition (Wang *et al.* 2021a; Chen *et al.* 2023; Valenzuela-Cantú *et al.* 2024). Wang *et al.* (2021a) revealed that the biochar addition in the process of anaerobic co-digestion of food waste and dewatered activated sludge could enrich the electroactive *Syntrophomonas* and *Methanosarcina*. As a potential redox-active medium, biochar can stimulate the potential direct electron transfer between species and inhibit the hydrogen synthase pathway (Wang *et al.* 2021a). Cui *et al.* (2021) found that biochar addition could increase the relative abundance of bacteria involved in syntrophic interactions, such as *Syntrophomonas* and *Syntrophobacter*, under high ammonia stress. Wang *et al.* (2021c) confirmed that methane production increased by 35–37% when the dosage of biochar increased from 0.6 to 1.2 g/g-TS in the algae anaerobic digestion process. However, the excess biochar may inhibit the growth of methanogens due to the alkali metals and functional groups on the surface (Shen *et al.* 2016). Shen *et al.* (2016) found that the cumulative methane production with the biochar addition of 12 g/L was 18.25% higher than that with a biochar dosage of 50 g/L. Luo *et al.* (2022) also demonstrated that the highest specific methane production of 553.0 mL/g-VS had been gained with a biochar dosage of 10 g/L rather than a higher dosage. However, Altamirano-Corona *et al.* (2021) found that the methane yield with a biochar dosage of 10 g/L had decreased by 10.7% with the control group.

Therefore, this study aimed to investigate the effects of biochar addition with different dosages on the anaerobic digestion process of food waste. The effect of biochar addition on methane yield was evaluated, and the changes in microbial community structure and methanogenic pathways were also explored.

2. MATERIALS AND METHODS

2.1. Preparation and characteristics of biochar

Poplar sawdust, purchased from Lianyungang City (China), was selected as the feedstock for biochar preparation. Biochar was prepared through the pyrolysis of poplar sawdust in a muffle furnace at 700 °C for 2 h under oxygen-limited conditions (Shanmugam *et al.* 2018). The yield of biochar is the mass ratio of biochar to initial dry biomass (Yang *et al.* 2020a, 2020b). The pH value of biochar was measured in a 10% (W/V, weight/volume) suspension in ultrapure water prepared by shaking at 150 rpm under ambient temperature for 24 h using a pH meter (PHB-4, LEICI, China, Xu *et al.* 2020). Ash contents were determined using the ASTM method (D-1762-84, Bagul *et al.* 2017). A scanning electron microscope (SEM, MIRA LMS, TESCAN, The Czech Republic) was used to observe the surface morphology of biochar. Energy-dispersive spectroscopy (Xplore 30, Oxford, UK) was used to examine the elemental composition of biochars. An automated surface area and Porosity Analyzer (TriStar II 3020, Quantachrome, USA) were used to determine the Brunauer–Emmett–Teller (BET) surface area and the pore structure of biochars. Fourier transform infrared (FTIR) (SENSOR-27, Bruker, Germany) was used to analyze the functional groups of biochars.

2.2. Substrate and inoculum

The food waste used in this study was composed of rice (20%), meat (20%), vegetables (30%), legumes (15%), and fats (15%) (Fisgativa *et al.* 2016). An anaerobic digestion inoculum (total solids (TS) of 10.98 ± 0.25 g/L, volatile solids (VS) of 6.65 ± 0.15 g/L) was collected from a laboratory-scale continuous stirred tank reactor that operates stably at 35 ± 1 °C for the anaerobic digestion of sewage sludge.

2.3. Batch food waste anaerobic digestion experiments

An automatic biomethane potential testing system (AMTPS-II, Bioprocess Control Company, Sweden) was used to perform anaerobic digestion batch experiments. A total of 400 mL digesters are filled with 300 mL of inoculum sludge and 10.71 g of food waste, and then biochar with the dosages of 0 g/L (BC00), 2.5 g/L (BC25), 5.0 g/L (BC50), 7.5 g/L (BC75), and 10.0 g/L (BC100) was added, respectively. Each test was made in triplicate; all bottles were added with distilled water to a constant volume of 400 mL and sealed. Subsequently, nitrogen was introduced into all batch digesters to keep an anaerobic state. Then, all digesters were cultured under 35 ± 1 °C until no biogas was produced. The specific meanings of some of the abbreviations used in this paper are given in Table S1 of the Supplementary Material.

2.4. Analytical methods

pH, TS, VS, and ammonia nitrogen of the mixed culture were measured according to standard methods (Yirong *et al.* 2017). 16S rRNA gene sequencing and polymerase chain reaction (PCR) amplification were used to analyze bacterial and archaeal domains. The mixed culture samples of BC00, BC25, BC50, BC75, and BC100 were collected from the digesters at the end of the anaerobic digestion process. Then, samples were stored at -20 °C before being delivered to Allwegene Co., Ltd (Allwegene, Beijing, China) for high-throughput sequencing. PCR extraction of these samples was performed using the Agencourt AMPure XP as per the manufacturer's instructions. The V3–V4 hypervariable regions of 16S rRNA from bacteria and archaea were amplified using universal primer sets of 338–806 (ACTCCTACGGGAGGCAGCAG, GGACTACHVGGGTWTCTAAT) and 344–806 (ACGGGGYGCAGCAGGCGCGA, GGACTACVSGGGTATCTAAT), respectively. Subsequently, the Illumina MiSeq was used to sequence PCR products. The Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used to analyze the functional gene prediction pathways.

