Effect of Biochar on Methane Production and Structural Characteristics in the Anaerobic Digestion (AD) of Rape Straw

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This study investigated the mesophilic and thermophilic anaerobic fermentation of rape straw with biochar addition. The effects of biochar on the biogas yield, degradation of lignocellulose, bacterial community, and crystallinity were explored. The results showed that the biogas yield and methane content increased as the biochar concentration was increased. The biochar concentration of 5.0% resulted in a high biogas yield in mesophilic and thermophilic anaerobic digestion at 142.2 mL/g and 193.5 mL/g, respectively, which were 40.5% and 21.0% improvements compared with the control. The corresponding methane contents were 59.4% and 57.0%, respectively. For the lignocellulose degradation, the cellulose content in the mesophilic AD decreased from 54.0% in the pretreated rape straw to between 18.7% and 25.0%. The microbial community results showed that as the biochar concentration was increased, the relative abundance of *Firmicutes* initially increased before it decreased. Among the microbial community results, the relative abundances of *Firmicutes* and *Bacteroides* in the biogas residue of the mesophilic anaerobic digestion were the highest in the biogas residue with the 5.0% biochar concentration sample in the mesophilic AD, at 27.06% and 39.20%, respectively. This result revealed the mechanism of biochar to improve the biogas production of rape straw in anaerobic fermentation.

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Keywords: Rape straw; Biochar; Biogas yield; Microbial community; Crystallinity

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INTRODUCTION

Biomass energy is an important energy source to replace fossil fuels. Biogas, hydrogen, and pyrolysis syngas are biologically and chemically produced (Elsayed *et al.* 2018). Anaerobic digestion (AD) for biogas production from lignocellulosic materials is a very mature technical method. Lignocellulose is the most abundant, widely sourced, and renewable resource in nature (Xu *et al.* 2022). Lignocellulosic biomass (rice straw, wheat straw, and rape straw, *etc*.) is comprised of 32% to 47% cellulose, 19% to 27% hemicellulose, and 5% to 24% lignin. However, biogas production is unstable due to the complex fermentation conditions. Thus, cellulose and hemicellulose utilization efficiency are relatively low (Li *et al.* 2018).

A new electron transfer process was discovered in the anaerobic methanogenesis system, namely, direct interspecies electron transfer (DIET). Microorganisms directly exchange electrons through nano-pili, cytochromes, and conductive particles (Zhao *et al.*

2015). DIET is efficient and synergistic with interspecies hydrogen transfer for methane production. Therefore, the DIET pathway has received a great deal of interest (Kang *et al.* 2021; Zhang *et al.* 2019).

Conductive materials promote the construction of DIET and increase the methane production rate of mutual metabolic, such as magnetite, biochar, zero-valent iron, carbon cloth, *etc*. (Pan *et al.* 2019; Gahlot *et al.* 2020; Xu *et al.* 2020; Cerrillo *et al.* 2021). Ma *et al.* (2021) investigated the effect of magnetite on AD treating saline wastewater and found that the addition of magnetite improved the methane yield by 16.5% to 36.3%. In the anaerobic digestion for methane production with the additive biochar, the microorganisms can attach to the surface of biochar, relying on its high conductivity and surface area to achieve the electron and energy exchange for enhancing the methane production. The activated carbon, biochar, carbon fiber cloth, and single-walled carbon nanotubes can promote the DIET process in anaerobic fermentation of biomass. Moreover, the feedstock and carbonization temperature can affect the characteristics of biochar, including pH, surface area, and electrical conductivity (Zhao *et al.* 2015).

Park *et al.* (2018) analyzed the methanogenesis behavior of activated carbon in promoting microbial DIET from the perspective of metagenomic, and the methane production increased by 30 to 70%. The 16S rDNA sequencing analysis found that the proportion of *Methanosarcina* decreased, and the proportion of *Methanosaeta* increased. *Geobacter* strains were analyzed from metagenomic analysis. Both *pila* and *omcS* gene abundances were greatly reduced, indicating that additional methane was obtained by the carbon dioxide reduction pathway caused by direct interspecies electron transfer. Therefore, biochar played an important role in process performance and enhancing biogas production. To date, there have been limited studies on microbial communities and cellulose crystallinity. It is necessary to explore the relationship between biogas production, microbial flora, and cellulose crystallinity.

This study used biochar to promote the conversion efficiency of fiber degradation and biogas yield of rape straw in AD. The biochar was used to enhance a direct interspecies electron transfer process, improve the microbial community structure, promote the metabolism of volatile fatty acids, and form a stable anaerobic fermentation system for biogas production. The effect of biochar concentration on biogas production, methane content, and lignocellulose degradation were investigated. The changes in the microbial community structure, crystallinity, and apparent structure during the anaerobic fermentation were analyzed to reveal the effect of biochar on the biogas yield and fiber degradation of rape straw.

