Effect of Biochar Addition on the Microbial Community and Methane Production in the Rapid Degradation Process of Corn Straw

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Abstract: Anaerobic digestion with corn straw faces the problems of difficult degradation, long fermentation time and acid accumulation in the high concentration of feedstocks. In order to speed up the process of methane production, corn straw treated with sodium hydroxide was used in thermophilic (50 °C) anaerobic digestion, and the effects of biochar addition on the performance of methane production and the microbial community were analyzed. The results showed that the cumulative methane production of all treatment groups reached over 75% of the theoretical methane yield in 7 days and the addition of 4% biochar increased the cumulative methane production by 6.75% compared to the control group. The addition of biochar also decreased the number of biogas and methane production peaks from 2 to 1, and had a positive effect on shortening the digestion start-up period and reducing the fluctuation of biogas production during the digestion process. The addition of 4% biochar increased the abundance of the bacterial family *Peptococcaceae* throughout the digestion period, promoting the hydrolysis rate of corn straw. The dominant archaeal genus *Methanosarcina* was significantly more abundant at the peak stage and the end of methane production with 4% biochar added compared to the control group.

Keywords: biochar; corn straw; anaerobic digestion; methane; microbial community

1. Introduction

In recent decades, the world has witnessed an alarming increase in the utilization of energy, rising from 8.5889 billion tons in 1995 to 13,147.3 million tons in 2015 [1]. The growing global demand for energy and the shortage of fossil fuel mineral reserves have recently promoted efforts for environmentally friendly renewable energy alternatives. Furthermore, some of the organic wastes produced in industry, agriculture and people's daily life have not been properly disposed of. The accumulation of these organic wastes needs to be paid attention to globally, and more effective and sustainable methods are needed to deal with these wastes, otherwise this will not only cause a great waste of resources, but also pose a potential threat to the ecological environment and human health [2]. Anaerobic digestion (AD) refers to a process of converting organic waste into methane and carbon dioxide, i.e., biogas, via a microbial process consisting of four steps: hydrolysis, acidogenesis (primary fermentation), acetogenesis (secondary fermentation),

and methanogenesis. Biogas is an important end product of anaerobic digestion with great utilization value, and it is a clean and environmental-friendly substitute for fossil fuel. It is estimated that biogas usage in the world will double in the coming years, rising from 14.5 gigawatts (GW) in 2012 to 29.5 GW in 2022 [3–5]. It is considered to be a clean and environmentally friendly method of recycling resources, reducing environmental pollution and mitigating greenhouse gas emissions from landfills [6].

Various kinds of organic materials, including food waste, municipal organic waste, sewage sludge and agricultural waste, have been utilized as feedstock in AD. Lignocellulosic biomass, as a sustainable source of natural carbohydrate polymers, is one of the most common substrates utilized in the AD process because of its availability and abundance [7]. Lignocellulosic materials have promising energy potential, and the content of cellulose and hemicellulose in its cell wall can reach 55% and 35%, which can be hydrolyzed by microorganisms to produce energy products such as biogas or biofuels [8]. However, due to the high C/N ratio and the special structure of lignocellulose, it is prone to volatile fatty acid (VFA) accumulation in the early stage of the relatively long duration of the AD process.

Several methods, such as co-digestion with nitrogen-rich feedstocks, the addition of urea and optimization of the F/I ratio, have proved to be beneficial to prevent acid inhibition and to shorten the digestion period [9–13]. VFA accumulation may also be mitigated by adding biochar to increase syntrophic action in an anaerobic reactor [14]. Biochar has several advantageous properties—high specific surface area, porosity, and cation exchange capacity (CEC) [15]—that make it an effective material for removing contaminants, and its addition in the AD process has been demonstrated to be capable of promoting the immobilization of microorganisms, the buffering capacity, cumulative CH_4 yields and direct interspecies electron transfer (DIET) between bacteria and methanogens [16–20]. Several possible explanations for these advantages of biochar, are that it can alleviate the acid inhibition caused by VFA accumulation at the early stage of digestion and enhance the organic acid utilization efficiency of microorganisms [19,21,22]. It is, however, difficult to implement the recycle of biochar in the anaerobic digestion process, leading to the economic infeasibility of introducing plentiful biochar into an AD engineering [23].

In order to improve the stability and shorten the digestion period of anaerobic digestion using corn straw as the only feedstock, this study examined a batch AD process using corn straw, with an F/I ratio (based on volatile solid) of 1 and varying amounts of biochar addition per working volume. The effects of biochar addition on CH_4 production, VFA concentration and microbial population distribution were studied in detail.

2. Materials and Methods

2.1. Experimental Materials

Biochar derived from corn straw was purchased from Guangzhou Yiyineng Biotechnology Co., Ltd. (Room C71, 3rd floor, 108 North Keyun Road, Guangzhou, China), Pyrolysis was performed in a charcoal furnace, heated to 500 °C at 8.5 °C per minute. The whole carbonization process took about 10 h. The BET surface area of biochar was 20.195 m²/g.