3. RESULTS AND DISCUSSION

3.1. Physicochemical properties of biochars

SEM images (Figure 1) showed that biochar was composed of irregularly shaped particles with highly porous structures and rough surfaces due to the escape of volatile gases during carbonization (Das *et al.* 2021). Biochar had a micro-pore volume of 0.01 cm³/g, a total pore volume of 0.08 cm³/g, and a BET surface area of 297.68 m²/g (Table 1). The large surface area and

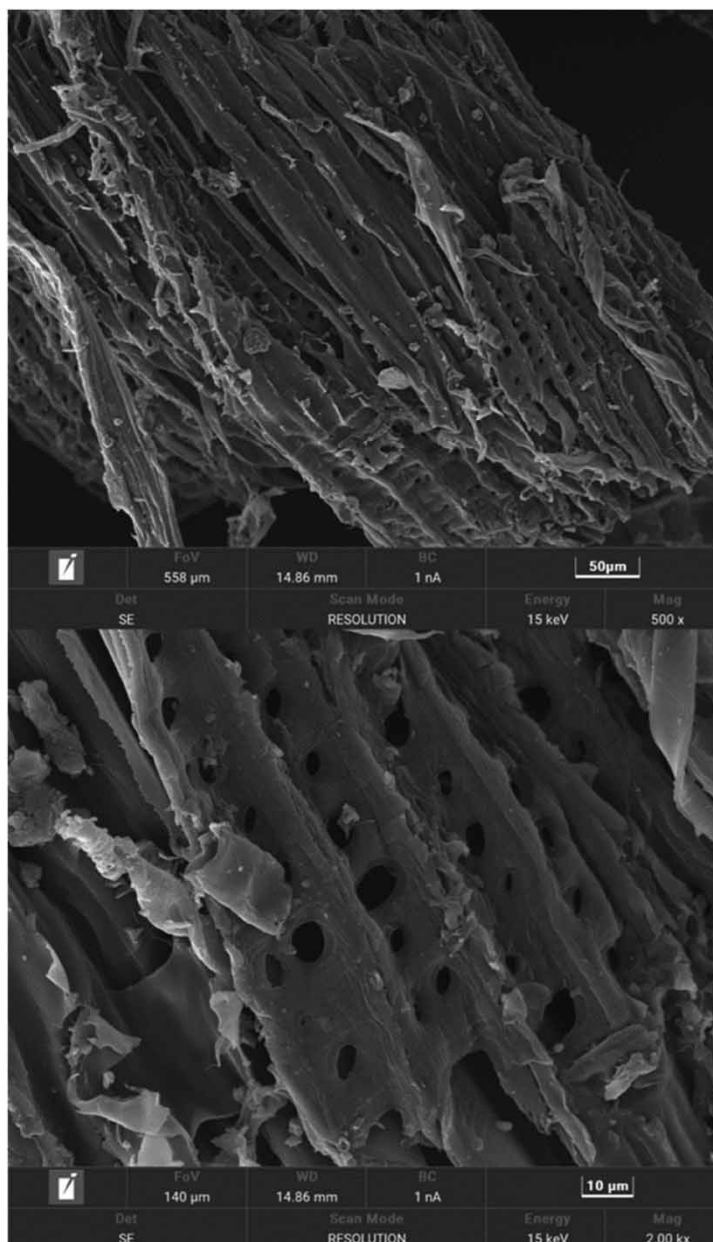


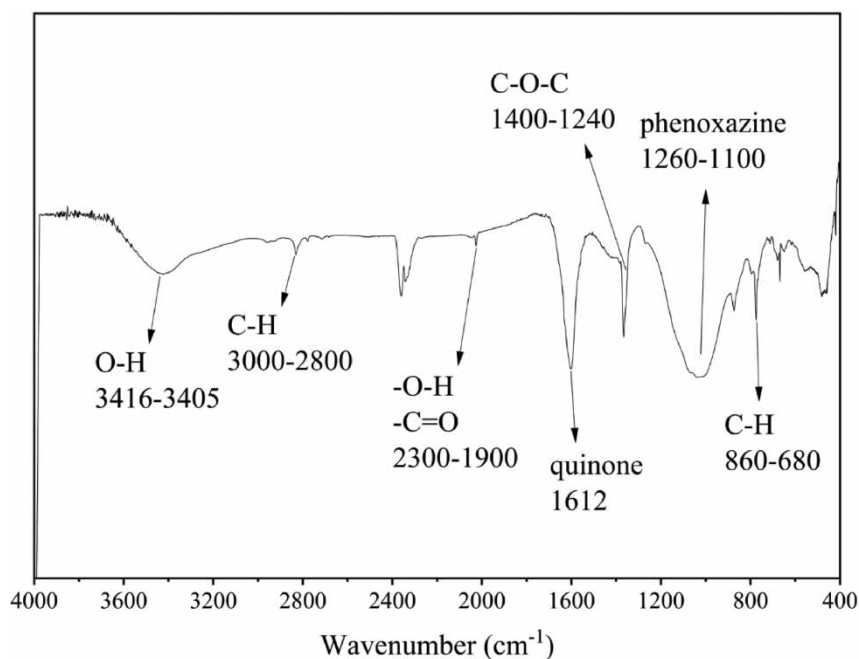
Figure 1 | SEM of biochar (500 and 2,000 times).

the excellent porous structure of biochar could create favorable conditions for the formation of microbial biofilms and provide a suitable surface for acid adsorption (Pan *et al.* 2019). The carbon content of biochar was 85.21%, and a high carbonization degree usually results in relatively high conductivity (Sun *et al.* 2017; Lam *et al.* 2018).

