ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

Biochemical Process and Microbial Evolution in the Conversion of Corn Straw Combined with Coal to Biogas

Wei Zhang, Zebin Wang, Hongyu Guo,* Libo Li, Minglu Zhang, Wen Zhang, Xiaoguang Sun, Shixuan Sun, Congliang Kou, and Weizhong Zhao

Cite This: *ACS Omega* 2022, 7, 31138−31148

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: The combined anaerobic fermentation of coal and straw can increase the production of biogas. To explore the mechanism of adding corn straw to increase methane production, coal with different metamorphic degrees and corn straw were collected for biogas production simulation experiments under different substrate ratios. The changes in liquid products, the structure of lignocellulose in corn straw, and microbial evolution were monitored. The results showed that the combined fermentation of bituminous coal A with corn straw and bituminous coal C with corn straw at a mass ratio of 2:1 each ((AC-2) and (CC-2)) and that of bituminous coal B and corn straw at a mass ratio of 3:1 (BC-3) had the best gas production, and methane yields reached 17.28, 12.51, and 14.88 mL/g, respectively. The fermentation liquid had organic matter with more types and higher contents during the early and peak stages of gas production, and fewer types of organic matter were detected in the terminal stage. The degradation of lignocelluloses in the corn straw of AC-2 was higher. With the increase in fermentation time, the carbohydrates in the fermentation system increased and the degradation rate of cellulose decreased gradually. The abundance of genes related to nitrate reduction gradually increased, while that of sulfate reduction was on the contrary. Bacteria in the cofermentation system mainly metabolized carbohydrates. During cofermentation with high metamorphic coal, corn straw would be preferentially degraded. The structure of the archaea community changed from *Methanosarcina* and *Methanothrix* to *Methanobacterium*.

1. INTRODUCTION

Under the development trend of global green energy and low-carbon transition, maintaining energy security and coping with global climate change have become major challenges for the world. Considering the cleaner combustion characteristics and higher calorific value of coalbed methane, it has gained much attraction compared to coal.[1] The production of biomethane from coal includes three processes: (1) release of soluble intermediates from coal, (2) degradation of soluble intermediates into substrates that can be used by methanogens, and (3) generation of methane.[2−4] The presence of effective microorganisms that convert available carbon in coal to methane and an in situ environment support the reproduction and metabolism of microorganisms.[5−7] Converting biomass organic matter into biogas is an inexpensive, highly feasible, and environmentally friendly treatment method.[8,9] Biomass with carbon-neutral properties can reduce greenhouse gas emissions during energy production.[10,11] The co-conversion of coal with straw can increase methane production several times compared to the bioconversion of single coal or straw.[12,13]

In the initial stage of the fermentation process, extracellular enzymes produced by hydrolysis microorganisms degrade complex organic aggregates into simple soluble monomers or dimers. This step is characterized by low gas production efficiency and lack of fermentation stability due to some hazardous substances such as volatile fatty acids (VFAs), NH_3−N, or free NH_3.[14,15] However, studies have shown that combining coal and straw fermentation reduces the production of toxic substances in the fermentation liquid, optimizes the community structure of hydrolysis, and accelerates hydrolysis.[13] Therefore, it is crucial to study the pathway of fatty acid biosynthesis and nitrate and sulfate reduction during fermentation.

Received: May 28, 2022
Accepted: August 10, 2022
Published: August 23, 2022

https://doi.org/10.1021/acsomega.2c03331
ACS Omega 2022, 7, 31138−31148

Table 1. Proximate and Ultimate Analyses of the Samples[a]

sample	M_{ad}/%	A_{ad}/%	V_{ad}/%	$R_{o,ran}$/%	C_{daf}/%	H_{daf}/%	N_{daf}/%	O_{daf}/%	S_{daf}/%
bituminous coal A	0.97	21.33	14.36	1.67	78.32	3.94	0.51	17.11	0.21
bituminous coal B	0.99	13.98	17.64	1.41	57.34	4.07	0.21	38.12	0.26
bituminous coal C	2.83	3.98	26.95	0.79	55.53	4.49	0.40	39.31	0.27
corn straw	11.14	11.29	61.56		63.12	5.48	1.07	30.13	0.20

[a]*M*: moisture; *A*: ash yield; *V*: volatile matter; ad: air-dry basis; $R_{o,ran}$: vitrinite random reflectance; daf: dry ash-free basis; C: carbon; H: hydrogen; N: nitrogen; O: oxygen; and S: sulfur.

The degradation of cellulose, hemicellulose, and lignin, which are important constituents of straw, also affects methane production.[16,17] Lignin in straw tightly enwraps cellulose and hemicellulose, making it difficult for cellulase enzyme to contact cellulose and hemicellulose, resulting in a slow hydrolysis rate.[18,19] Therefore, microorganisms and their extracellular enzymes do not easily combine with it.[20,21] Infrared spectroscopy can be used for the analysis and functional characterization of the lignocellulose structure by determining its various groups.[22,23] Thus, monitoring the infrared spectrum characteristics during the process of coal and straw cofermentation can determine the degradation degree of straw.

In this study, corn straw and coal samples were used in gas production simulation experiments. The intermediate liquid products, infrared spectral characteristics of corn straw, microbial community changes, and the pathways of fatty acid biosynthesis and nitrate and sulfate reduction were monitored during the gas production process. The mechanism of adding corn straw to increase methane production in coal anaerobic fermentation was discussed.

2. EXPERIMENTAL MATERIALS AND METHODS

2.1. Experimental Materials. The coal samples were collected from bituminous coal A of Wangjialing mine in Yuncheng, Shanxi (China), bituminous coal B of Shoushan No. 1 mine in Pingdingshan, Henan (China), and bituminous coal C of Meiyukou mine in Datong, Shanxi (China). Corn straw was collected from Yongxingtun village, Jiaozuo City, Henan Province (China). Each coal sample was crushed and sieved through an 80−100 mesh sieve, and the straw was sieved through a 30 mesh sieve. The fermentation liquid domesticated for multiple generations in the laboratory was selected as the source of microorganisms. The ultimate and proximate analyses were carried out in accordance with the standards ISO 17247−2013 and ISO 17246−2010. The analysis results are presented in Table 1.

2.2. Methanogen Enrichment Culture. The enrichment medium of methanogens can be obtained through ref 13. Prior to the experiment, a re-enrichment culture of the fermentation liquid for multigenerational acclimatization was required to stimulate the activity of the methanogens. The prepared medium was sterilized in a sterilizer at 121 °C for 20 min. The pH of the medium was adjusted to 7 with 1 mol/L HCl and 0.5 mol/L NaOH. The acclimatized fermentation liquid was mixed with the medium at a volumetric ratio of 1:5, and N_2 was passed through it for 5 min to remove all of the air. It was then sealed quickly using a sealing film and allowed to enrich in a constant-temperature incubator at 35 °C for 7 days.

2.3. Experimental Methods. *2.3.1. Biogas Production Simulation Experiment.* The pre-prepared experimental samples and 200 mL of enriched acclimatized fermentation liquid were added into a 250 mL conical flask sterilized using high-pressure sterilization. The air in the flask was replaced with high-purity nitrogen for 3 min to ensure a strict anaerobic environment. The bottles were quickly sealed with a sealing film, and a gas collection bag was attached to the bottles. These were then placed in a constant-temperature incubator at 35 °C for static fermentation. In addition, the experiments of anaerobic fermentation of single coal and single corn straw under the optimal gas production combination were carried out as a comparative analysis. Information on different samples is presented in Table 2. Three replicates were set up for each

Table 2. Fermentation Combinations with Different Proportions of Substrates

sample number	coal type	straw type	coal (g)	corn straw (g)	proportion of coal and corn straw
AC-2	bituminous coal A	corn straw	4	2	2:1
AC-3	bituminous coal A	corn straw	6	2	3:1
AC-4	bituminous coal A	corn straw	8	2	4:1
BC-2	bituminous coal B	corn straw	4	2	2:1
BC-3	bituminous coal B	corn straw	6	2	3:1
BC-4	bituminous coal B	corn straw	8	2	4:1
CC-2	bituminous coal C	corn straw	4	2	2:1
CC-3	bituminous coal C	corn straw	6	2	3:1
CC-4	bituminous coal C	corn straw	8	2	4:1

gas production experiment, and the gas production period was set as 30 days. The gas and fermentation liquid sample was collected for products' analysis.