Corn straw (CS) was collected from farmland in Donghai county, Lianyungang city, Jiangsu province, China. It was first homogenized and then sifted through a 20-mesh sieve, pretreated by 0.5 MNaOH at room temperature for 6 h (ratio of solid to liquid was 1:10), washed with clean water until the washing solution was neutral, pressed and dried and stored in a refrigerator at 4 °C. The inoculum was originally taken from a long-running cow manure anaerobic digestion project on a dairy farm in Kaiping County, Jiangmen City, Guangdong Province, and was used after domestication in the laboratory. The total solid (TS) and VS of the inoculum were 5.19% and 3.68%, respectively (i.e., 70.91% VS of TS), and the pH was 7.90.

2.2. Setup and Design of Biochar-Amended Corn Straw Anaerobic Digestion Experiments

The experiments were designed to investigate the effects of biochar on corn straw mono-digestion and carried out with the self-assembled anaerobic digestion reactors.
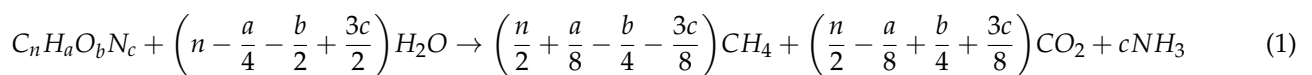
The reactor was a 500 mL glass bottle with a working volume of 350 mL. The agitation system of BPC Instruments was connected to the top of the reactor to provide a stirring function, while the biogas was collected through a silicone tube, three-way valve and 2 L AnaeroPack. The ratio of C/N in the substrates was adjusted to 25 by adding urea and pretreated corn straw in proportion, and the TS concentration of the AD system was 8% with a $VS_{inoculum}/VS_{substrate}$ of 1. Different amounts of biochar per working volume (*w/v*) were added for studying the effects of biochar on the AD process of CS. The groups included the control group (0%), the A group (1%), the B group (2%) and the C group (4%). In addition, the blank control group containing only inoculum was set up, and the biogas (biomethane) produced by the blank group was subtracted from the results of other groups. The substrates, inoculum and biochar were mixed evenly and filled with nitrogen for 5 min to guarantee anaerobic conditions. Throughout the AD period, the temperature of the digesters was maintained by a water-bath pot at $50 \pm 1°C$. When the biogas production was less than 1% of the cumulative biogas yield for three consecutive days, that point was taken as the end of the digestion, so the digestion period was 10 days. All the experiments were conducted in triplicate. Samples of biogas and liquid from digesters were collected periodically until the AD process ceased.

2.3. Analytical Methods

Characterization and compositional analysis of the corn straw was performed before the digestion began. The TS, VS and ash content were determined by the standard methods [24]. Elemental analysis (C, H and N) was performed using the Vario EL cube. The volume of biogas in each reactor was measured by releasing the biogas pressure with 100-mL glass syringes and the gas composition was analyzed with a gas chromatograph (GC; Agilent 7890A, Agilent Technologies, Inc., Wood Dale, IL, USA). The measured biogas and methane production was corrected to volumetric production under STP conditions (273 K and 1 atm pressure) according to the ideal gas law [25]. A 5-mL liquid sample was collected on Days 0, 1, 3, 5, 7 and 10 for liquid analysis. The collected digestate samples were centrifuged at 12,000 rpm for 10 min and subsequently filtered with a 0.45-μm membrane filter to analyze the soluble chemical oxygen demand (SCOD) and individual VFAs. SCOD was analyzed by a DR-1900 spectrophotometer (HACH, Loveland, Colorado, USA). VFAs was analyzed with a high-performance liquid chromatograph (GC9790 Plus, Zhejiang Fuli Analytical Instruments Co., Ltd, Zhejiang, China).

2.4. Theoretical Methane Production

The theoretical methane production (TMP, mL/g-VS) of corn straw was calculated according to Equations (1) and (2) below [26]:

$$C_nH_aO_bN_c + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3c}{2}\right)H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right)CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8}\right)CO_2 + cNH_3 \qquad (1)$$

$$TMMY = \frac{22.4 \times 1000 \times \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right)}{12n + a + 16b + 14c} \qquad (2)$$

2.5. Kinetic Study

The modified Gompertz model has been widely used for the kinetic analysis of methane production process and has been proved to fit the process well [27], which can be described as follows [28]:

$$M_{(t)} = M_{max} \exp\left\{-\exp\left[\frac{R_{max} \times e}{M_{max}} \times (\lambda - t) + 1\right]\right\} \qquad (3)$$

where $M_{(t)}$ represents experimental specific methane yield (mL/gVS) for digestion time t (day); M_{max} represents the simulative specific methane yield (mL/gVS); Rmax represents the simulative maximum daily methane yield (mL/gVS/day); λ represents the

simulative lag phase (day) of each group; e represents the natural logarithm: value, approximately 2.718.