The FTIR spectra of biochar is presented in Figure 2. The absorbance peaks at wavenumbers of 3,416–3,405, 3,000–2,800, and 2,300–1,900 cm^{-1} were due to the stretching vibration of hydroxyl groups O–H (Xin *et al.* 2017), C–H of aliphatic compounds (Hu *et al.* 2021), hydroxyl and carbonyl functional groups, respectively. Wang *et al.* (2021b) found that biochars with high absorption peaks of redox-active organic functional groups (such as O–H, C = O, and C–H) usually showed good ability to act as a shuttle or electron mediator. The absorbance peaks at wavelength of 1,400–1,240 cm^{-1} were due to the presence of the ether group (C–O–C) in cellulose (Das *et al.* 2009). The absorbance peaks at wavenumbers of 1,612 and 1,260–1,100 cm^{-1} were the characteristic peaks of quinone C = C or C = O (Zhang *et al.* 2019) and associated with the presence of phenazine (Shanmugam *et al.* 2018). The reversible electron transfer capacity of electroactive oxygen functional groups (quinone

Table 1 | Physio-chemical characteristics of biochar

Analysis	Parameter	Biochar
Proximate analysis	Biochar yield (wt. %)	26.88 ± 0.10
	Ash content (wt. %)	21.64 ± 0.56
Physio-chemical	pH	9.72 ± 0.01
Property	BET surface area (m ² /g)	297.68
	Micro-pore volume (cm ³ /g)	0.01
	Total pore volume (cm ³ /g)	0.08
Ultimate analysis	C (wt.%)	85.21
	O (wt.%)	12.18
	Na (wt.%)	0.08
	Mg (wt.%)	0.19
	K (wt.%)	0.81
	Ca (wt.%)	1.26
	Mn (wt.%)	0.07
	Fe (wt.%)	0.2

**Figure 2** | FTIR spectra of biochar.

and phenazine) on biochar surfaces could promote potential direct interspecies electron transfer (DIET) for the degradation of volatile fatty acids (Binh & Kajitvichyanukul 2019; Wang *et al.* 2020a). The absorbance peaks at wavelength of 860–680 cm⁻¹ were due to the stretching vibration of the aromatic C–H bond (Wang *et al.* 2020b).

3.2. Effect of biochar addition on anaerobic digestion

3.2.1. Variation of pH and NH₃-N concentration in the anaerobic digestion system

The pH values and NH₃-N concentrations during the anaerobic digestion process are shown in Figure 3. The pH of all groups reached its lowest values on the third day and then gradually increased until the end of the test. The pH values of the test groups were higher than those of the control group, probably because the addition of biochar could provide carriers for