EXPERIMENTAL

Materials

Rape straw was collected in a suburb of Huaian, China. The straw was air-dried, ground in a hammer mill, and passed through a 20-mesh standard screen. The ground straw was sealed in plastic bags and stored at room temperature. The inoculum sludge was taken from a 5,000 m^3 anaerobic digester, which used swine manure and wheat straw as substrate and operated for two years.

The following feedstock characteristics were based on weight percentages. The total solids (TS) and volatile solids (VS) of the rape straw were 88.0% and 90.9%, respectively. The rape straw was composed of 48.9% cellulose, 12.6% hemicellulose, and

11.6% lignin. The TS and VS of the sludge were 6.9% and 68.6%, respectively. The sludge was composed of 31.3% cellulose, 15.2% hemicellulose, and 22.4% lignin. Biochar was obtained from fixed pyrolysis reactor with only cattle manure at 550 °C. The biochar was ground and passed through a 100-mesh standard sieve. The pH of biochar was 10.35, and the electrical conductivity (EC) value 8150 μS/cm. The BET (Brunauer, Emmet and Teller) surface area was 3.28 m^2/g.

Anaerobic Digestion

Before AD, the rape straw was pretreated with sodium hydroxide of 1% concentration (w/w). The temperature, solids loading, and time of the pretreatment were 60 ℃, 10% (w/w), and 48 h, respectively. After the pretreatment, the solid sample was watered using 1 L of deionised water and dried at 105 ℃. The pretreated rape straw was stored in plastic bags at room temperature.

To study the effect of the biochar concentration on the biogas yield, the lignocellulose degradation, the crystallinity, the surface structure of rape straw, and the microbial community structure during the AD, the biochar concentration was set to 0, 2.5%, 5.0%, 7.5%, and 10.0% based on total solids. A 0.5 L distillation flask was used as a batch anaerobic digestion reactor. Eight grams of pretreated rape straw were added to 240 g of fresh sludge and 150 mL of deionised water. The working volume and total solid concentration were 0.4 L and 6%, respectively. To avoid the acidification of bioreactor systems, $NaHCO_3$ was added with 1.0 g/L in each run. All reactors were capped with rubber stoppers and put into water bath at 37 °C (mesophilic AD) and 55 °C (thermophilic AD), respectively. Prior to the AD test, the reactors were flushed with nitrogen to remove oxygen from the headspace and maintain an anaerobic environment. To minimize the effect of random errors, each run was duplicated, and the average data was reported. The biogas volume was determined by the drainage method, and the biogas was collected by a 1 mL syringe for the gas component analysis. The biogas production and methane content were measured at 24 h intervals.

Analytical Methods

The TS and VS were measured according to standard methods (APHA 1998). The samples were heated overnight at 105 °C to determine the TS and burned at 550 °C to determine the VS. The fiber composition was measured as described (Sluiter *et al.* 2008). The methane content in the biogas was determined by gas chromatography (GC) (SP-7860; Nuoxi, Shanghai, China) equipped with a thermal conductivity detector (TCD) and a stainless-steel packed column. The temperatures of the injector, detector, and oven were maintained at 100, 100, and 130 °C, respectively. Nitrogen was the carrier gas. The average methane content was calculated by the average of all methane content in whole fermentation process.

The microbial diversity of the fermentation liquor was analyzed. A total of 10 mL of fermentation liquor was sampled. After extracting the genomic DNA with D3142 kit (Shanghai MEIJI Biotechnology Co., Shanghai, China), the 16S rDNA was amplified with barcode-specific primers, 341F (CCTACGGGNGGCWGCA) and 806R (GGACTACHV-GGGTATCTAAT). The purified amplified product (namely amplicon) was connected to the sequencing adapter to construct a sequencing library and sequenced on Illumina.

X-ray diffraction was conducted using a Bruker D8 Advance (Karlsruhe, Germany) device. Scans for rape straw and biogas residues were collected from 5 to 60° (2θ scale) using Cu Kα radiation (40 kV, 40 mA) with a scanning speed of 10°/min. The surface morphology of the solid samples was analyzed using a scanning electron microscope (FEI QUANTA FEG250, Portland, OR, USA).