The characteristic changes in liquid products, the solid-phase structure of corn straw, enzyme activity, and microbial structure were studied during the fermentation of the optimal combination of coal and straw. The process analysis includes the early stage (AC-2(E), BC-3(E), and CC-2(E)), peak stage (AC-2(P), BC-3(P), and CC-2(P)), and terminal stage (AC-2(T), BC-3(T), and CC-2(T)) of fermentation.

2.3.2. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Liquid Products. The liquid products were analyzed using a gas chromatograph/mass spectrometer (Agilent GC-MS 7890−5977A). About 50 μL of concentrated HCl was added to 20 mL of the extract. Then, the mixture was poured into a 50 mL centrifuge tube, 6 g of NaCl was added (using the salting-out effect), and the mixture was stirred. Then, 10 mL of dichloromethane was added to a 50 mL centrifuge tube and the contents were subjected to vortex extraction for 10 min at room temperature, followed by static

https://doi.org/10.1021/acsomega.2c03331
ACS Omega 2022, 7, 31138−31148

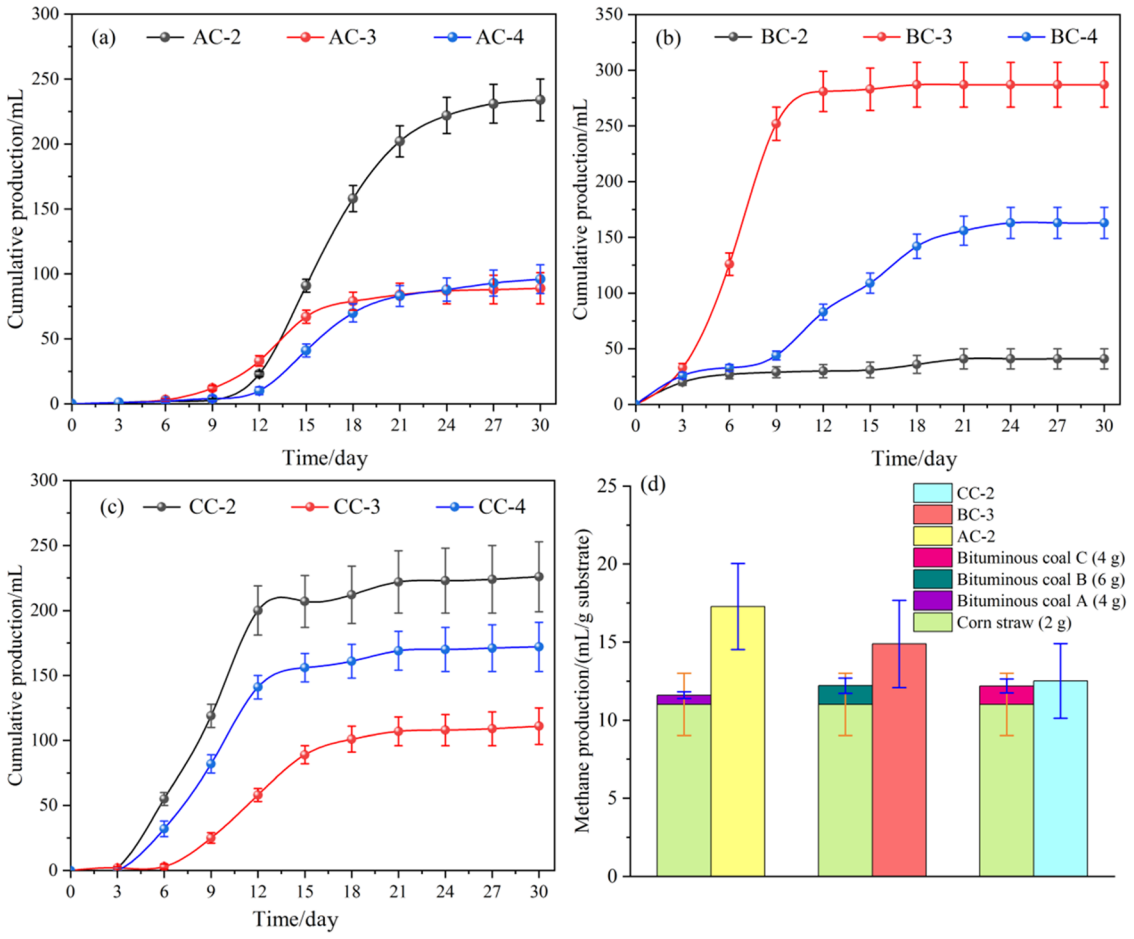

Figure 1. Cumulative gas production and methane production by different fermentation combinations: (a−c) the cumulative gas production of combined fermentation of bituminous coal A, bituminous coal B, and bituminous coal C with corn straw, respectively, and (d) methane production by the optimal gas production combination.

treatment for 10 min, and the lower organic phase was collected. This procedure was repeated twice. The extracts were combined three times and concentrated to dryness under a stream of nitrogen gas. Two microliters of dichloromethane was added, and the vortexed mixture was dried over an anhydrous Na_2SO_4 cartridge and analyzed by GC/MS.

2.3.3. Fourier Transform Infrared (FT-IR) Analysis of Corn Straw. The corn straw samples were crushed for 2 min and passed through 100 mesh filters. After drying at 65 °C for 12 h, 1 mg of the sample and 50 mg of potassium bromide (KBr) were evenly ground using an FW-5 tablet press at a pressure of 1 MPa for 30−60 s. The compounds were pressed into a sheet with a diameter of approximately 13 mm. The changes in the infrared spectra of straw samples were analyzed by a Fourier transform infrared spectrometer (Nicolet iS50, Thermo Fisher Scientific Inc.) using the pressed slice method.

2.3.4. Microbial Community Structure Test. The total genomic DNA samples of bacteria and archaea were extracted using a Rapid DNA Rotation Extraction Kit (MP Biomedicals, Santa Ana, CA). The quantity and quality of extracted DNA were measured by a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Specific primers were used for the PCR amplification of the bacterial 16S rDNA V3−V4 region and the PCR amplification of the archaea mcrA region. NEB Q5 DNA polymerase was used for the PCR.

The microbial function was predicted by PICRUSt software. Based on the Kyoto encyclopedia of genes and genomes (KEGG) methane metabolic pathway (Map: 00061, 00910, and 00920), the metabolic genes with predicted abundance greater than 100 were selected for further evaluation of fatty acid biosynthesis and nitrate and sulfate reduction pathways. Refer to https://www.genome.jp/kegg/ for the query of gene functions related to metabolism.