2.6. High-Throughput Sequencing of Bacterial and Archaeal Communities

Samples centrifuged at 120,000 rpm for 10 min on Days 0, 1, 3 and 10 were stored at −80 °C prior to total genomic DNA extraction. Concentration and purity of extracted DNA were determined with TBS-380 and NanoDrop2000, respectively. DNA extract quality was checked on 1% agarose gel. High-throughput DNA sequencing was performed on an Illumina MiSeq (San Diego, CA, USA).

2.7. Statistical Analysis

The average and standard deviation of the experimental data are calculated by using the solver function of Microsoft Excel. The kinetic parameters of modified Gompertz model were simulated by Origin 8.0 software.

3. Results and Discussion

3.1. Characteristics of Corn Straw

The TS and VS of unpretreated CS were 94.17 and 86.62, respectively (i.e., 91.98% VS of TS), while the TS and VS of pretreated CS were 34.47 and 33.40, respectively (i.e., 96.9% VS of TS), indicating that CS was high in organic content but that NaOH pretreatment could increase the content of organic matter relative to TS in the raw materials. The cellulose, hemicellulose and lignin content of CS is shown in Table 1. The theoretical methane production of pretreated CS was 206 mL/VS. The contents of cellulose and hemicellulose increased by 41.44% and 14.65% respectively, while the content of lignin decreased by 23.47% after NaOH pretreatment. The removal of lignin is believed to be beneficial to increasing the yield of methane; [29] reported that the methane yield of CS pretreated with 8% NaOH could be increased to 188.7 mL/g VS, 84.2% higher than that of untreated raw materials. SEM images of surface scans for raw and pretreated corn straw are shown in Figure 1. In the untreated image, the surface of the raw feedstocks is hard and smooth. In the image of pretreated materials, it can be observed that the surface crystallinity decreased and the cellulose crystallinity transformed from a smooth rigid structure to a messier condition with a broken and rough surface. Other research [30,31] has previously pointed out that the crystallinity of cellulose can affect the adsorption effect of cellulase components cellobiose hydrolase Cel7A.

Table 1. Characteristics of corn straw used for anaerobic digestion.

Parameters	Raw Corn Straw	Pretreated Corn Straw
TS (%, WM)	94.17	34.47
VS (%, WM)	86.62	33.40
C (%, TS)	43.58	43.01
H (%, TS)	6.02	6.38
O (%, TS)	35.91	47.11
N (%, TS)	1.11	0.41
Cellulose (%, TS)	30.91	43.72
Hemicellulose (%, TS)	22.53	25.83
Lignin (%, TS)	19.43	14.87
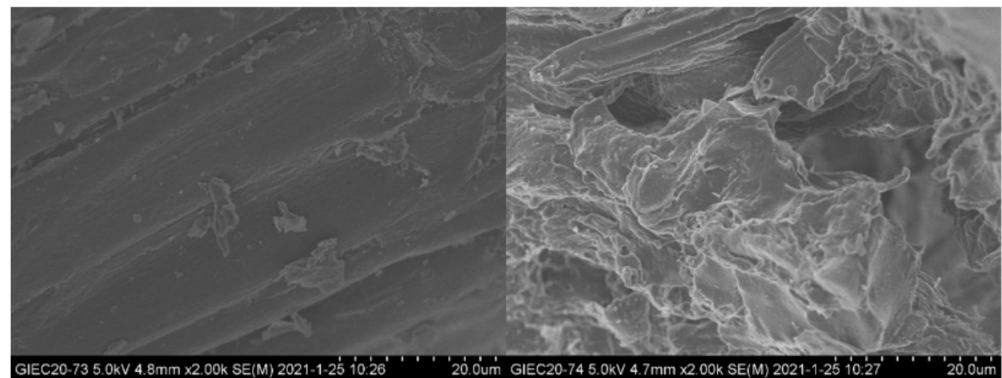
Figure 1. SEM images of raw corn straw (CS) and pretreated CS.