RESULTS AND DISCUSSION

Biogas Production, Methane Yield, and Average Methane Content

In the batch experiment, the cumulative biogas production of rape straw with biochar was compared with that of control (Fig. 1). Biogas production stabilized after 20 days. The results indicated that cumulative biogas production was obviously affected by biochar concentration. For the mesophilic AD (Fig. 1a), the cumulative biogas production of control (without biochar) was only 809 mL. With the increase of biochar concentration, the biogas production first increased, and then decreased. This was probably because an appropriate concentration of biochar was beneficial to direct interspecies electron transfer (DIET), and excessive biochar affected heat and mass transfer in AD. The cumulative biogas production at 5.0% biochar concentration was highest in mesophilic AD, 1173 mL, which was 45.0% higher than that of control.

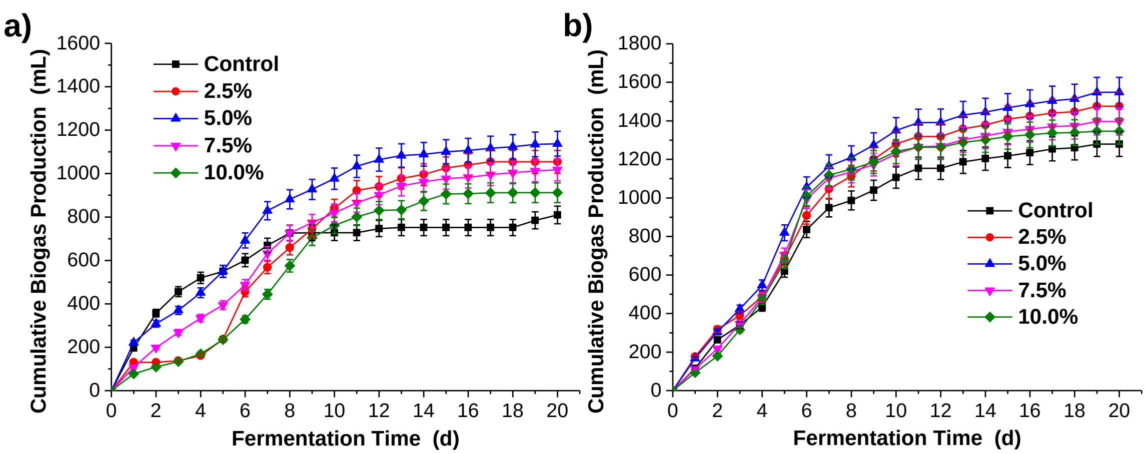


Fig. 1. The effect of biochar on biogas production of rape straw in the a) mesophilic and b) thermophilic AD

For the thermophilic AD (Fig. 1b), the trend of cumulative biogas production was consistent with mesophilic AD. The cumulative biogas production from 5.0% biochar concentration was higher than those from the control, 2.5%, 7.5%, and 10.0% additions. For the 5.0% biochar concentration, the cumulative biogas production in thermophilic AD was 1548 mL, which was 21% higher than that of control. Moreover, biogas production in thermophilic AD was higher than that in mesophilic AD.

In this process, biochar acted as a conductor material to promote direct interspecies electron transfer of microorganisms (Kang *et al.* 2021). The hydrogen ions, electrons, and carbon dioxide were converted to methane through direct interspecies electron transfer ($8H^+ + 8e^- + CO_2 = CH_4 + 2H_2O$). Therefore, it was judged that the biogas production of rape straw was increased during mesophilic and thermophilic AD.

Methane Yield and Average Methane Content

As shown in Fig. 2a, the biogas yield and average methane content of the control in the mesophilic AD were only 101.2 mL/g and 28.9%, respectively, which indicated that the pre-treated rape straw ought not to be used for biogas production directly due to low biogas yield and methane content. With the increase of the biochar concentration, the biogas yield was increased. Compared with the control, biogas yield and methane yield at 5.0% biochar concentration increased by 40.5% and 188.8%, respectively, and the average methane content was 59.4%. When the biochar concentration was 7.5% and 10.0%, the biogas and methane yield decreased slightly compared to the 5.0% biochar concentration. This indicated that biochar played an important role in the mutual metabolism of acid-producing and methanogenic microorganisms.

The biogas yield of the control in the thermophilic AD was 160.0 mL/g, which was 58.1% higher than the control of the mesophilic AD. As the biochar concentration increased from 2.5% to 10.0%, the biogas yields were 184.5, 193.5, 174.7, and 168.3 mL/g, respectively, which represented increases by 15.3%, 21.0%, 9.2%, and 5.2% compared with the control, respectively. In the thermophilic AD, the biogas yield of the 5.0% biochar concentration was the best, and it was higher than that of the mesophilic AD, which indicated that biochar was suitable for both mesophilic and thermophilic AD of rape straw.

In the mesophilic and thermophilic AD, the average methane contents in the biochar-added experiments were higher than those of the control. An appropriate biochar concentration can obtain high methane content. This is because biochar can strengthen the direct inter-species electron transfer process of methanogenesis, which increases the path of methanogenesis (Pan *et al.* 2019).