3. RESULTS AND DISCUSSION

3.1. Gas Production by Different Combinations of Coal and Corn Straw via Anaerobic Fermentation. Figure 1 shows the cumulative gas production and methane production by anaerobic fermentation of bituminous coal A, B, and C with corn straw at ratios of 2:1, 3:1, and 4:1 (specific data are shown in Tables S1 and S2). It was observed that bituminous coal A and C resulted in the highest gas production with corn straw at a ratio of 2:1 and therefore was considered the optimal gas production combination. Bituminous coal B and corn straw at a ratio of 3:1 was also considered the optimal combination for gas production. The cumulative gas production values of AC-2 and CC-2 were 234 and 226 mL, which were higher than those of AC-3 and CC-3 by 162.92 and 103.60%, respectively. The gas production by bituminous coal B showed a significant difference under different ratios, with BC-3 resulting in 287 mL of cumulative gas production, which was 6 times higher than that of BC-2. At the same time, methane production by each combination was also different.

https://doi.org/10.1021/acsomega.2c03331
ACS Omega 2022, 7, 31138−31148

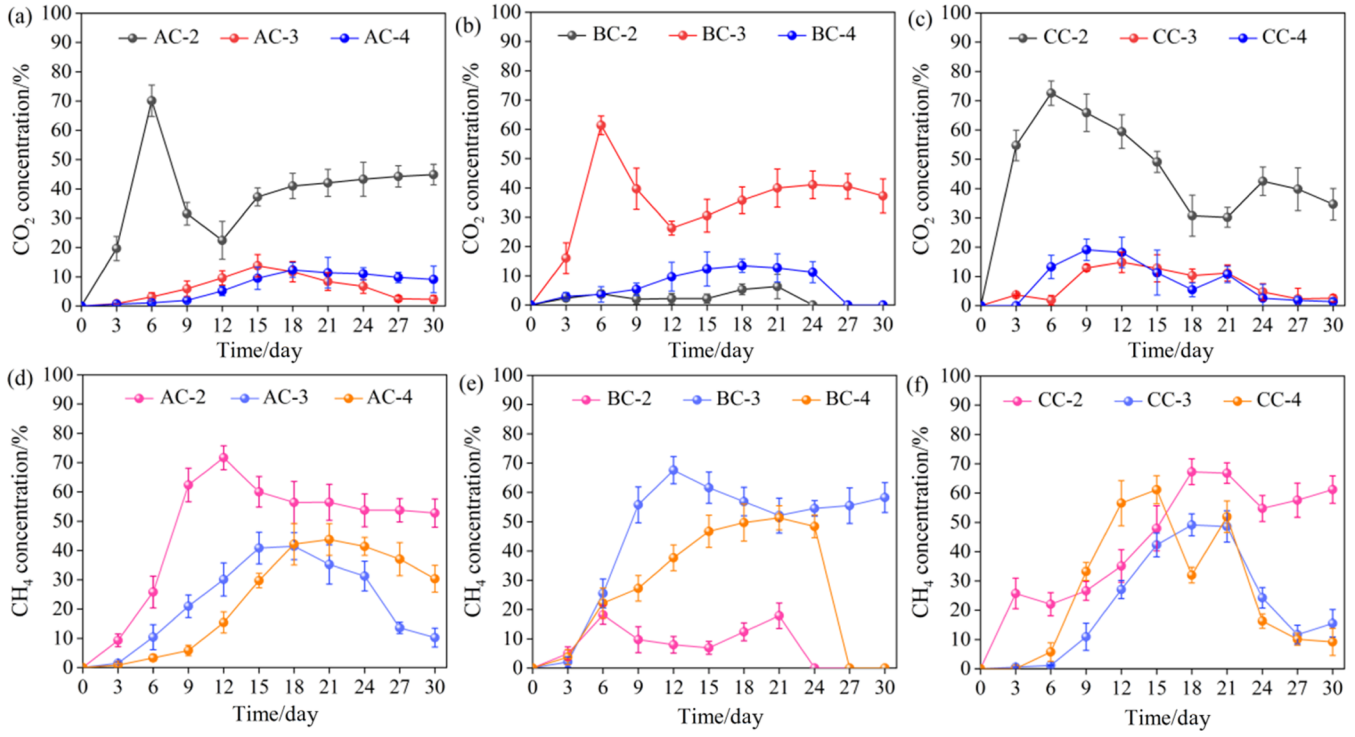

Figure 2. CH_4 and CO_2 concentration changes with gas production time: (a−c) CO_2 concentration changes during combined fermentation of bituminous coal A, bituminous coal B, and bituminous coal C with corn straw, respectively, and (d−f) CH_4 concentration changes during combined fermentation of bituminous coal A, bituminous coal B, and bituminous coal C with corn straw, respectively.

The optimal combinations for methane production corresponded to the optimal combinations for cumulative gas production, which were AC-2, BC-3, and CC-2. Methane production by the optimal gas production combination was higher than the sum of methane yield of single coal and single corn straw (Figure 1d). Methane productions by AC-2, BC-3, and CC-2 were 17.28, 14.88, and 12.51 mL/g, respectively.

According to the modified Buswell equation (eq 1),[24] the theoretical biomethane potential (BMP) values of corn straw, coal, and their combination were calculated separately.

$$\begin{aligned} &C_aH_bO_cN_dS_e + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{8} + \frac{e}{4}\right) \times H_2O \\ &\rightarrow \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} + \frac{e}{4}\right) \times CO_2 \\ &\quad + \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} + \frac{3d}{8} - \frac{e}{4}\right) \times CH_4 + d \times NH_3 \\ &\quad + e \times H_2S \end{aligned} \tag{1}$$

According to the ultimate analysis of corn straw and coal (Table 1), the value for eq 1 was obtained by dividing the percentage content of the element by the corresponding molar mass. The sum of each coal and corn straw value was used as the value of their combination. Therefore, the chemical formulas for corn straw, bituminous coal A, bituminous coal B, bituminous coal C, and their combination were $C_{5.255}H_{5.437}O_{1.883}N_{0.076}S_{0.006}$, $C_{6.521}H_{3.909}O_{1.069}N_{0.036}S_{0.007}$, $C_{4.774}H_{4.038}O_{2.383}N_{0.015}S_{0.008}$, $C_{4.623}H_{4.455}O_{2.457}N_{0.029}S_{0.008}$, $C_{11.776}H_{9.346}O_{2.952}N_{0.112}S_{0.013}$, $C_{10.029}H_{9.475}O_{4.266}N_{0.091}S_{0.014}$, and $C_{9.878}H_{9.892}O_{4.340}N_{0.105}S_{0.014}$, respectively. Based on the $C_aH_bO_cN_dS_e$ of eq 1, eq 2 became

$$\mathrm{BMP} = \frac{\left[\left(\frac{a}{2}\right) + \left(\frac{b}{8}\right) - \left(\frac{c}{4}\right) - \left(\frac{3d}{8}\right) - \left(\frac{e}{4}\right)\right] \times 22\,400}{(12a + b + 16c + 14d + 32e)} \left[\frac{\mathrm{mL}}{g_{vs}}\right] \tag{2}$$

Using eq 2, the BMP values of 629.293, 776.662, 512.922, 502.461, 703.019, 571.208, and 565.982 mL/g_{vs} were obtained for the digested corn straw, bituminous coal A, bituminous coal B, bituminous coal C, and their combination, respectively. According to the results, the BMP values of corn straw, coal, and their combination were much greater than methane production. This result is closely related to the complex lignin structure of corn straw and the complex aromatic structure of coal.[5,22] The BMP values of the combination of bituminous coal A, bituminous coal B, bituminous coal C, and corn straw were consistent with the methane trends of AC-2, BC-3, and CC-2.

The change in CH_4 and CO_2 concentrations with gas production time during cofermentation is shown in Figure 2. With the increase in gas production time, the CO_2 concentration of each combination gradually increased to a peak and then slowly decreased to different concentration levels. The CO_2 concentration of the optimal gas production combinations, AC-2, BC-3, and CC-2, were the first to reach the peak, and the peak values were 70.11, 61.41, and 72.59%, respectively. During the subsequent slow decrease of CO_2 concentration, compared with other combinations, AC-2, BC-3, and CC-2 maintained a high concentration level. The change in CH_4 concentration was consistent with the change in CO_2 concentration. The maximum concentration of CH_4 was also observed with AC-2, BC-3, and CC-2, reaching 71.69,