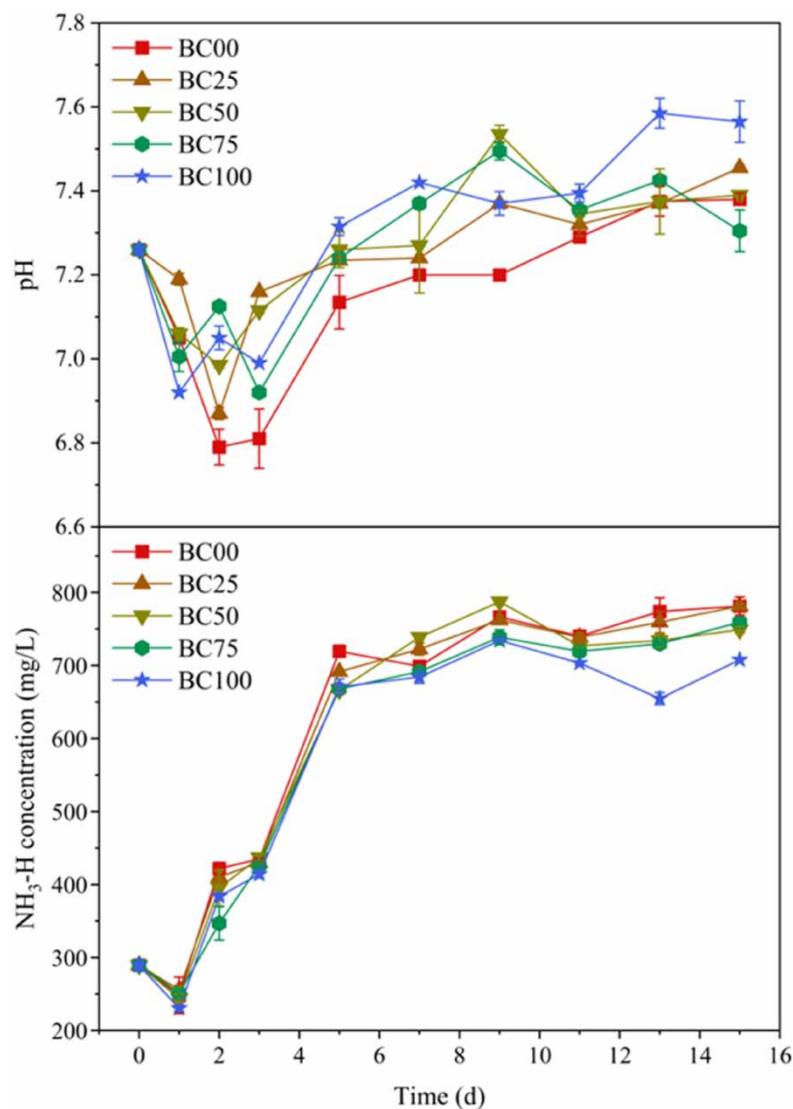


Figure 3 | pH value and NH₃-N concentrations vary with time.

microorganisms and promote the growth of methanogens, thereby accelerating the consumption of organic acids. In addition, the presence of a large number of alkaline groups (Figure 2) and alkali or alkaline earth metals (Table 1) could also play a buffering role (Luo *et al.* 2015). The pH value of the BC100 group was relatively higher than the other groups, which could mitigate the effect of volatile fatty acid accumulation on methanogenic microbes. The NH₃-N concentration was between 230.57 and 781.34 mg/L. The NH₃-N concentration in BC100 was lower than in the other groups, which could be attributed to the fact that biochar addition could promote the growth and reproduction of the microbes that consume NH₃-N (Luo *et al.* 2015).

3.2.2. Profiles of methane production

Figure 4 shows the effect of biochar addition on methane production. The highest cumulative methane volume was 759.35 ± 13.15 mL for BC100, followed by BC50 (397.50 ± 28.80 mL), BC25 (393.03 ± 1.79 mL), BC75 (384.7 ± 0.30 mL), and BC00 (332.5 ± 0.50). The cumulative methane production of each experimental group showed the same trend in the first 5 days, and methane was produced mainly from small molecular and easily degradable organic matter. BC00, BC25, BC50, and BC75 showed significant biogas production peaks on the second day, which were 218.00, 243.60, 247.20, and 197.00 mL/d, respectively. BC100 had two significant biogas production peaks, 218.00 (day 2) and 89.60 mL/d (day 10). The first peak of

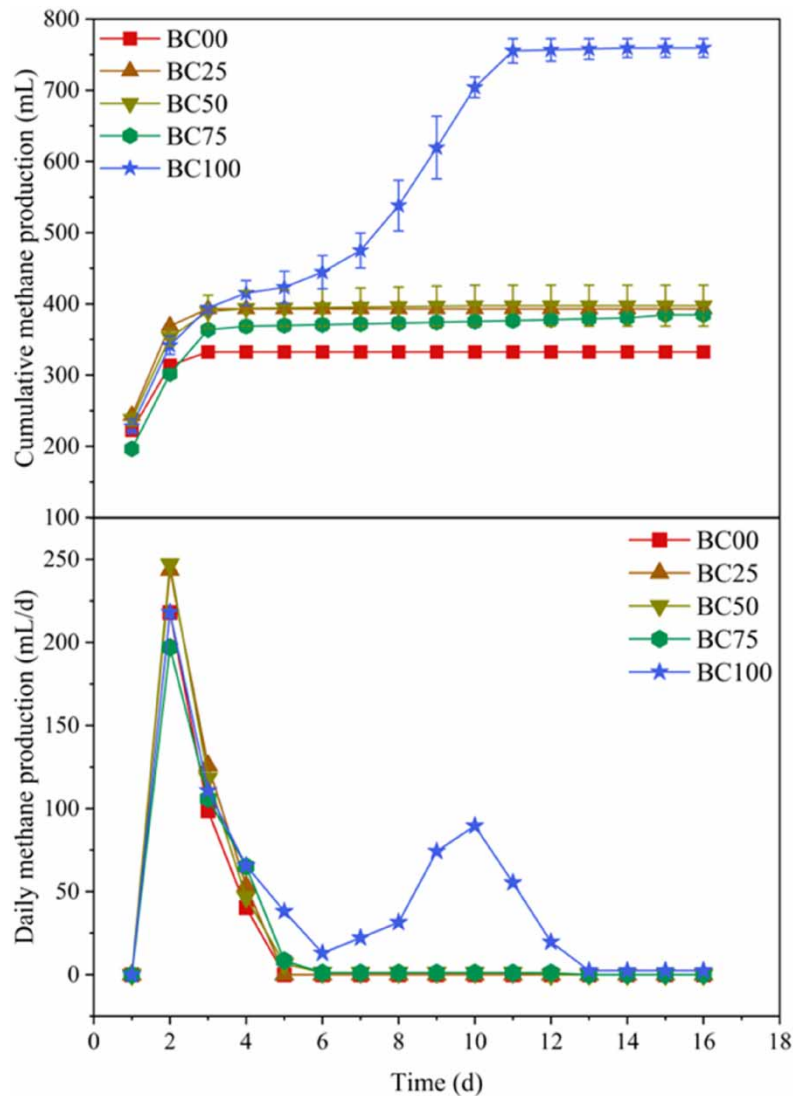


Figure 4 | Cumulative and daily methane production varies with time.