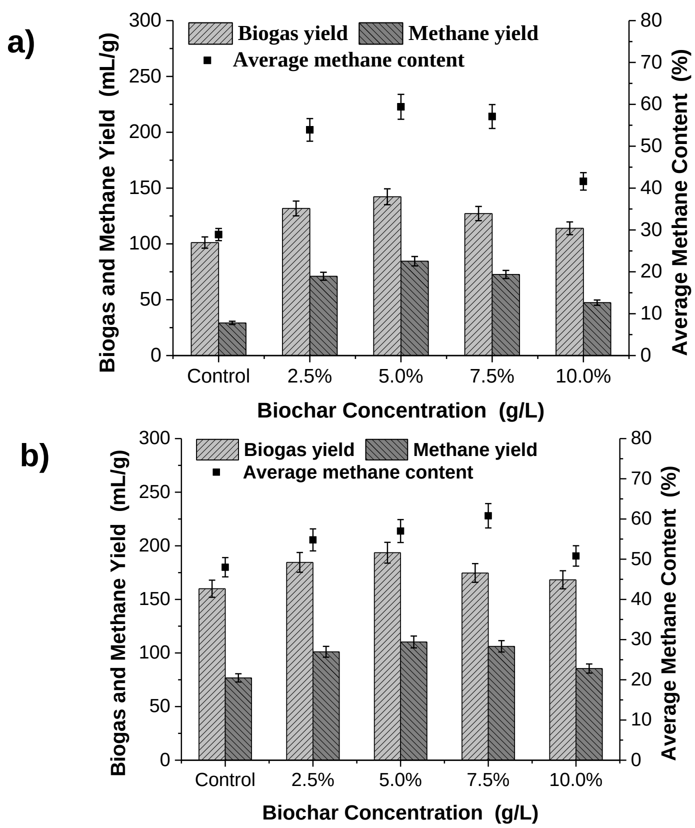


Fig. 2. The effect of biochar on biogas yield, methane yield, and average methane content of rape straw in the a) mesophilic and b) thermophilic AD

Changes in the Lignocellulose Content During AD

The contents of the cellulose, hemicellulose, and lignin in the raw rape straw were 48.9%, 12.6%, and 11.6%, respectively. The cellulose and hemicellulose together accounted for 61.6% of the total raw rape straw content. After the pretreatment (Table 1), the hemicellulose content was reduced to 9.1%. The reduction of the hemicellulose resulted in the increase of cellulose and lignin. The cellulose and lignin were increased to 54.0% and 15.9%, respectively. The combined cellulose and hemicellulose content made up 63.1% of the pretreated rape straw. The pretreatment increased the available fiber content and destroyed the fiber structure (Alper *et al.* 2021). This was conducive to the degradation of cellulose and hemicellulose.

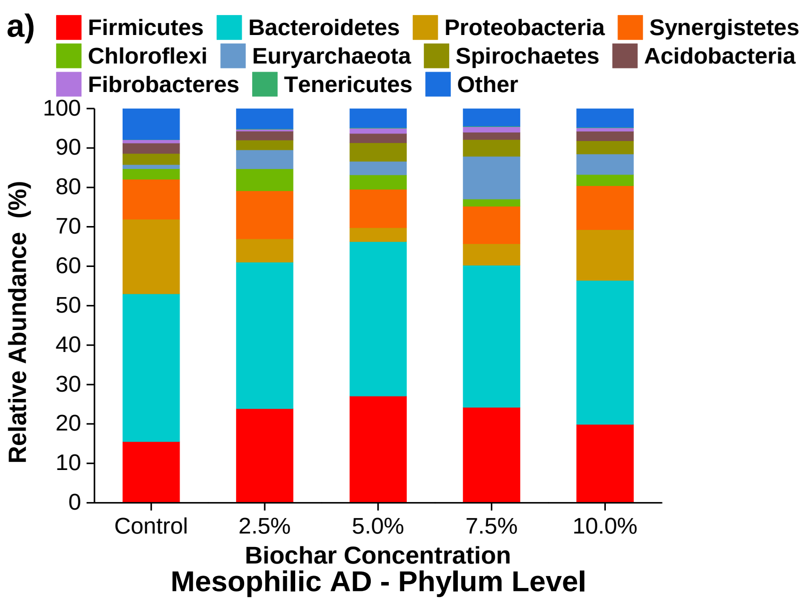
After the mesophilic AD, the cellulose and hemicellulose content of the pretreated rape straw was reduced. The cellulose content varied from 18.7% to 25.0%, and the hemicellulose content ranged from 12.9% to 15.5%. This illustrated that the process of AD for methane production mainly degraded the cellulose and hemicellulose. The cellulose and hemicellulose contents of the optimal experimental group for biogas production with a concentration of 5.0% biochar were 18.7% and 12.9%, respectively. Due to the degradation of cellulose and hemicellulose, the lignin content of all the experimental groups increased obviously (Tian *et al.* 2017), ranging from 26.6% to 32.6% after AD. This indicated that the addition of biochar can effectively promote the degradation of cellulose and hemicellulose. The addition of biochar strengthened the direct electron transfer between microbials for the methanogenesis process and enriched the microbial flora. Further, the cellulose and hemicellulose completely degraded and improved the production of biogas. The lignocellulose degradation trend of thermophilic AD was consistent with that of mesophilic AD, and the cellulose content of each group was higher than that of the mesophilic AD. This was because more cellulose was converted into volatile fatty acids and other small molecule substances, and the refractory cellulose content increased.