https://doi.org/10.1021/acsomega.2c03331
ACS Omega 2022, 7, 31138−31148

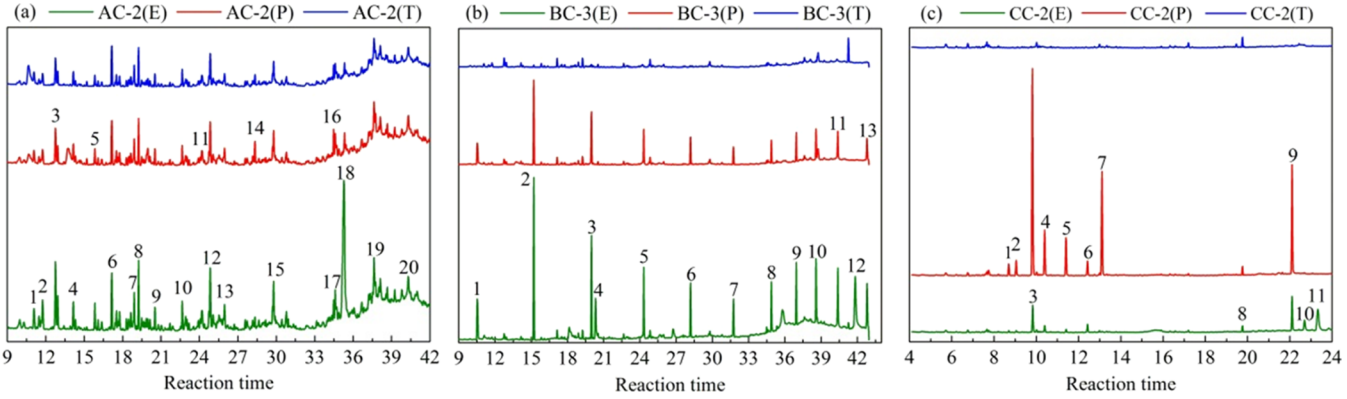

Figure 3. GC-MS results of different fermentation stages in (a) AC-2, (b) BC-3, and (c) CC-2.

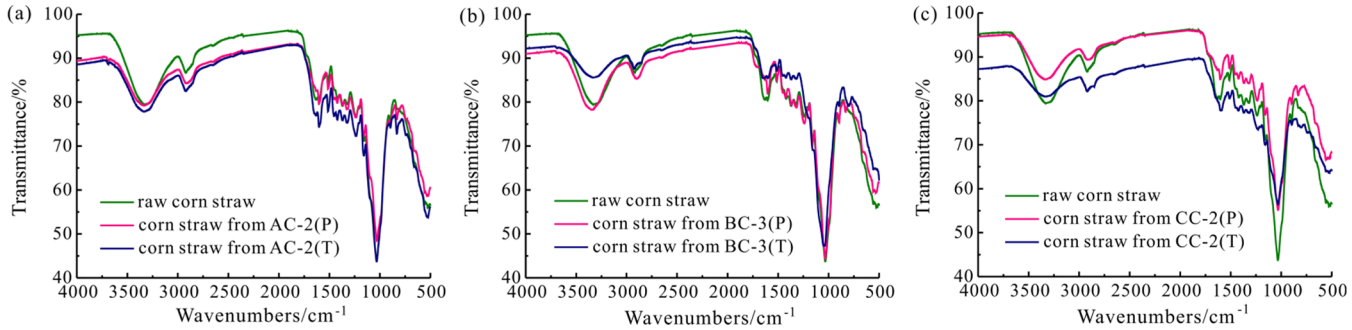

Figure 4. FT-IR spectra of corn straw at different fermentation stages: (a) corn straw from the combined fermentation of bituminous coal A and corn straw; (b) corn straw from the combined fermentation of bituminous coal B and corn straw; and (c) corn straw from the combined fermentation of bituminous coal C and corn straw.

67.62, and 67.27%, respectively. However, the peak concentration of CH_4 appeared later than that of CO_2. Since some CO_2 remains dissolved in the fermentation liquid, the other part is used by the methanogens to convert it into biomethane.[25] With the increase in CO_2 concentration, more CO_2 can be used by methanogens, resulting in a lag followed by the peak of CH_4. When other conditions are quantitative and the material ratio is the only variable, the two achieve the best gas production effect only when the material ratio is appropriate.

3.2. Analysis of the Main Controlling Factors during the Fermentation of the Optimal Gas Production Combination. *3.2.1. Variation Characteristics of Liquid Products.* The GC-MS results showing different metabolites produced during the fermentation at different periods are shown in Figure 3, and Table S3 presents the compounds corresponding to different peak numbers in Figure 3. Table S4 represents the area percentage and quantitative value of the organic matter for each number.

In the early stage of anaerobic fermentation, the abundance of organic compounds such as heterocyclic compounds, benzene derivatives, long-chain alkanes, and substituted alkane, ethers, esters, and organic compounds containing oxygen and nitrogen increased in the liquid phase with the increase in the metamorphic degree of coal. Thereafter, the newly generated VFAs were detected in the liquid phase of CC-2(P). These were mainly butyric acid and acetic acid. VFAs can be used as intermediates for methane production, and methyl compounds can be converted into biomethane through the methylotrophic pathway of methanogens. The abundance of organic compounds in the liquid phase of AC-2(P) and BC-3(P) decreased. Esters and ethers were detected in BC-3(P), including phosphonoacetic acid, 3TMS derivative, 3-chloropropane-1,2-diol, bis(*tert*-butyldimethylsilyl) ether, and 2,5-dihydroxybenzoic acid. The contents of heterocyclic compounds and benzene derivatives decreased in BC-3(P). There was still a higher content of dodecane and heptadecane and a lower content of short-chain alkanes and substitute alkanes in the liquid products of AC-2(P). The analysis results of liquid-phase products at the peak period showed that the fermentation solution had high gas production potential at this time, and it corresponded to the peak stage of gas production. At the end of the gas production experiments, little liquid products were detected in each group. The types of liquid products of BC-3(T) and CC-2(T) significantly decreased, while that of AC-2(T) also decreased but had no overall significant change. During the whole fermentation process, the content and species of liquid products in AC-2 were higher than those in BC-3 and CC-2, which may be one of the causes for its higher biogas and methane production. The comprehensive analysis showed that the types and contents of organic matter in the fermentation liquid of different optimal gas production combinations for the combined fermentation of corn straw and coals were quite different. The fermentation liquid for the optimal gas production combination had organic matter with more types and higher contents during the early and peak stages of gas production. However, in the terminal stage of gas production, fewer types of organic matter were detected in the fermentation liquid during optimal gas production with low-rank coal.

https://doi.org/10.1021/acsomega.2c03331
ACS Omega 2022, 7, 31138−31148

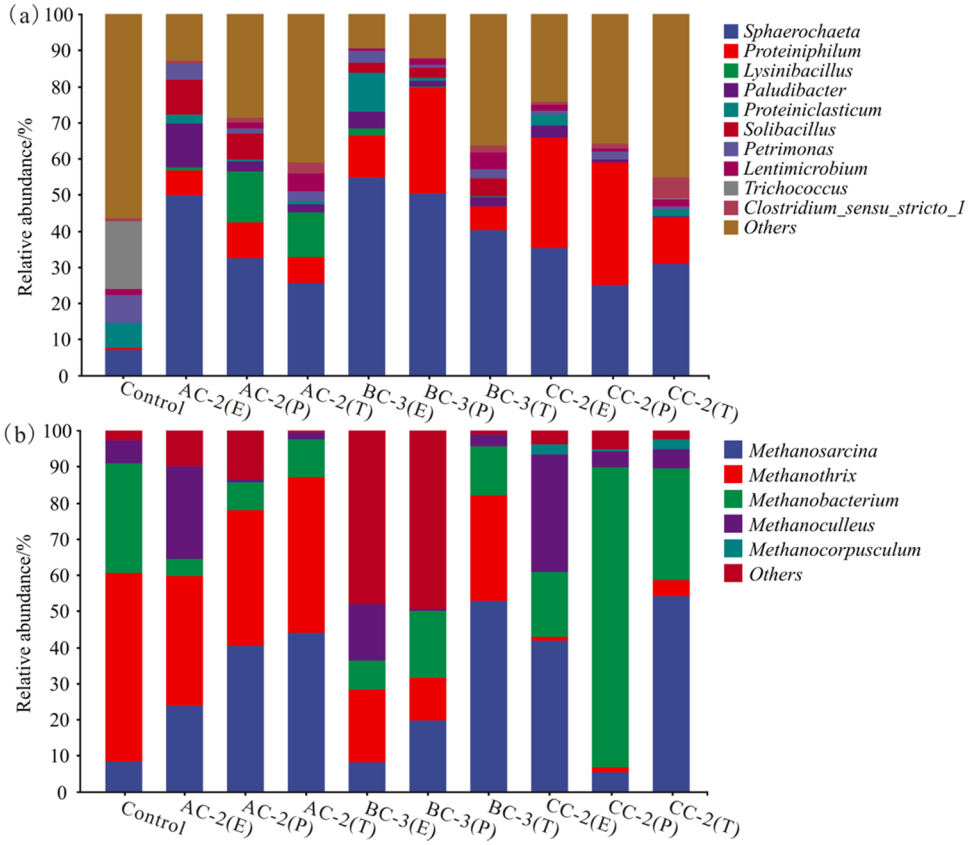

Figure 5. Diagrams of (a) bacterial community structure and (b) archaea community structure.