biogas production was mainly due to the digestion of degradable substances by methanogenic bacteria (Kaur *et al.* 2020). At the same time, macromolecular organic matter was decomposed into organic acids through hydrolysis and acidification, which led to a decrease in pH value (Figure 3), and the daily methane production dropped sharply in the following days. The daily methane production of BC100 was significantly higher than that of other groups in the late stage of the anaerobic digestion process, mainly due to the digestion of refractory macromolecular organic matter.

3.2.3. Microbial community structure

The microbial community structures of different experimental groups are shown in Figure 5. Bacteroidetes are often associated with the decomposition and fermentation of polysaccharides (Rivière *et al.* 2009; Lim *et al.* 2020). The relative abundances of Bacteroidetes decreased by 31.10% (BC25), 28.40% (BC50), 43.50% (BC75), and 65.93% (BC100), respectively, compared with that in BC00, mainly due to the components of the substrate having a relatively low content of polysaccharide. Meanwhile, the addition of biochar increased the relative abundance of Chloroflexi. Chloroflexi could degrade monosaccharides and polysaccharides with the production of acetic acid (Rivière *et al.* 2009). The relative abundances of Chloroflexi in BC25, BC50, BC75, and BC100 increased by 271.61, 252.72, 169.54, and 197.63%, respectively, compared with that in BC00.

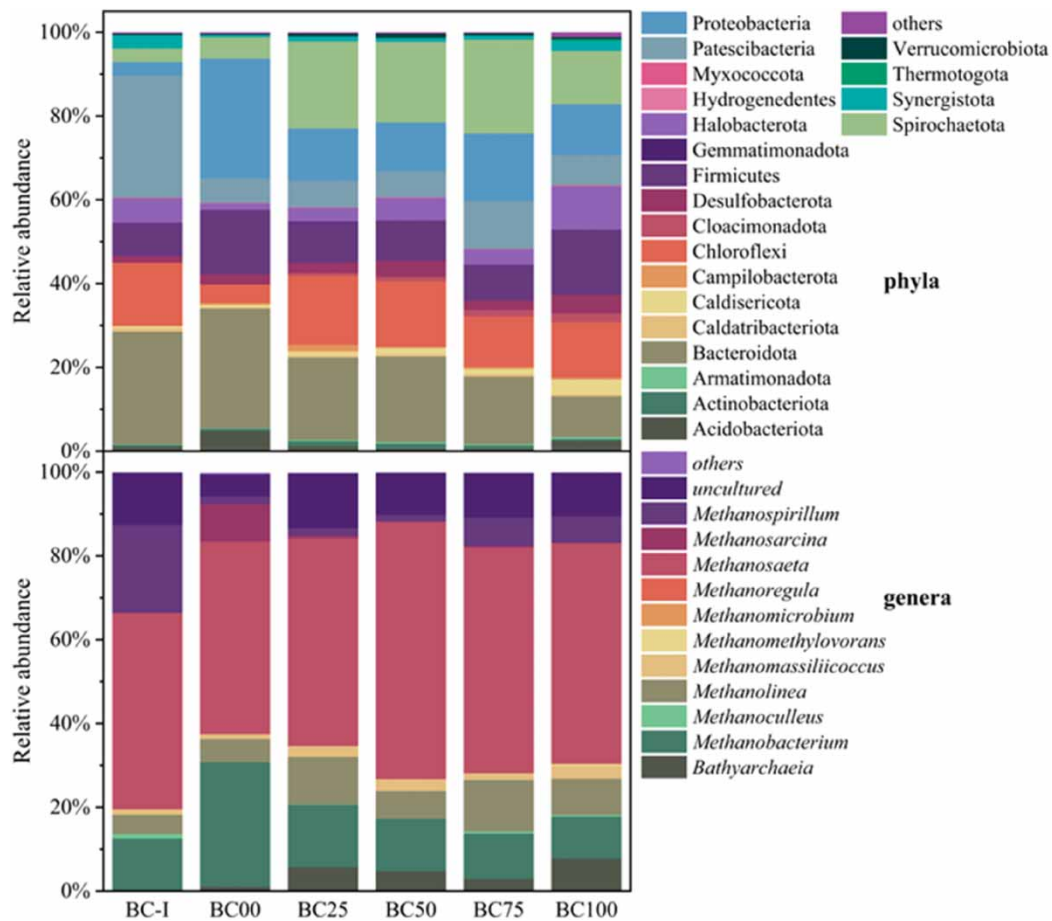


Figure 5 | Relative abundance of various bacterial phyla and archaeal genera.