Table 1. Changes of the Lignocellulose Composition of Rape Straw in AD

Group	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Rape straw	48.92 ± 2.45	12.63 ± 0.63	11.58 ± 0.58
Pretreated rape straw	54.00 ± 2.70	9.12 ± 0.46	15.90 ± 0.80
Mesophilic AD			
Control	23.03 ± 1.15	15.39 ± 0.77	26.60 ± 1.33
2.5%	22.50 ± 1.13	15.52 ± 0.78	28.36 ± 1.42
5.0%	18.69 ± 0.93	12.94 ± 0.65	32.58 ± 1.63
7.5%	23.01 ± 1.15	14.24 ± 0.71	28.60 ± 1.43
10.0%	25.02 ± 1.25	13.61 ± 0.68	27.08 ± 1.35
Thermophilic AD			
Control	31.82 ± 1.59	9.86 ± 0.49	23.17 ± 1.16
2.5%	24.88 ± 1.24	13.09 ± 0.65	28.15 ± 1.41
5.0%	21.80 ± 1.09	12.48 ± 0.62	24.75 ± 1.24
7.5%	25.77 ± 1.29	12.84 ± 0.64	26.04 ± 1.30
10.0%	27.03 ± 1.35	12.94 ± 0.65	31.59 ± 1.58

Microbial Community Structure

Figure 3a shows that at the phylum level, the biogas residues of the control sample in mesophilic AD primarily contained *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Synergistetes*, with relative abundances of 15.5%, 37.5%, 18.9%, and 10.1%, respectively. The relative abundances of *Chloroflexi*, *Euryarchaeota*, *Spirochaetes*, *Acidobacteria*, and *Fibrobacteres*, at 2.67%, 1.02%, 2.82%, 2.64%, and 0.83%, respectively. Consistent with other studies (Park *et al.* 2018), the *Firmicutes* and *Bacteroides* were the dominant microbial community in the anaerobic fermentation systems. When the biochar concentration increased from 2.5% to 10.0%, the relative abundance of *Firmicutes* increased initially before decreasing. The relative abundances of *Firmicutes* and *Bacteroides* in the biogas residue with a biochar concentration of 5.0% were the highest, at 27.1% and 39.2%, respectively. The relative abundances of the *Proteobacteria*, and *Synergistetes* were also affected by biochar. Compared with other groups, the *Proteobacteria* in the biogas residue of the 5.0% biochar concentration group had a low abundance, and the abundance of the *Chloroflexi* was relatively high. This reflected the balance of interdependence and mutual restriction between the microbial community in AD for methane production.