3.2.2. FT-IR Change Characteristics of Corn Straw. The infrared spectrum characteristics of the corn straw samples at different fermentation stages for the optimal gas production combination are shown in Figure 4. The infrared spectra of straw samples were basically similar, with obvious absorption peaks appearing at wavenumbers close to 3333, 2910, 1620, 1510, 1435, 1340, 1220, 1035, and 897 cm^{-1}. At the same time, there were differences in the absorption intensity of some characteristic peaks, and the attribution of each peak is shown in Table S5. The spectral transmittance of each corn straw sample was small near 3333 and 1035 cm^{-1}, which showed that O−H stretching vibration and C−H and C−O bending vibration were intense. During the peak period of gas production, the spectral characteristics of the three straw samples were not the same as those of raw straw. The spectral transmittance values of the three straw samples from AC-2(P), BC-3(P), and CC-2(P) were enhanced at 500−1800 cm^{-1}, indicating that the vibration intensity of the functional groups had weakened. The spectral transmittance of the straw samples from AC-2(P) and BC-3(P) decreased at 1800−4000 cm^{-1}, indicating that the vibrational intensity of the functional groups increased. The microorganisms were more active at this time. The functional group structure, molecular vibration mode, and symmetry of the straw changed after the attack by enzymes. This resulted in the change in transition dipole moment and the intensity of infrared absorption spectra increased. After gas production ended, the vibration peaks in the lignin benzene ring structure, lilac ring, and guaiacyl ring were enhanced in the straw samples of AC-2(T), BC-3(T), and CC-2(T), confirming that some of the cellulose and hemicellulose were degraded by microbes. Lignin is a complex polymer that is difficult to degrade, so the vibration peak was more significant with the increase in the proportion. At the same time, the spectral transmittance of the three straw samples was weakened near 2910 cm^{-1}, which showed that the vibration peaks of methyl, methylene, and methine were enhanced. Therefore, the degradation by microorganisms caused the chemical bonds between straw molecules to break, thus reducing the linear alkanes and increasing the branched alkanes.[22,23,26] Comparing the spectral characteristics of the three straw samples after gas production ended, the transmittance of AC-2 at each peak was lower than those of BC-3 and CC-2. The degradation of lignocelluloses in corn straw of AC-2 was higher, which may be one of the reasons for its higher methane production.

3.2.3. Variation Characteristics of the Microbial Community Structure. The species composition of the microbial community is important for the continuous development of the anaerobic fermentation system.[27] Therefore, the sequence distribution (relative abundance > 5%) of archaea and bacteria in the original bacterial solution without a substrate was control. Microorganisms in the fermentation liquid during different fermentation stages were analyzed at the genus level. This helped to better understand the succession of the microbial community structure during the fermentation of coal and corn straw.

3.2.3.1. Analysis of Bacterial Structure. Figure 5a shows the community abundance at the bacterial genus level. The main bacterial genera were *Sphaerochaeta* and *Proteiniphilum*, accounting for 30.46 and 12.48% of the total sequencing bacterial genera, respectively. *Sphaerochaeta* converted carbohydrates into acetate, ethanol, hydrogen, and carbon dioxide.[28]

https://doi.org/10.1021/acsomega.2c03331
ACS Omega 2022, 7, 31138−31148

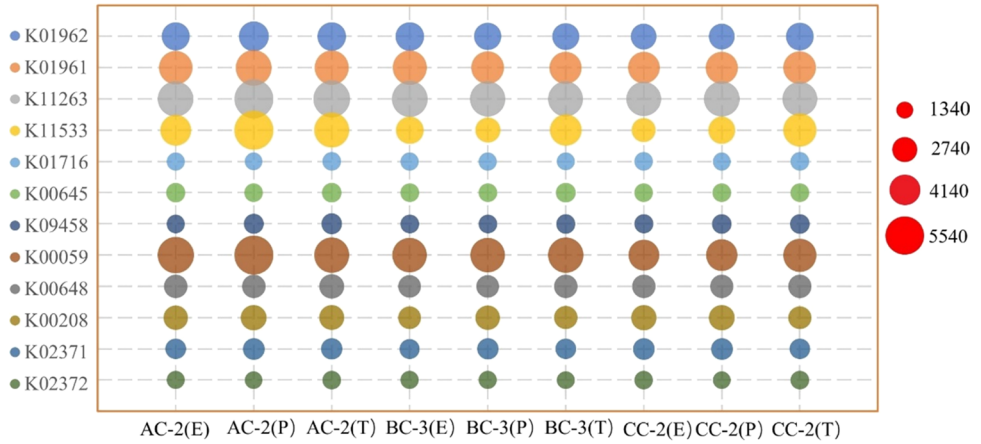

Figure 6. Homologous gene abundance of fatty acid biosynthesis pathways. The gene names represented by KO values in the figure and their explanations are shown in Table 3.

Table 3. Description of the Annotated Homologous Genes from Fatty Acid Biosynthesis

KO	gene name	description
K01962	*accA*	acetyl-CoA carboxylase carboxyl transferase subunit α [EC:6.4.1.2]
K01961	*accC*	acetyl-CoA carboxylase, biotin carboxylase subunit [EC:6.4.1.2 6.3.4.14]
K11263	*bccA*	acetyl-CoA/propionyl-CoA carboxylase, biotin carboxylase, biotin carboxyl carrier protein [EC:6.4.1.2 6.4.1.3 6.3.4.14]
K11533	*fas*	fatty acid synthase [EC:2.3.1.-]
K00648	*fabH*	3-oxoacyl-[acyl-carrier-protein] synthase III [EC:2.3.1.180]
K00647	*fabB*	3-oxoacyl-[acyl-carrier-protein] synthase I [EC:2.3.1.41]
K00645	*fabD*	[acyl-carrier-protein] S-malonyltransferase [EC:2.3.1.39]
K09458	*fabF*	3-oxoacyl-[acyl-carrier-protein] synthase II [EC:2.3.1.179]
K00059	*fabG*	3-oxoacyl-[acyl-carrier protein] reductase [EC:1.1.1.100]
K01716	*fabA*	3-hydroxyacyl-[acyl-carrier protein] dehydratase/trans-2-decenoyl-[acyl-carrier protein] isomerase [EC:4.2.1.59 5.3.3.14]
K02372	*fabZ*	3-hydroxyacyl-[acyl-carrier-protein] dehydratase [EC:4.2.1.59]
K00208	*fabI*	enoyl-[acyl-carrier protein] reductase I [EC:1.3.1.9 1.3.1.10]
K02371	*fabK*	enoyl-[acyl-carrier protein] reductase II [EC:1.3.1.9]
K01071	*MCH*	medium-chain acyl-[acyl-carrier-protein] hydrolase [EC:3.1.2.21]

Proteiniphilum converted organic matter, such as yeast extract and peptone, into acetic acid and carbon dioxide. When the substrate is exhausted, hydrogen and carbon dioxide can also be used to catalyze the synthesis of acetic acid.[29] In summary, with the increase in fermentation time, the trend of bacterial community change in the three experimental groups was similar. After adding the fermentation substrate, the carbohydrates in the fermentation system increased, which increased the abundance of *Sphaerochaeta*. Furthermore, the contents of hydrogen and carbon dioxide also increased, making the *Proteiniphilum* abundance reach a maximum at the fermentation peak. With time, the degradation rate of cellulose decreased gradually, and the decrease in carbohydrates led to the decrease in *Sphaerochaeta* abundance. *Sphaerochaeta* and *Proteiniphilum* established a significant subordinate relationship. At the early stage of fermentation, the ratios of *Sphaerochaeta* to *Proteiniphilum* in AC-2(E), BC-3(E), and CC-2(E) were 730.98, 472.74, and 114.91%, respectively. In addition, AC-2(E) had the *Paludibacter* genus that was not detected in the other two fermentation systems, which could degrade a variety of monosaccharides and disaccharides into propionic acid, acetic acid, and a small amount of butyric acid.[30] It can be seen that with the increase in the metamorphic degree of coal, bacteria in the cofermentation system mainly metabolized carbohydrates. This indirectly reflected that during the cofermentation with high metamorphic coal, corn straw would be preferentially degraded, thus significantly improving the activity of carbohydrates-degrading bacteria. Moreover, the macromolecular substances in the fermentation system of AC-2 were abundant, and the effective conversion rate of the substrate to methane also increased.