Firmicutes can produce extracellular enzymes (such as cellulase, lipase, and protease) and play an important role in the catabolic metabolism of cellulose, lipids, proteins, sugars, and amino acids (Zhao *et al.* 2017). The relative abundances of Firmicutes in BC25, BC50, and BC75 decreased by 36.58, 37.62, and 44.21%, respectively, compared with that in BC00, while that in BC100 increased by 1.76%. Biochar addition increased the relative abundance of Halobacterota, Hydrogenedentes, Patescibacteria, Spirochaetota, and Synergistota. Halobacterota includes methanogenic species that use acetic acid as an electron donor (Fan *et al.* 2022). Hydrogenedentes can syntrophically degrade glycerol and lipids by expressing genes encoding triacylglycerol extracellular hydrolysis (Nobu *et al.* 2015; Gaspari *et al.* 2023). The relative abundances of Halobacterota in BC25, BC50, BC75, and BC100 increased by 100.69, 242.16, 130.79, and 558.11%, respectively, while that of Hydrogenedentes in BC25, BC50, BC75, and BC100 increased by 90.00, 80.00, 100.00, and 400.00%, respectively, compared with that in BC00. Spirochaetota can convert carbohydrates into volatile fatty acids (Yang *et al.* 2020a, 2020b; Borth *et al.* 2022). The relative abundance of Spirochaetota in BC25, BC50, BC75, and BC100 increased by 303.11, 271.79, 332.81, and 146.51%, respectively, compared with that in BC00. Synergistota plays an important role in the acidification process of anaerobic digestion (Park *et al.* 2016). The relative abundance of Synergistota in BC25, BC50, BC75, and BC100 increased by 146.15, 81.45, 88.69, and 375.57%, respectively, compared with that in BC00.

Bathyarchaeia, *Methanobacterium*, *Methanoculleus*, *Methanolinea*, *Methanomassiliicoccus*, *Methanomethylovorans*, *Methanomicrobium*, *Methanosaeta*, *Methanosarcina*, and *Methanospirillum* were dominant methanogenic archaea, and their relative abundances in different samples were 94.14% (BC00), 86.59% (BC25), 89.75% (BC50), 89.07% (BC75), and 89.45% (BC100), respectively. *Bathyarchaeia* is a multifunctional methanogenic archaeon that can promote the degradation of carbohydrates, proteins, volatile fatty acids, and methyl compounds and use H₂ and CO₂ to synthesize acetate and lactate (Khan *et al.* 2022). *Bathyarchaeia* could also enhance the activity of methyl coenzyme M and accelerate methanogenesis

(Li *et al.* 2021). The relative abundance of *Bathyarchaea* in BC25, BC50, BC75, and BC100 increased by 457.14, 361.68, 188.89, and 659.18%, respectively, compared with that in BC00. *Methanobacterium* (Jing *et al.* 2017), *Methanoculleus* (Dong *et al.* 2022), and *Methanospirillum* (Zhou *et al.* 2014) are hydrogenotrophic methanogenics that can consume H₂ with CO₂ or formic acid to produce methane. The relative abundances of *Methanobacterium*, *Methanoculleus*, and *Methanospirillum* in BC100 increased by 66.31, 1430.77, and 261.36%, respectively, compared with that in BC00. *Methanolinea* can produce methane primarily from H₂ and formic acid as substrates (Li *et al.* 2018b), and its relative abundances in BC100 increased by 58.13% compared with that in BC00. *Methanomassiliicoccus* is a methyl methanogenic bacterium that can produce methane primarily from methanol (or methylamine) with hydrogen as an electron donor (Becker *et al.* 2016). The relative abundances of *Methanomassiliicoccus* in BC100 increased by 112.90% compared with that in BC00. The addition of biochar increased the relative abundance of *Methanomicrobium* and *Methanosaeta*. In BC25, BC50, BC75, and BC100, the relative abundances of *Methanomicrobium* increased by 80.00, 60.00, 160.00, and 400.00%, respectively, compared with that in BC00. *Methanosaeta* could participate in the DIET by receiving electrons directly from bioelectric connections or by some conductive materials directly accepting electrons to produce methane (Zhao *et al.* 2018; Zhang *et al.* 2020). And the relative abundances of *Methanosaeta* increased by 14.68% compared with BC00.

BC-I represented various bacteria in initial inoculation sludge; BC00, BC25, BC50, BC75, and BC100 represented bacteria in mixed culture with biochar dosages of 0, 2.5, 5.0, 7.5, and 10.0 g/L, respectively.

3.2.4. Functional genes of PICRUSt metabolism

Figure 6 shows the four main functional genes of KEGG of archaea, including metabolism (71.26–75.49%), genetic information processing (20.90–24.73%), environmental information processing (1.33–1.78%), and cellular process (1.65–2.44%). The secondary metabolic pathways of metabolism mainly included amino acid metabolism, the metabolism of cofactors and vitamins, and carbohydrate metabolism. Moreover, the relative abundances of functional genes related to amino acid metabolism in BC25 and BC75 increased by 4.99 and 4.97%, respectively, compared with that in BC00. The relative abundances of genes related to cofactors and vitamin metabolism in BC25, BC50, BC75, and BC100 increased by 6.61, 11.72, 7.38, and 16.67%, respectively, compared with that in BC00. The relative abundances of genes related to carbohydrate metabolism in BC25, BC50, and BC100 increased by 3.49, 6.25, and 3.63%, respectively, compared with that in BC00. The secondary metabolic pathways of genetic information processing were mainly replication and repair, folding, sorting and