3.2. Biomethanization Performance

Cumulative biogas/methane production and daily methane production rate are shown in Figure 2. Notably, the short digestion period of 10 days indicated a rather rapid degradation of CS when compared to previous studies [32], where the digestion period of corn straw was 60 days and the methane production rate decreased significantly after 15 days with the F/I ratio of 2 (TS-based). Moreover, the cumulative methane production of all treatment groups reached over 75% of the theoretical methane yield in 7 days, indicating that the methane production potential of CS was released to a great extent. This might be because the high inoculum (F/I ratio of 1, VS-based) guaranteed a higher utilization rate of SC by microorganisms. The ultimate methane production amounts in the 0%, 1%, 2% and 4% groups were in the range of 155.51–167.00 mL-CH_4/g VS added. Compared with the control group, the cumulative methane yield increased by only 4.21% and 6.75% in the 1% and 4% groups, respectively, while it decreased by 0.6% in the 2% group—low enough to be negligible. Interestingly, although the addition of biochar did result in a slight increase in the methane yield, it did not seem to be as significant as the improvement reported in a previous study [33], possibly because of the various metal elements released from the biochar, other characteristics of the biochar, or an insufficient dosage of biochar in our research. A 1% addition of biochar has been proved to be able to cause a two-fold increase in methane production when compared with the control group and is beneficial for maintaining syntrophy in the anaerobic digestion process [34]. Another reason for the different effect of biochar addition may be that the improvement of the biodegradability of corn straw by NaOH pretreatment and a relatively suitable digestion environment guaranteed the rapid biodegradation of feedstocks in different groups. However, with an increase in the amount of added biochar, the number of peaks of biogas and methane production decreased from 2 to 1, and the biomethanization peak was advanced from the 4th day (0% treatment) to the 3rd day (1%, 2% and 4% groups). The results calculated using the modified Gompertz equation for methane production are shown in Table 2. Compared with the 0% group, the addition of biochar increased Rmax from 48.08 mL/d to 56.58 mL/d (1%), 61.65 mL/d (2%) and 65.37 mL/d (4%), respectively. Moreover, although the addition of biochar led to the advance of the biomethanization peak, as far as the fitting results were concerned, the addition of biochar increased the lag time (λ) of methanogenesis in varying degrees.
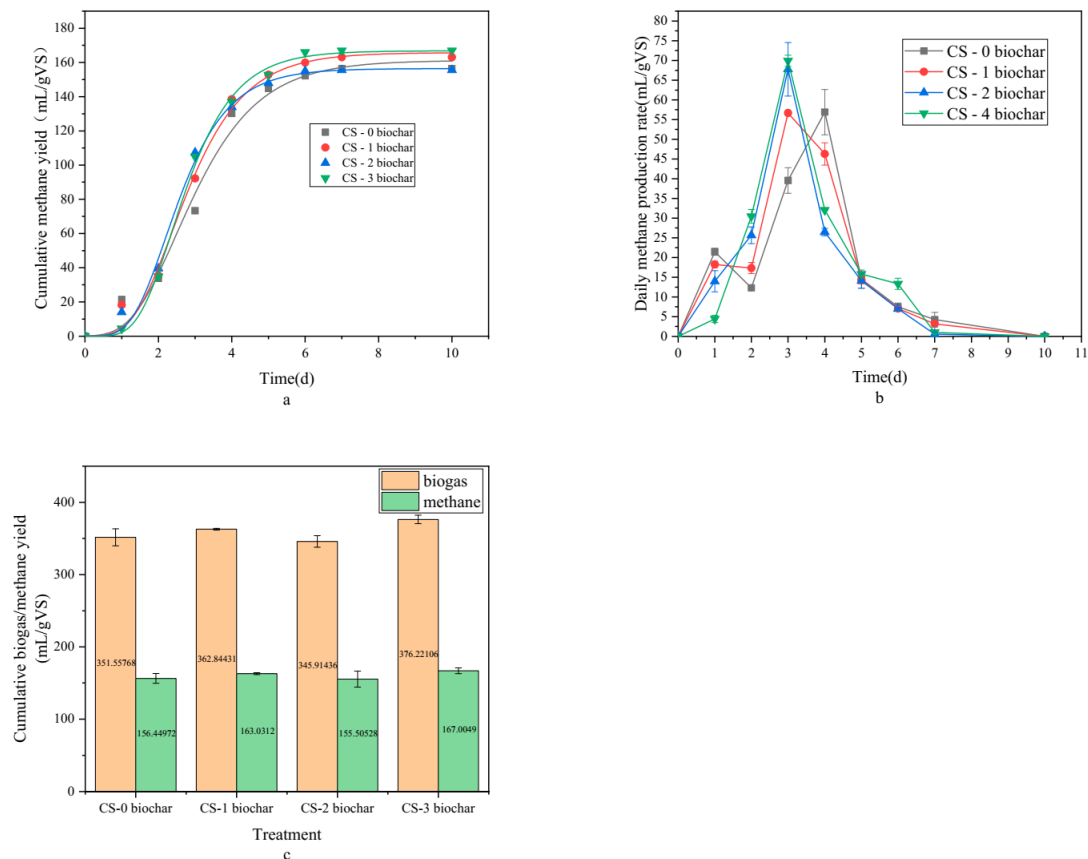
Figure 2. (**a**) Cumulative methane yield and the fitting curve using the modified Gompertz model; (**b**) daily methane production rate; (**c**) ultimate biogas/methane production, during anaerobic digestion.

Table 2. Calculated results using the modified Gompertz equation for methanogenesis in different treatments.