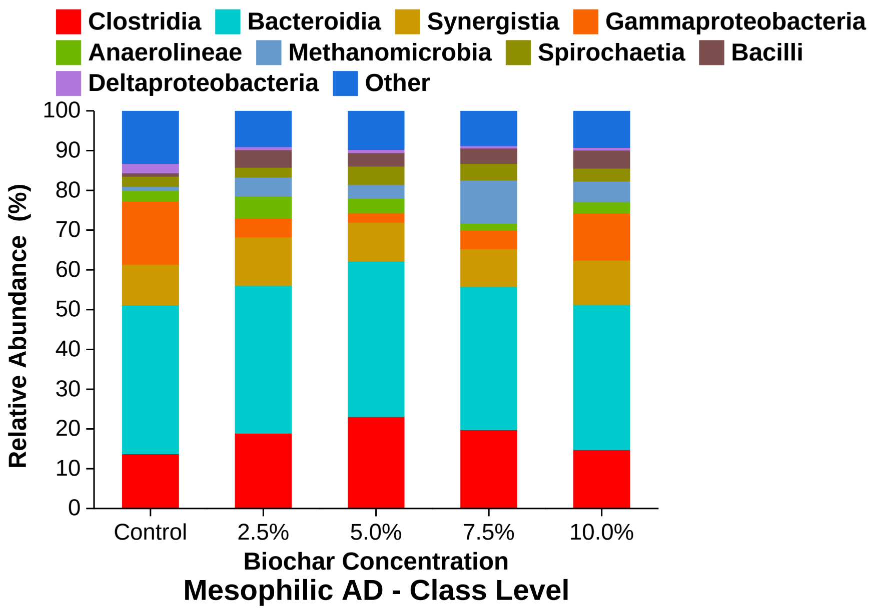
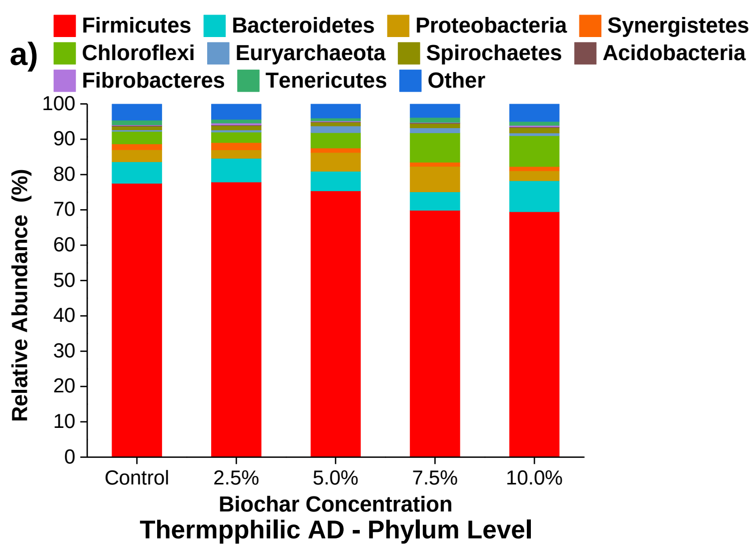


Fig. 3. The effect of the biochar on the microbial community in the mesophilic AD of rape straw at the a) phylum level and the b) class level

At the class level, the relative abundance of *Clostridia* also showed a similar trend of increasing before it decreased. The relative abundance of *Clostridia* for the concentration of 5.0% biochar was high (23.1%), and the relative abundance of *Bacteroidia* was the highest (39.1%). The amount of *Gammaproteobacteria* in the biogas residues of the biochar was lower than in those in the control sample, which indicated that the conductor materials had an important impact on the *Gammaproteobacteria* in the anaerobic fermentation system. The relative abundance of *Methanomicrobia* for the biochar was the high (3.4% to 10.7%), which was consistent with the previous results of biogas production. The relative abundance of the *Bacilli* belonging to *Firmicutes* for the biochar was 3.35% to 4.52%. This was higher than that of the control (0.89%).

a)



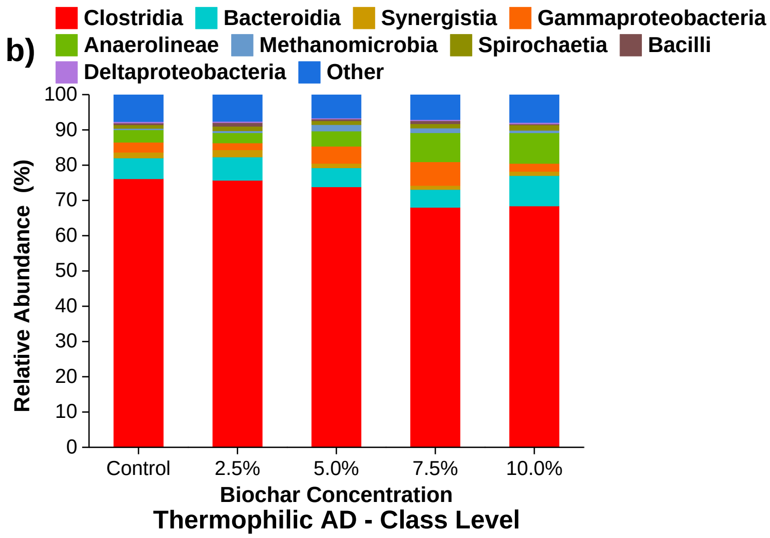


Fig. 4. The effect of the biochar on the microbial community in the thermophilic AD of rape straw at the a) phylum level and the b) class level

For the thermophilic AD (Fig. 4), the dominant microbials changed. At the phylum level, the relative abundance of *Firmicutes* reached 69.5% to 77.9%. Compared with the mesophilic AD, the relative abundance of *Bacteroidetes* phylum decreased to between 5.2% and 8.7%. The relative abundance of *Proteobacteria* and *Synergistetes* was greatly reduced, while the relative abundance of *Chloroflexi* increased. At the class level, the changes of the microbial abundance were consistent with the phylum level. The *Clostridia* was the dominant microbial. In addition, the relative abundance of *Methanomicrobia* in *Euryarchaeota* was high for biochar groups, which was similar with the mesophilic AD. When the biocahr concentration ranged from 2.5% to 10.0%, the relative abundance of *Methanomicrobia* was between 0.56% and 1.80%.