3.2.3.2. Analysis of Archaeal Structure. No significant difference was observed in the dominant archaea before or after adding the fermentation substrate to the acclimated bacterial solution. *Methanothrix*, *Methanosarcina*, *Methanobacterium*, and *Methanoculleus* were the main species (Figure 5b). *Methanothrix* is one of the acetotrophic archaea and its content decreases with the decrease in the metamorphic degree of coal.[31] Recent studies have reported that direct interspecific electron transfer (DIET) is more advantageous than interspecific hydrogen transfer (IET) during synergistic fermentation. *Methanosarcina* directly accepts electrons from electron donors.[32] The content of *Methanosarcina* in AC-2(E), BC-3(E), and CC-2(E) increased by 431.35, 320.26, and 403.22%, respectively, compared with that in the control group. With the increase in fermentation time, *Methanosarcina* continued to grow, which proved the superiority of the cofermentation system of coal and corn straw. As can be seen from Figure 5b, the structure of the archaea community also changed from the mixed-type (*Methanosarcina*) and aceticlastic (*Methanothrix*) methanogens to hydrogenotrophic methanogens (*Methanobacterium*) as the substrate changed from high

https://doi.org/10.1021/acsomega.2c03331
ACS Omega 2022, 7, 31138−31148

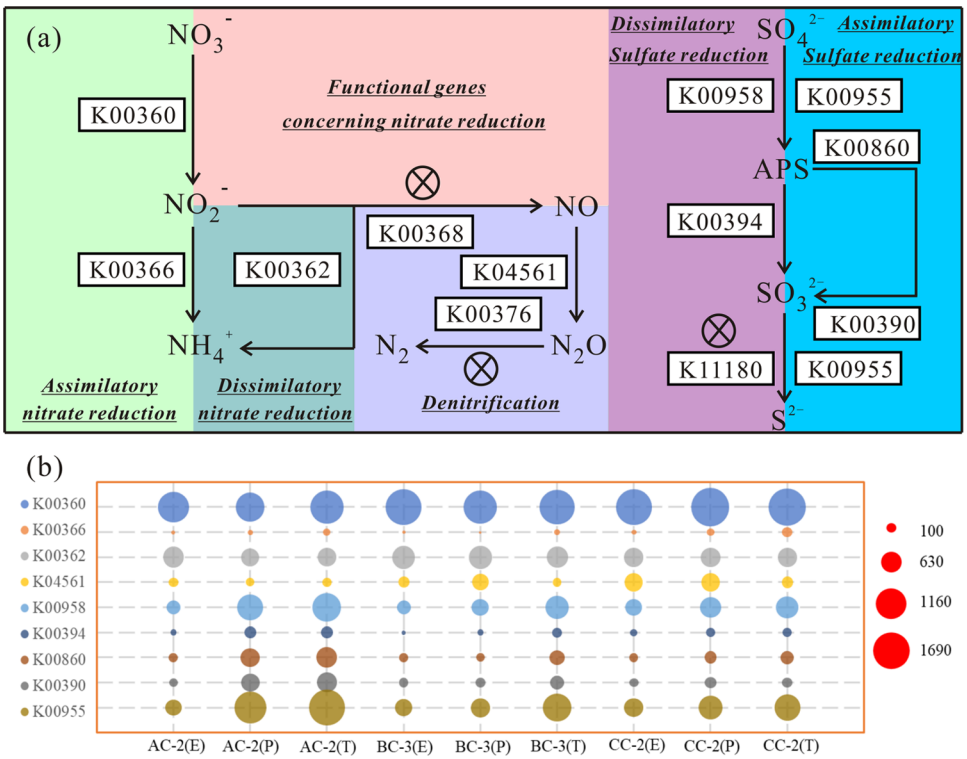

Figure 7. (a) Homologous genes of the nitrate and sulfate reduction pathway and (b) genes abundance. The gene names represented by KO values in this figure and their explanations are shown in Table 4.

Table 4. Description of the Annotated Homologous Genes Concerning Nitrate Reduction and Sulfate Reduction Pathways

KO	gene name	description
K00360	*nasB*	assimilatory nitrate reductase electron transfer subunit [EC:1.7.99.-]
K00366	*nirA*	ferredoxin-nitrite reductase [EC:1.7.7.1]
K00362	*nirB*	nitrite reductase (NADH) large subunit [EC:1.7.1.15]
K00368	*nirK*	nitrite reductase (NO-forming) [EC:1.7.2.1]
K04561	*norB*	nitric oxide reductase subunit B [EC:1.7.2.5]
K00376	*nosZ*	nitrous-oxide reductase [EC:1.7.2.4]
K00958	*sat*	sulfate adenylyltransferase [EC:2.7.7.4]
K00955	*cysNC*	bifunctional enzyme CysN/CysC [EC:2.7.7.4, 2.7.1.25]
K00860	*cysC*	adenylylsulfate kinase [EC:2.7.1.25]
K00394	*aprA*	adenylylsulfate reductase, subunit A [EC:1.8.99.2]
K00390	*cysH*	phosphoadenosine phosphosulfate reductase [EC:1.8.4.8 1.8.4.10]
K11180	*dsrA*	dissimilatory sulfite reductase α subunit [EC:1.8.99.5]

metamorphic coal to low metamorphic coal.[33] This was also the reason for high CO_2 production in the CC-2 cofermentation process. Significantly, *Methanoculleus* was detected in all three archaea communities, using H_2/CO_2 or sodium formate as the methanogenic substrate.[34] However, this archaeon completely disappeared in the peak period, which may be due to its strong sensitivity to the fermentation environment where the pH decreased due to the formation of VFAs. During the fermentation process, the abundance of *Methanosarcina*, *Methanothrix*, and *Methanobacterium* in BC-3 was low, resulting in underutilization of substrates, thereby limiting the generation of methane. The archaea community of CC-2(P) was relatively single, mainly dominated by hydrogenotrophic methanogens (accounting for 83.15% of the total archaea community), and the available metabolic substrates were few, slowing down the process of the methanogenic metabolism of archaea. Significantly, the single archaea community of CC-2 caused a large accumulation of acetic acid, which reduced the pH of the whole fermentation system (Figure S1). The activities of some pH-sensitive hydrolytic bacteria and archaea also decreased, which may be one of the reasons for the low methane production.

3.2.4. Fatty Acid Biosynthesis and Nitrate and Sulfate Reduction Pathways. The homologous genes of the fatty acid biosynthesis pathway in cofermentation of coal with different metamorphic degrees and corn straw were analyzed (Figure 6 and Table 3). The results showed that there were significant differences in 14 homologous genes of each sample. Acetyl-CoA carboxylase (EC: 6.4.1.2) is related to various organic metabolic pathways (such as pyruvate metabolism to produce acetic acid and butyric acid),[35] generally corresponding to *accA*, *accC*, *bccA*, and *fas*. As shown in Figure 6, the abundances of *accA*, *accC*, *bccA*, and *fas* involved in carbohydrate and energy metabolism were higher in AC-2(P), which was consistent with the rich liquid products and higher biomethane production in AC-2.

https://doi.org/10.1021/acsomega.2c03331
ACS Omega 2022, 7, 31138−31148

It is well known that various hydrolases and oxidoreductases are used in fatty acid synthesis. *fabA* and *fabZ* are typical hydrolases, which act on C−N and C−O, respectively. The contents of C−N and C−O in coal decreased with the increase in the metamorphic degree of coal. The abundances of *fabA* and *fabZ* in AC-2 were lower than those in the other experimental groups. *fabF* and *fabH*, as acyltransferases, play key roles in fatty acid synthesis. Meanwhile, *fabK*, *fabI*, and *fabG* are critical oxidoreductases involved in the electron transfer between NAD(P)H/NAD(P)$^+$. Therefore, the higher abundance of these genes in AC-2 accounted for its efficient methane production.