Treatments	Rmax (mL·d^{-1})	λ (d)	Mmax (mLCH$_4$)	Ultimate CH$_4$ Yield (mL)	R2
0%	48.07616 ± 7.00503	1.25275 ± 0.2469	161.19639 ± 6.57806	156.45	0.98048
1%	56.57962 ± 6.09878	1.29596 ± 0.16176	165.72529 ± 4.29053	163.03	0.99076
2%	61.65405 ± 5.93145	1.26393 ± 0.12675	156.39771 ± 3.04981	155.51	0.99375
4%	65.37325 ± 3.94515	1.43229 ± 0.07937	166.78352 ± 2.13146	167.00	0.99761

The pH varied from 7.0 to 8.3 and no acidified phenomena were observed over all the treatments. There were evident differences in the SCOD and pH among different treatment groups in the first two days. With the increase of the dosage of biochar, the reduction of pH decreased at the incipient stage of AD, as did the dissolution of COD. For the profiles of VFA concentrations, acetic acid, propionic acid and butyric acid were the main VFAs produced in the digestion process (Table 3). The VFAs on the first day varied significantly among the different groups: 7275.90 mg/L (0%), 6442.53 mg/L (1%), 5368.36 mg/L (2%) and 3653.62 mg/L (4%), mainly reflected in the content of acetic acid. At the same time, the addition of biochar inhibited the formation of butyric acid, but had no obvious effect on the content of propionic acid. A sharp decrease in the VFAs was witnessed from Day 3 to Day 5 while propionic acid accumulated and became dominant. VFAs were further utilized from Day 5 to Day 7. A previous study showed a similar trend of VFA degradation, where the VFA concentrations, consisting mainly of acetic acid, butyric acid and propionic acid, were high on Day 0, and both acetic acid and butyric acid were almost completely consumed between Day 7 and Day 30 while propionic acid remained [35,36].

Table 3. Volatile fatty acid (VFA) concentration in different treatments during anaerobic digestion (AD).

Treatments	Time (d)	Acetic	Propionic	Butyric	VFAs
0%	1	6699.73	419.13	157.03	7275.90
	3	4723.80	947.08	486.55	6157.43
	5	162.51	1150.72	ND	1313.24
	7	80.53	98.53	ND	237.28
	10	81.50	139.52	ND	338.50
1%	1	5894.01	470.53	77.98	6442.53
	3	5536.27	1055.42	613.10	7204.79
	5	119.66	1125.03	ND	1244.68
	7	ND	136.66	ND	188.40
	10	ND	133.73	ND	224.83
2%	1	4900.81	467.56	ND	5368.36
	3	3539.47	1046.33	342.04	4927.83
	5	122.53	1080.15	ND	1261.34
	7	89.63	142.36	ND	306.34
	10	ND	121.14	ND	181.32
4%	1	3178.53	475.09	ND	3653.62
	3	4240.03	1397.01	369.73	6006.76
	5	160.18	1107.15	ND	1267.33
	7	82.90	143.23	ND	316.81
	10	88.40	129.70	ND	288.36

ND: under the detected value.

The differences in the gas production performance of different treatment groups in the initial stage could be explained by the pH value, SCOD and VFAs changes. The results showed that the addition of biochar alleviated the disturbance of the hydrolysis process to the liquid environment of the reactor at the initial stage of digestion, so that the accumulation trend of SCOD and VFAs decreased obviously, and the pH value of the system was relatively stable. Compared with the phenomenon whereby gas production and methane production in the 0% and 1% groups increased first and then decreased in the first three days, the higher addition of biochar made the production and utilization process of the digestion intermediate, like VFAs, relatively coordinated, and the smaller environmental fluctuation provided favorable conditions for the growth and reproduction of microorganisms, especially methanogenic bacteria, thus making the peak time of methane production one day earlier and the maximum daily methane production higher. In conclusion, the results indicated that the addition of biochar had a positive influence on reducing the fluctuation of biogas production during the digestion process and advancing the time of peak methane production.

3.3. Microbial Community Characteristics Analysis

High-throughput sequencing of bacterial and archaeal communities was employed to investigate the microbial community structures and dynamics in the AD systems. Four samples were collected for each group at Day 0 (inoculum), Day 1 (hydrolytic acidification stage), Day 3 (main methane production stage) and Day 10 (the end of the AD process). An average of 51,107,418.9 clean reads were obtained per sample, accounting for an average of 98.06% of raw reads.

The distribution of bacteria at the phylum level is shown in Figure 3. Similar bacterial phyla compositions were observed among the four treatment groups but the relative abundance of each phyla varied in time and treatments. *Firmicutes*, *Proteobacteria*, *Chloroflexi* and *Bacteroidetes* were the four major phyla found throughout the AD process for all treatments, covering over 80% of the bacterial community in all experimental samples. The four predominant phyla were mainly hydrolytic bacteria, although these showed different distributions during AD. For the 0% treatment, with the progress of digestion, the abundance of *Firmucutes* was relatively high from Day 1 (84.03%) to Day 3 (77.52%), but decreased significantly to 48.48% on Day 10, a result positively correlated with methane