In summary, the changes and differences in the microbial community structure played an important role in the organic matter degradation and biogas production (Gahlot *et al.* 2020). The biochar can help regulate the dominant microbials and improve the efficiency and stability of AD.

XRD Analysis of Rape Straw

To reveal the degradation mechanism of cellulose and hemicellulose for the conversion of waste biomass to methane during additive biochar, X-ray diffraction was used to determine the samples' Cr I at the different stages in the whole process. X-ray diffraction images was shown in Fig. 5. The diffraction peak intensity and Cr I for each sample was calculated and given in Table 2. The results showed that the Cr I of the rape straw was 46.8%. After pretreatment, the Cr I increased to 49.53% due to the removal of lignin and other components in alkaline pretreatment. The results in this study were in agreement with previous results (Wang *et al.* 2019). In the mesophilic AD, the Cr I of the solid digestate decreased to 37.0% and 28.1% for the control and 5.0% biochar group, respectively. And the intensity of crystalline and amorphous peaks become weaker in solid digestate. This indicated that the cellulose and hemicellulose in crystalline region and amorphous region were degraded for biogas production. For the thermophilic AD, the Cr I at 5.0% biochar was increased to 52.6%. This was because the most of cellulose and hemicellulose was removed, and the remaining cellulose was recalcitrant. This resulted the increase of Cr I compared with that of before fermentation, revealing that the biochar can promote the lignocelluloses to biogas production.

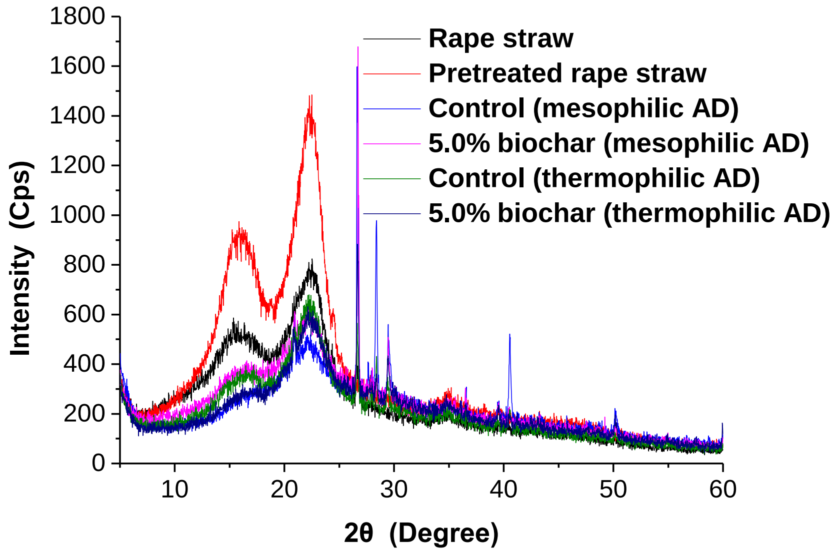


Fig. 5. X-ray diffraction images of rape straw

Table 2. Changes of X-ray Diffraction Peaks and Crystalline Index of Rape Straw

Group	2θ=22.5° peak intensity (Cps)	2θ=18° peak intensity (Cps)	Cr I (%)
Rape straw	439	825	46.79
Pretreated rape straw	704	1395	49.53
Mesophilic AD			
Control	304	483	37.06
5.0% biochar	417	580	28.10
Thermophilic AD			
Control	361	619	41.68
5.0% biochar	282	591	52.58

SEM Analysis

The solid residues after mesophilic and thermophilic AD with 5.0% biochar were examined using scanning electron microscopy (SEM) to study the apparent structure of rape straw, as shown in Fig. 6. Raw rape straw was smooth and rich in silicon. In the pretreatment, the lignin and silicon were removed, and the fiber structure was broken. After mesophilic AD with 5.0% biochar, the degradation effect was obvious. After AD of rape straw, most of cellulose and hemicellulose were degraded. The fiber surface was rough, porous, and irregular. This is consistent with the previous conclusion in lignocellulose analysis process. After thermophilic AD with 5.0% biochar, the fiber become more fragmented, and the fiber surface and pores contained a small amount of biochar particles. This also indicated that biochar could promote the degradation of fibers.