Methane is usually an end-product of accepting electrons in the oxidation of butyrate and acetate during the process of anaerobic fermentation. However, nitrates and sulfates can also accept electrons, which may even exceed methane production. The homologous genes of the nitrate and sulfate reduction pathway were analyzed (Figure 7a and Table 4). All of the genes showed lower abundance in the cofermentation of coal and corn straw (Figure 7b). The abundance of K00372 and K00363 in nitrate reduction and the abundance of K00958, K00955, and K00956 in sulfate reduction are higher. To further confirm the enrichment of nitrate and sulfate in anaerobic fermentation, the abundance of genes related to nitrate and sulfate reduction in different stages was analyzed. It should be noted that the enrichment culture contains only sulfate and no nitrate. The abundance of genes related to nitrate reduction gradually increased with the increase in the metamorphic degree of coal, while that of sulfate reduction was on the contrary. The results confirmed that sulfate may be enriched in anaerobic fermentation while considering that nitrate can be exhausted, but their differences were not obvious, which also accounted for the decrease in methane concentration. However, the abundance of genes involved in nitrate reduction in AC-2 was 1.67 and 2.01 times higher than those in BC-3 and CC-2, respectively. As a substitute for methane production, nitrate and sulfate reduction can also stimulate the copiotrophic metabolism of VFAs,[36] which also confirmed the existence of high methane production in AC-2. Therefore, the coexistence of nitrate and sulfate reduction and methane production was theoretically possible, which is consistent with the other researchers' findings.[37]

In addition, some studies have confirmed that the soluble calcium ions contained in the fermentation substrate can occupy specific surface functional groups of lignin, preventing extracellular enzymes to adsorb onto lignin, increasing the efficiency and stability of the anaerobic fermentation system.[38,39] The lower Ca^{2+} content in bituminous coal C caused more extracellular enzymes to adsorb on the surface of lignin, decreasing the bioavailability of extracellular enzymes. This is one of the reasons why the yield of biomethane and the content of metabolic enzymes from CC-2 are lower than those of other experimental groups.

4. CONCLUSIONS

The results showed that the methane production followed the order AC-2 (17.28 mL/g) > BC-3 (14.88 mL/g) > CC-2 (12.51 mL/g). The fermentation liquid had organic matter with more types and higher contents during the early and peak stages but fewer types of organic matter in the terminal stage. Lignocellulose and benzene derivatives in the corn straw of AC-2 were degraded more than those of BC-3 and CC-2 after the gas production ended. Bacteria in the cofermentation system mainly metabolized carbohydrates after adding the fermentation substrate. The archaea community changed from the mixed-type (*Methanosarcina*) and aceticlastic (*Methanothrix*) methanogens to hydrogenotrophic methanogens (*Methanobacterium*) as the substrate changed from high metamorphic coal to low metamorphic coal. The higher gene abundance from fatty acid biosynthesis, nitrate reduction, and sulfate reduction in AC-2 accounted for efficient methane production. In summary, the combined fermentation of high-grade metamorphic coal and corn straw had an excellent degradation effect in the fermentation process, which provides scientific evidence for clean and efficient conversion of coal and straw.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c03331.

Gas production of single coal and single corn straw; attribution of peak numbers of liquid-phase products; pH at different times of gas production; and assignment of each peak of the FT-IR spectrum of corn straw (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Hongyu Guo − *School of Energy Science and Engineering, Henan Polytechnic University, Jiaozuo 454000, China;* orcid.org/0000-0002-2259-110X; Email: ghy1026@126.com

Authors

Wei Zhang − *China University of Mining and Technology, Xuzhou 221018, China; PetroChina Coalbed Methane Company Limited, Beijing 100028, China*

Zebin Wang − *PetroChina Coalbed Methane Company Limited, Beijing 100028, China*

Libo Li − *PetroChina Coalbed Methane Company Limited, Beijing 100028, China*

Minglu Zhang − *School of Energy Science and Engineering, Henan Polytechnic University, Jiaozuo 454000, China*

Wen Zhang − *PetroChina Coalbed Methane Company Limited, Beijing 100028, China*

Xiaoguang Sun − *PetroChina Coalbed Methane Company Limited, Beijing 100028, China*

Shixuan Sun − *PetroChina Coalbed Methane Company Limited, Beijing 100028, China*

Congliang Kou − *PetroChina Coalbed Methane Company Limited, Beijing 100028, China*

Weizhong Zhao − *Department of Environmental Engineering, Technical University of Denmark, DK-2800 Lyngby, Denmark*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c03331

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (41972178, 42172195, 42172199, 42072193), the Excellent Youth Science Foundation of Henan Province (202300410168), the Distinguished Young

Scholars Science Foundation of Henan Province (222300420008), and the Fundamental Research Funds for the Universities of Henan Province (NSFRF220301).