production, while those of *Proteobacteria*, *Chloroflexi* and *Synergistetes* increased from 3.2%, 2% and 0.93% to 8.34%, 12.02% and 6.5% from Day 1 to 10, respectively. Moreover, the abundance of *Bacteroidetes* decreased on Day 3 and then increased on Day 10. As a result, a higher methane production rate was achieved near Day 3, at which time the methanogens effectively consumed the intermediate products of hydrolysis, such as acetate. A similar microbial dynamic was reported by Wachemo et al. [37], who found that the abundance of Firmicutes and *Bacteroidetes* increased and decreased, respectively, when the digestion process approached its biomethanization peak stage. Notably, the abundance of the main acidifying bacteria including *Firmicutes*, *Proteobacteria*, *Chloroflexi* and *Bacteroidetes* decreased from Day 3 to Day 10. The same trend has been found by Pan, S.Y. et al. [38] and was explained as the result of substrate being continuously consumed in the process of AD and the bacteria functioning in hydrolysis and acidogenesis are gradually replaced by other bacteria such as *Actinobacteria* and *Cloacimonetes*. Compared with the control group, the abundance of *Firmucutes* was relatively low at the initial stage of fermentation, gradually increased on Day 3 and exceeded that of the control group at the end of digestion, indicating that the addition of 4% biochar can alleviate the rapid release of VFAs at the initial stage of digestion to prevent acidification of the system, promote the hydrolysis of straw and increase the cumulative methane yield by the end of the AD period. The relative differences in the bacterial composition were studied by principal coordinates analysis (PcoA) and the results are shown in Figure 4. A highly similar community dynamics among the 0%, 1% and 2% groups was identified at the beginning of the experiment (Day 1), while the 4% group shifted away from the others. Furthermore, the bacteria differed in different groups at the end of digestion (Day 10) but showed similar community dynamics at the biomethanization peak (Day 3).
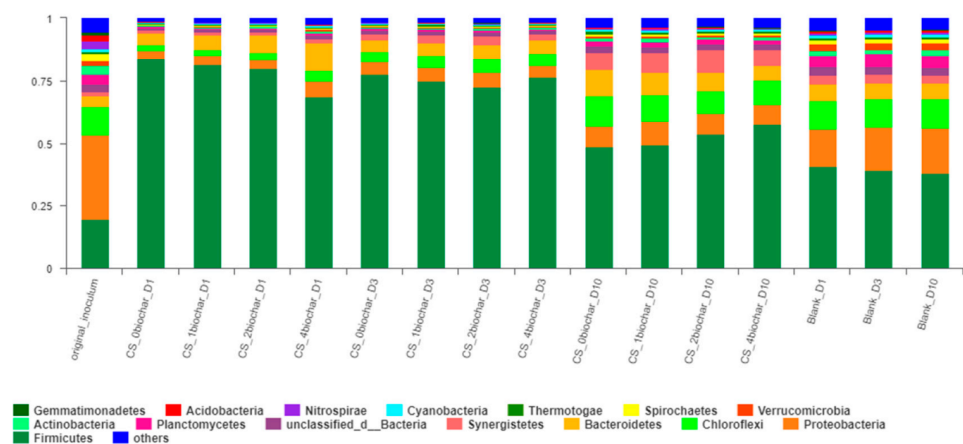

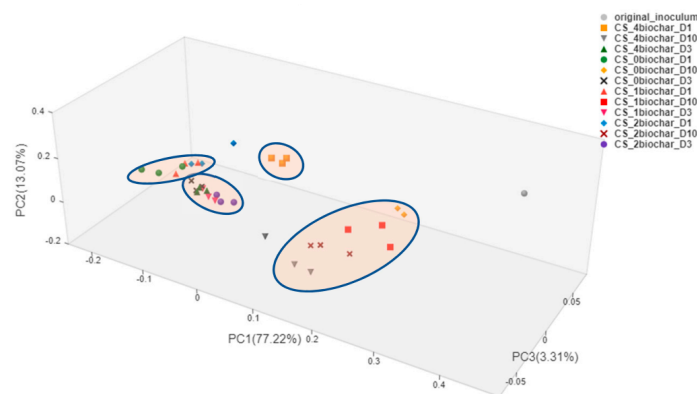**Figure 3.** Taxonomic distribution of bacteria at phylum level.

Figure 4. 3D-Principal coordinates analysis (PCoA) biplots of Bray-Curtis distances of bacteria during AD.