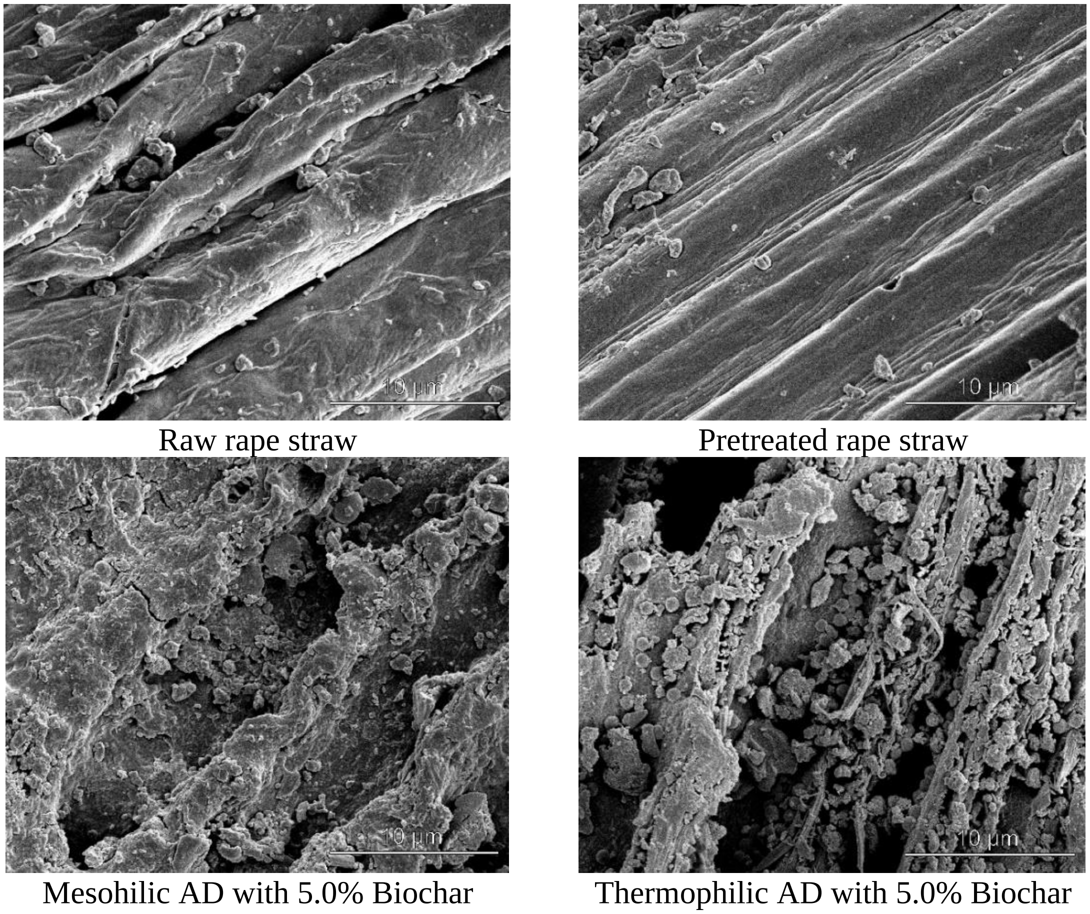


Fig. 6. SEM images of rape straw in different stages

CONCLUSIONS

1. The biochar improved the biogas yield of rape straw and the degradation of lignocellulose. With the addition of biochar, the biogas yield and the average methane content increased obviously. When the biochar concentration was 5.0%, the biogas yields in the mesophilic and thermophilic AD were 142.2 mL/g and 193.5 mL/g, respectively, which was 40.5% and 21.0% higher than those of the control. Therefore, a biochar concentration of 5.0% was a suitable condition for AD.
2. For the lignocellulose degradation, the cellulose content in the mesophilic AD decreased from 54.0% in the pretreated rape straw to between 18.7% and 25.0%.
3. The sequencing results showed that as the biochar concentration increased, the relative abundance of *Firmicutes* initially increased before it decreased. From the sequencing results, the relative abundances of *Firmicutes* and *Bacteroides* were high in the biogas residue with the 5.0% biochar concentration sample in the mesophilic AD, at 27.1% and 39.2%, respectively. The relative abundance changes of *Clostridia* in the microbials at the class level were consistent with the phylum level. The microbial community structure revealed the mechanism of biochar to promote the biogas production of rape straw.

4. The use of biochar to improve the methane yield was a promising method. In industrial anaerobic digestion process, the recovery of biochar to reduce costs should be focused.

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