REFERENCES

(1) Senthamaraikkannan, G.; Gates, I.; Prasad, V. Development of a multiscale microbial kinetics coupled gas transport model for the simulation of biogenic coalbed methane production. *Fuel* **2016**, *167*, 188−198.
(2) Su, X. B.; Zhao, W. Z.; Xia, D. P. The diversity of hydrogen-producing bacteria and methanogens within an in situ coal seam. *Biotechnol. Biofuels* **2018**, *11*, No. 245.
(3) Strąpoć, D.; Mastalerz, M.; Dawson, K.; Macalady, J.; Callaghan, A. V.; Wawrik, B.; Turich, C.; Ashby, M. Biogeochemistry of microbial coal-bed methane. *Annu. Rev. Earth Planet. Sci.* **2011**, *39*, 617−656.
(4) Su, X. B.; Hong, J. T.; Xia, D. P.; Zhao, W. Z. The variety and transition of key intermediate liquid products during the process of coal-to-biohydrogen fermentation. *Int. J. Energy Res.* **2018**, *43*, 568−579.
(5) Shao, P.; Wang, A. K.; Wang, W. F. Effect of chemical structure of lignite and high-volatile bituminous coal on the generation of biogenic coalbed methane. *Fuel* **2019**, *245*, 212−225.
(6) Wang, B. B.; Yu, Z. S.; Zhang, Y. M.; Zhang, H. X. Microbial communities from the Huaibei Coalfield alter the physicochemical properties of coal in methanogenic bioconversion. *Int. J. Coal Geol.* **2019**, *202*, 85−94.
(7) Jones, E. J. P.; Voytek, M. A.; Warwick, P. D.; Corum, M. D.; Cohn, A.; Bunnell, J. E.; Clark, A. C.; Orem, W. H. Bioassay for estimating the biogenic methane-generating potential of coal samples. *Int. J. Coal Geol.* **2008**, *76*, 138−150.
(8) Liotta, F.; Esposito, G.; Fabbricino, M.; Hullebusch, E. V. D.; Pirozzi, F. Methane and VFA production in anaerobic digestion of rice straw under dry, semi-dry and wet conditions during start-up phase. *Environ. Technol.* **2016**, *37*, 505−512.
(9) Janke, L.; Weinrich, S.; Leite, A. F.; Terzariol, F. K.; Nikolausz, M.; Nelles, M.; Stinner, W. Improving anaerobic digestion of sugarcane straw for methane production: Combined benefits of mechanical and sodium hydroxide pretreatment for process designing. *Energy Convers. Manage.* **2017**, *141*, 378−389.
(10) Haghighi Mood, S.; Golfeshan, A. H.; Tabatabaei, M.; Jouzani, G. S.; Najafi, G. H.; Gholami, M.; Ardjmand, M. Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. *Renewable Sustainable Energy Rev.* **2013**, *27*, 77−93.
(11) Raslavičius, L.; Striugas, N.; Felneris, M. New insights into algae factories of the future. *Renewable Sustainable Energy Rev.* **2018**, *81*, 643−654.
(12) Yoon, S. P.; Jeon, J. Y.; Lim, H. S. Stimulation of biogenic methane generation from lignite through supplying an external substrate. *Int. J. Coal Geol.* **2016**, *162*, 39−44.
(13) Guo, H. Y.; Dong, Z. W.; Su, X. B.; Liu, S.; Jia, J. B.; Yu, H. F.; Xia, D. P. Synergistic biodegradation of coal combined with corn straw as a substrate to methane and the prospects for its application. *Energy Fuels* **2018**, *32*, 7011−7016.
(14) Neves, L.; Goncalo, E.; Oliveira, R.; Alves, M. M. Influence of composition on the biomethanation potential of restaurant waste at mesophilic temperatures. *Waste Manage.* **2008**, *28*, 965−972.
(15) Shen, R. X.; Jing, Y.; Feng, J.; Luo, J.; Yu, J. D.; Zhao, L. X. Performance of enhanced anaerobic digestion with different pyrolysis biochars and microbial communities. *Bioresour. Technol.* **2020**, *296*, No. 122354.
(16) Gao, J.; Chen, L.; Yuan, K.; Huang, H. M.; Yan, Z. C. Ionic liquid pretreatment to enhance the anaerobic digestion of lignocellulosic biomass. *Bioresour. Technol.* **2013**, *150*, 352−358.
(17) Schroyen, M.; Vervaeren, H.; Van, H. S. W. H.; Raes, K. Impact of enzymatic pretreatment on corn stover degradation and biogas production. *Bioresour. Technol.* **2014**, *173*, 59−66.
(18) Zheng, Y.; Zhao, J.; Xu, F. Q.; Li, Y. B. Pretrealment of lignocellulosic biomass for enhanced biogas production. *Prog. Energy Combust. Sci.* **2014**, *42*, 35−53.
(19) Kaparaju, P.; Serrano, M.; Thomsen, A. B.; Kongjan, P.; Angelidaki, I. Bioethanol, bio-hydrogen and biogas production from wheat straw in a biorefinery concept. *Bioresour. Technol.* **2009**, *100*, 2562−2568.
(20) Camarero, S.; Sarkar, S.; Ruiz-Dueñas, F. J.; et al. Description of a versatile peroxidase involved in the natural degradation of lignin that has both Manganese peroxidase and lignin peroxidase substrate interaction sites. *J. Biol. Chem.* **1999**, *274*, 10324−10330.
(21) Gold, M. H.; Alic, M. Molecular biology of the lignin-degrading basidiomycete Phanerochaete chrysosporium. *Microbiol. Rev.* **1993**, *57*, 605−622.
(22) Ghaffar, S. H.; Fan, M. Structural analysis for lignin characteristics in biomass straw. *Biomass Bioenergy* **2013**, *57*, 264−279.
(23) Biswas, B.; Pandey, N.; Bisht, Y.; Singh, R.; Kumar, J.; Bhaskar, T. Pyrolysis of agricultural biomass residues: Comparative study of corn cob, wheat straw, rice straw and rice husk. *Bioresour. Technol.* **2017**, *237*, 57−63.
(24) Achinas, S.; Euverink, G. J. W. Theoretical analysis of biogas potential prediction from agricultural waste. *Resour.-Effic. Technol.* **2016**, *2*, 143−147.
(25) Guo, H. Y.; Gao, Z. X.; Xia, D. P.; Yin, X. J.; Jia, J. B.; Dou, Y. L. Biological methanation of coal in various atmospheres containing CO_2. *Fuel* **2019**, *242*, 334−342.
(26) Zhang, R. *Preparation and Application of Novel Biogas Fermentation Microbial Agent*; Nanjing for University: Nanjing, 2014.
(27) Dearman, B.; Marschner, P.; Bentham, R. H. Methane production and microbial community structure in single-stage batch and sequential batch systems anaerobically co-digesting food waste and biosolids. *Appl. Microbiol. Biotechnol.* **2006**, *69*, 589−596.
(28) Ritalahti, K. M.; Justicialeon, S. D.; Cusick, K. D.; Ramoshernandez, N.; Rubin, M.; Dornbush, J.; Löffler, F. E. Sphaerochaeta globosa gen. nov. sp. nov. and *Sphaerochaeta pleomorpha* sp. nov. free-living, spherical spirochaetes. *Int. J. Syst. Evol. Microbiol.* **2012**, *62*, 210−216.
(29) Chen, S. Y.; Dong, X. Z. *Proteiniphilum acetatigenes* gen. nov. sp. nov. from a UASB reactor treating brewery wastewater. *Int. J. Syst. Evol. Microbiol.* **2005**, *55*, 2257−2261.
(30) Qiu, Y. L.; Kuang, X. Z.; Shi, X. S.; Yuan, X. Z.; Guo, R. B. *Paludibacter jiangxiensis* sp. nov. a strictly anaerobic, propionate-producing bacterium isolated from rice paddy field. *Arch. Microbiol.* **2014**, *196*, 149−155.
(31) Li, E. Z.; Deng, T. C.; Yan, L.; Zhou, J. Z.; He, Z. L.; Deng, Y.; Xu, M. Y. Elevated nitrate simplifies microbial community compositions and interactions in sulfide-rich river sediments. *Sci. Total Environ.* **2021**, *750*, No. 141513.
(32) Feng, D.; Xia, A.; Liao, Q.; Nizami, A. S.; Sun, C. H.; Huang, Y.; Zhu, X. Q.; Zhu, X. Carbon cloth facilitates semi-continuous anaerobic digestion of organic wastewater rich in volatile fatty acids from dark fermentation. *Environ. Pollut.* **2020**, No. 116030.
(33) Ma, K.; Liu, X. L.; Dong, X. Z. *Methanobacterium beijingense* sp. nov. a novel methanogen isolated from anaerobic digesters. *Int. J. Syst. Evol. Microbiol.* **2005**, *55*, 325−329.
(34) Zellner, G.; Messner, P.; Winter, J.; Stackebrandt, E. *Methanoculleus palmolei* sp. nov. an irregularly coccoid methanogen from an anaerobic digester treating wastewater of a palm oil plant in North-Sumatra, Indonesia. *Int. J. Syst. Bacteriol.* **1998**, *48*, 1111−1117.
(35) Krivoruchko, A.; Zhang, Y. M.; Siewers, V.; Chen, Y.; Nielsen, J. Microbial acetyl-CoA metabolism and metabolic engineering. *Metab. Eng.* **2015**, *28*, 28−42.
(36) Gieg, L. M.; Fowler, S. J.; Berdugo-Clavijo, C. Syntrophic biodegradation of hydrocarbon contaminants. *Curr. Opin. Biotechnol.* **2014**, *27*, 21−29.
(37) Rezadehbashi, M.; Baldwin, S. A. Core sulphate-reducing microorganisms in metalremoving semi-passive biochemical reactors

https://doi.org/10.1021/acsomega.2c03331
ACS Omega 2022, 7, 31138−31148

and the co-occurrence of methanogens. *Microorganisms* **2018**, *6*, No. 16.
(38) Yang, B.; Wyman, C. E. BSA treatment to enhance enzymatic hydrolysis of cellulose in lignin containing substrates. *Biotechnol. Bioeng.* **2006**, *94*, 611−617.
(39) Yue, Z. B.; Ma, D.; Peng, S.; Zhao, X.; Chen, T. H.; Wang, J. Integrated utilization of algal biomass and corn stover for biofuel production. *Fuel* **2016**, *168*, 1−6.

https://doi.org/10.1021/acsomega.2c03331
ACS Omega 2022, 7, 31138−31148