The most abundant four archaea genera, accounting for over 70% of the total archaeal genera, were *Methanosarcina*, *Methanoculleus*, *Methanosaeta* and *Methanobacterium*, and *Methanosarcina* was the most abundant methanogen during the whole AD process in all treatment groups, as shown in Figure 5. *Methanosarcina*, which is capable of utilizing Methanol, Methylamine, H_2 and acetate for methane production [39], doubled from Day 1 to Day 10 in all groups. Moreover, the addition of biochar increased the abundance of *Methanosarcina* on Day 3, which may explain why the biomethanization peak was advanced from the 4th day (0% treatment) to the 3rd day (1%, 2%, 4% groups), for its multiple utilization of methanogenic substrate and effective consumption of the VFAs released during the previous stage [38]. *Methanoculleus* can use formic acid for growth, reproduction and methane production, while Methanosaeta is a highly efficient and specific acetotrophic methanogen. They both decreased gradually with the process of digestion, possibly due to competition from *Methanosarcina*. Hydrogenotrophic methanogen *Methanobacterium* increased during the digestion process, indicating that the methanogenic pathway is gradually transformed from utilizing acetic acid to hydrogen and carbon dioxide as the main substrates. The PcoA results of archaea genera is displayed in Figure 6 A rapid transformation in the microbial community structure from the initial inoculum was observed in each treatment group while the digestion proceeded, showing different community distribution on the 1st, 3rd and 10th day, but all the treatment groups shared a similar distribution of archaea during the same stage of AD process.
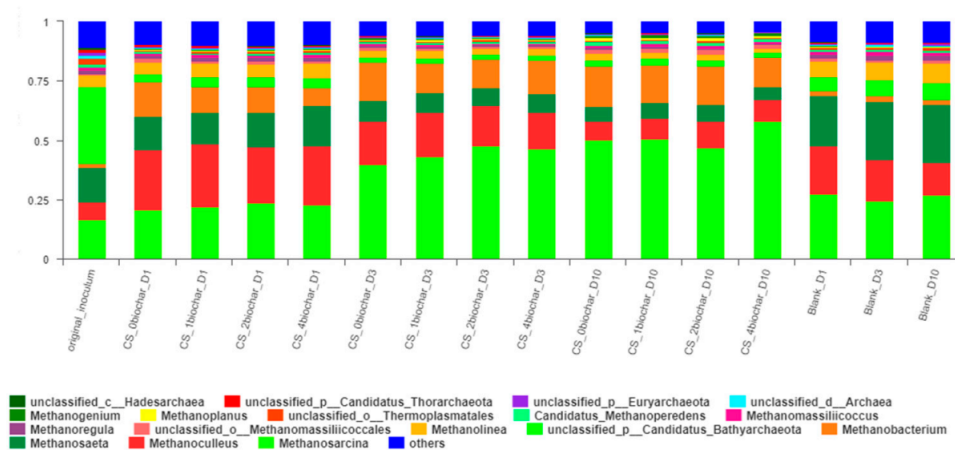

Figure 5. Taxonomic distribution of archaea at genus level.
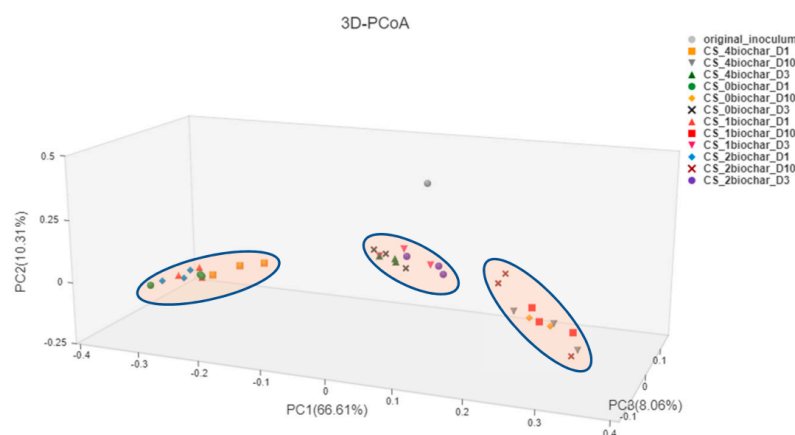

Figure 6. 3D-Principal coordinates analysis (PCoA) biplots of Bray-Curtis distances of archaea during AD.

To further explain the influence and the possible mechanism of biochar addition on the bacterial communities during the AD process in detail, the relative abundance of bacteria at