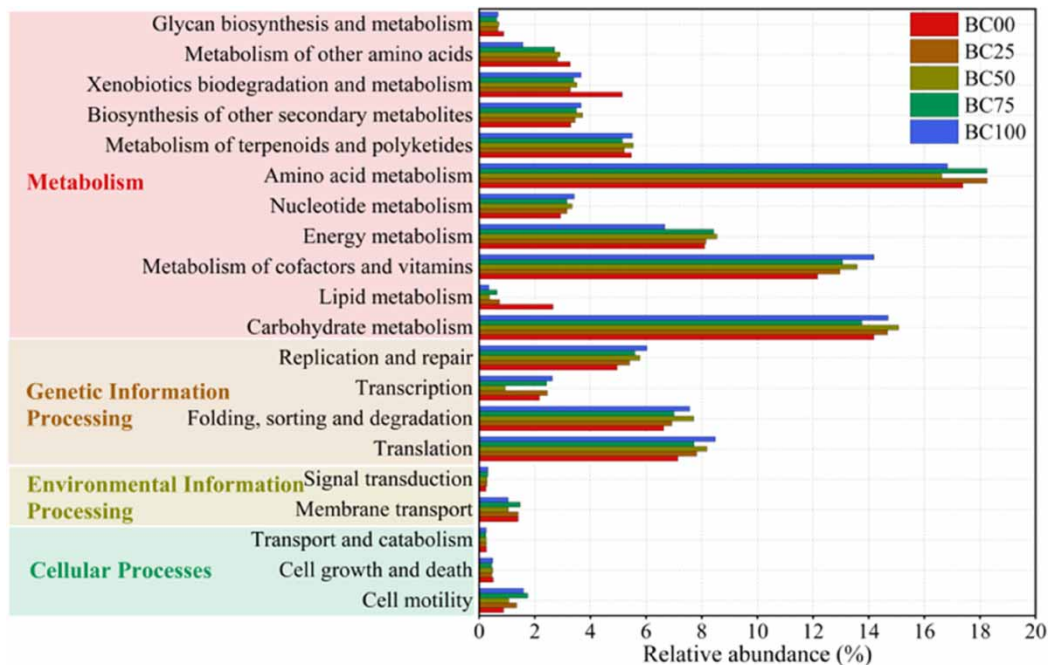


Figure 6 | Relative abundance of KEGG pathway level 2 in archaea.

degradation, and translation. The relative abundance of genes related to replication and repair in BC25, BC50, BC75, and BC100 increased by 8.93, 16.41, 12.80, and 21.54%, respectively, compared with that in BC00. The relative abundance of genes related to folding, sorting, and degradation in BC25, BC50, BC75, and BC100 increased by 4.41, 16.38, 5.65, and 14.16%, respectively, compared with that in BC00. The relative abundance of genes related to translation in BC25, BC50, BC75, and BC100 increased by 9.44, 14.59, 8.17, and 18.84%, respectively, compared with that in BC00. The secondary metabolic pathways of environmental information processing were mainly signal transduction and membrane transport. The relative abundance of genes related to signal transduction in BC25, BC50, BC75, and BC100 increased by 12.05, 12.99, 24.66, and 25.49%, respectively, compared with that in BC00. The relative abundance of genes related to membrane transport in BC75 increased by 5.14%, respectively, compared with that in BC00. Cellular processes are mainly involved cell motility, and the relative abundance of genes related to it in BC25, BC50, BC75, and BC100 increased by 54.18, 22.06, 99.55, and 82.00%, respectively, compared with that in BC00.

As carbohydrates and amino acids are the most important components of food waste, the differences in the relative abundances of functional genes related to carbohydrate metabolism and amino acid metabolism were investigated. It can be seen from Figure 7 that the relative abundances of genes related to C5-branched dibasic acid metabolism, pyruvate metabolism, citrate cycle (TCA cycle), and glycolysis/gluconeogenesis were highest in BC100, which were 11.38–26.27% higher than those in BC00. The relative abundances of genes related to histidine metabolism, lysine biosynthesis, and valine, leucine,