family level was calculated and the results are shown in Figure 7. *Ruminococcaceae*, *Peptococcaceae*, *Clostridiaceae*, *Defluviitaleaceae* and *Lachnospiraceae* were the five main families in the *Firmucutes* phylum. *Anaerolineaceae*, *Synergistaceae* and *Marinilabiliaceae* were the main families in the *Chloroflexi*, *Synergistetes* and *Bacteroidetes* phyla, respectively. *Ruminococcaceae* and *Defluviitaleaceae* are the main bacteria that decompose cellulose, which is a macromolecular substance that is easy to be hydrolyzed in the process of anaerobic digestion. Both their abundance in the 4% group on the first day was less than that of the control group, which may be one of the reasons why the addition of biochar reduces the growth of SCOD and VFAs at the initial stage of digestion. Moreover, *Defluviitaleaceae*, the abundance of which remained relatively high from Day 1 to Day 3 but decreased to nearly 0% on Day 10, seemed to be positively correlated with methane production. The abundance of *Marinilabiliaceae*, which can utilize hemicellulose as its sole carbon source and whose final product is mainly propionic acid, increased from 2.47–6.94% on Day 1 from treatments 0–4%, indicating that the addition of biochar may have a positive influence on hemicellulose biodegradation at the beginning of the anaerobic digestion period. Promoting DIET between bacteria and methanogens is an important effect of biochar addition in anaerobic digestion process. Notably, the abundance of *Peptococcaceae* increased with the process of digestion and the addition of biochar and DIET between *Peptococcaceae* and *Methanosarcina* was beneficial to their growth and metabolism during the digestion process, which could be the reason of the higher maximum methane production with biochar addition.
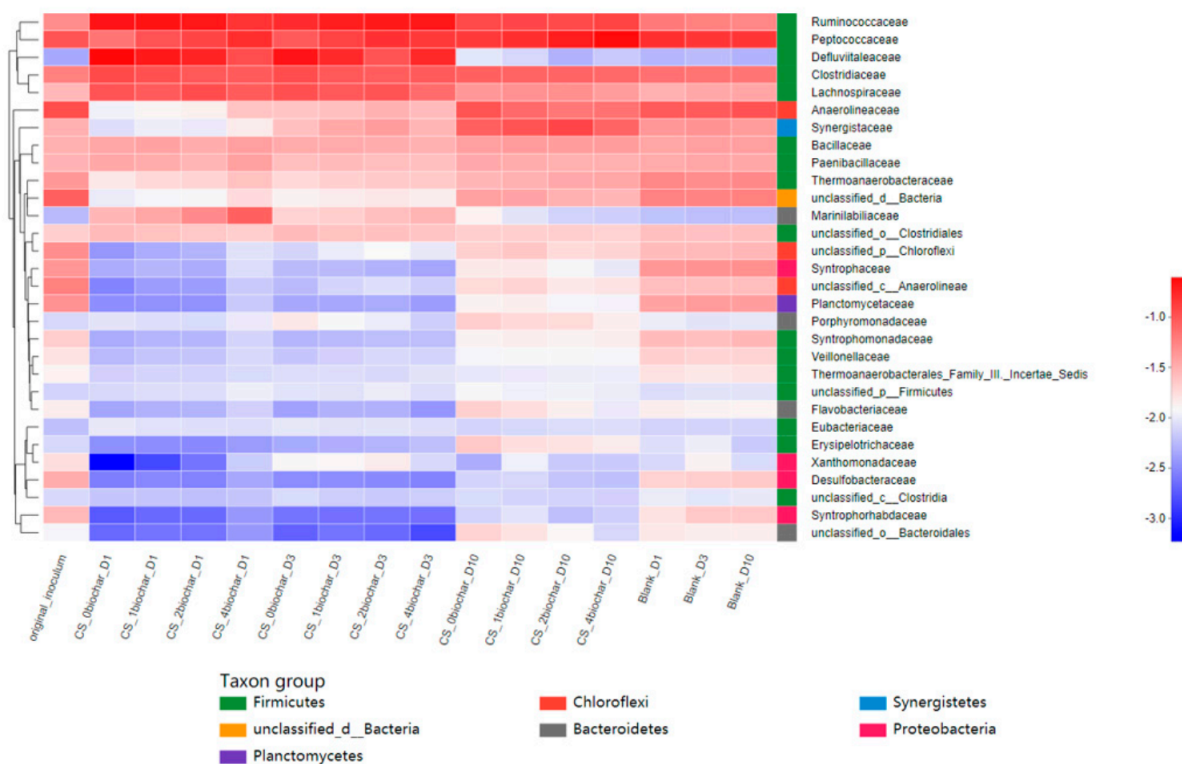

Figure 7. Heat map of sequence distribution bacteria at the family level.

4. Conclusions

In this work, a batch digestion test using corn straw was performed for 10 days. The highest final biogas and methane yields from CS were 376.22 and 167.00 mL/g VS, respectively. The microbial community structures were markedly changed at every critical stage but lacked significant differences among the groups. For bacteria, *Firmicutes Proteobacteria*, *Chloroflexi* and *Bacteroidetes* were the major phyla. From Day 1 to Day 10, the abundance of Firmicutes decreased while the abundance of *Proteobacteria* and *Chloroflexi* increased. For archaea, *Methanosarcina*, *Methanoculleus*, *Methanosaeta* and *Methanobacterium*

were the predominant genera during AD. *Methanosarcina* and *Methanobacterium* displayed a successive increase during the digestion period while *Methanoculleus* and *Methanosaeta* decreased. Moreover, the addition of biochar decreased the abundance of *Firmucutes* at the early stage of AD but promoted *Firmucutes* and *Methanosarcina* from Day 3 to Day 10. This study suggests that the addition of biochar has a positive effect on advancing the time of peak methane production and reducing the fluctuation of biogas production during the digestion process. The purpose of this paper is to provide a relatively simple energy utilization mode of corn straw, and in the further studies, the TS of the reactor will be gradually increased to 12% and 16% to explore the changes and possible problems in the process of gradually reducing the production of waste liquid in the digestion process.

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