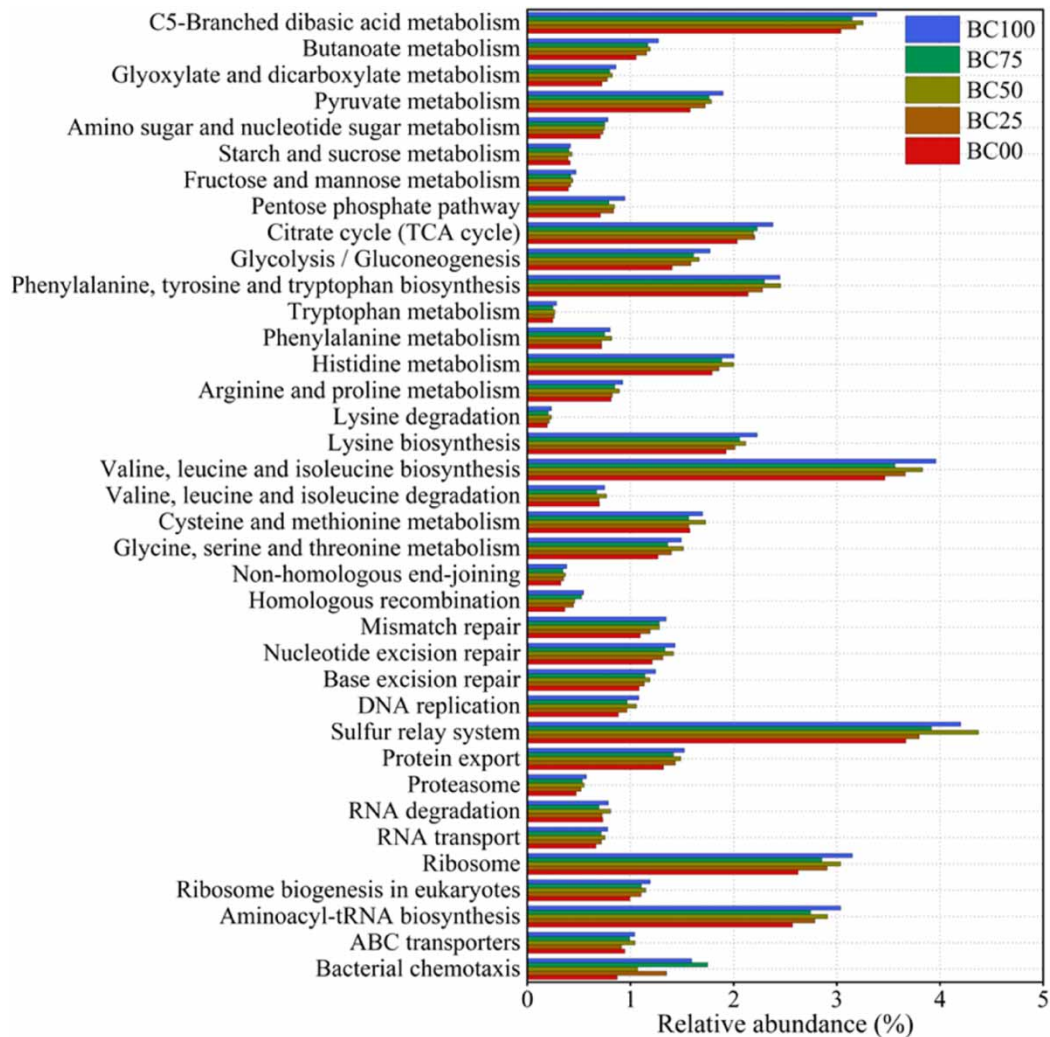


Figure 7 | Relative abundance of KEGG pathway level 3 in archaea.

and isoleucine biosynthesis were also highest in BC100, which were 11.96–15.71% higher than those in BC00. The relative abundance of genes related to phenylalanine, tyrosine, and tryptophan biosynthesis was highest in BC50, followed by BC100. The addition of biochar with 10 g/L increased the relative abundance of functional genes related to carbohydrate and amino acid metabolism. This may be related to *Bathyarchaeia*, which can increase the activity of methyl coenzyme M and promote the degradation of organic matter, such as carbohydrates and proteins (Li *et al.* 2021; Khan *et al.* 2022). Their abundance increased by 659.18% in the BC100 experimental group compared with the control group (Figure 5).

As the processing of genetic information and environmental information can ensure the transfer of information within or between species, the differences in the relative abundance of functional genes for replication and repair, folding, sorting and degradation, translation, membrane transport, and cell motility were analyzed. The relative abundances of genes related to mismatch repair, nucleotide excision repair, and base excision repair were highest in BC100, which were 14.76–22.76% higher than those in BC00. The relative abundances of genes related to the sulfur relay system and protein export were also highest in BC100, which were 14.47 and 15.40% higher than those in BC00, respectively. The relative abundances of genes related to ribosome and aminoacyl-tRNA biosynthesis were also highest in BC100, which were 19.95 and 18.15% higher than those in BC00, respectively. The relative abundance of ABC transporters was highest in BC50, followed by BC100, which was 10.44 and 10.02% higher than that in BC00, respectively. The relative abundance of genes related to bacterial chemotaxis was highest in BC75, followed by BC100, which were 100.76 and 83.09% higher than those in BC00, respectively. The results indicate that biochar addition with 10.0 g/L could promote metabolism and genetic information processing.

4. CONCLUSION

Biochar addition with different dosages could increase the cumulative methane production by 15.70–128.38%, with an optimal biochar dosage of 10.00 g/L. The methanogenesis performance was significantly improved due to the increase in major carbohydrate and amino acid metabolism and the selective enrichment of *Bathyarchaeia*, *Methanobacterium*, *Methanolinea*, *Methanoculleus*, and *Methanospirillum*. Meanwhile, it could also promote microbial replication and repair, folding, sorting, and degradation, ABC transporters, and bacterial chemotaxis by the microorganisms.

AUTHOR CONTRIBUTIONS

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by S.P., F.L., C.Q., J.L., and N.L.. The first draft of the manuscript was written by S.P., F.L., C.Q., and J.L., and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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