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The mechanisms of biogenic methane metabolism by synergistic biodegradation of coal and corn straw

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ABSTRACT

The mechanisms associated with the biomethane metabolism through the synergistic biodegradation of both coal and corn straw were explored to improve the utilization rate of corn straw. This applies to the filling of the goaf with corn straw and the production of biomethane using indigenous bacteria in the mine water with coal. The results showed that new macromolecular substances (e.g., Tetracosane and Pentacosane) were produced on the third day. A lower coal rank leads to a lower biodegradation rate of low-molecular-weight substances (e.g., butyric acid and valeric acid). Under the addition of coal samples, the biodegradation rate of cellulose, hemicellulose and lignin in corn straw could reached up to 29.82%, 35.79% and 6.16%, respectively. The addition of corn straw promoted the complementary advantages of archaeal genera (such as *Methanosarina* and *Methanospirillum*) and decreased the adverse bacterial genera (such as *Desulfovibrio* and *Pseudomonas*) in the fermentation system of single coal.

1. Introduction

Biogenetic coalbed methane is an important component of coalbed methane resources and is produced from coal that has been used as a carbon source and converted into small molecular substances (dominated by methane) by anaerobic microorganisms (Mayumi et al., 2016). However, coal as a complex organic-mineral resource that is difficult to biodegrade by microorganisms (Vandenbroucke and Largeau, 2007). Methods such as microbial pretreatment, chemical pretreatment and the addition of trace elements have been proposed to increase biomethane production. Xia et al. studied the effects of trace elements on biological metabolic processes and methane production of coal, observing that methane production can be increased by up to 37% with the appropriate addition of trace elements and that the transcription of the mcrA gene also increases (Schmidt et al., 2018; Xia et al., 2017; Capson-Tojo et al., 2018; Mirco et al., 2018). Pretreatment of coal samples also have an important impact on biogas production. After the coal sample is treated with HNO_3, H_2O_2, and fungi (such as white rot fungi), biomethane production increases to varying degrees (Tianyu et al., 2018; Haider et al., 2018). However, the above treatment is not conducive to future commercial utilization, as it promotes groundwater pollution and has high costs. Agricultural waste is a potential resource for anaerobic fermentation that is composed of cellulosic substances and is hydrogen-rich, with the substrates being more readily degraded by microorganisms if its H/C ratio is moderate (Yao and Chen, 2016). These wastes are degraded with coal, which can be complemented with respect to the H/C ratio to achieve better biogas production. It has been scholars have proposed that coal mine goaves can be filled with corn straw, which is combined with the residual coal and produce biomethane under the action of indigenous microorganisms from the coal mine water (Guo et al., 2018). After finishing the coal mining, when the gas flows into the goaf, the pressure will increase, which is conducive to the anaerobic fermentation of coal and corn straw (Lindeboom et al., 2016; Scamardella et al., 2019; Li et al., 2017). Moreover, filling the goaf with corn straw not only solves common problems of other filling materials (such as gangue and paste), such as low filling rate and high investment. This technique improves the utilization rate of corn straw, and also promotes the conversion of residual coal and corn straw into clean energy using biotechnology. This achieves resource reuse, energy conservation and emission reduction. However, the content of harmful elements in different coal samples differed. When underground water flows into the goaf, these harmful elements in the coal samples may accelerate and dissolve into the water under the addition of corn straw, thus destroying the underground aquatic environment need to be

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reduced and the theory needs to be applied on-site, which requires further comprehensive research.

Many scholars have confirmed that the synergistic degradation of coal and straw can increase biomethane production. Guo et al. showed that the greatest biomethane production occurred during the co-biodegradation of coal and rice straw (Guo et al., 2019). Yoon et al. observed that biomethane production from straw and lignite was much higher than that observed using single lignite, the optimum combination ratio of lignite and straw into biomethane is 3:1 (Yoon et al., 2016). Guo et al. selected three coal samples, corn straw and the indigenous bacteria from the coal mine water to carry out biological gas production experiments, observing that the optimal proportion of the synergistic biodegradation of lignite B, bituminous coal D and bituminous coal C with corn straw was 2:1, 3:1, and 3:1, respectively (Guo et al., 2018). The above reports demonstrate the high efficiency of the co-biodegradation of coal and straw for biogas production, but its underlying mechanism is still unclear.

Therefore, in this study, three coal samples with different degrees of metamorphism and corn straw as well as the intermediate liquid products, the cellulosic substances contents of corn straw and the microbial community structure were evaluated to investigate the underlying mechanism of the synergistic biodegradation of different coal samples and corn straw.

2. Experimental materials and methods

2.1. Sample collection and preparation

Lignite B (the Yimin mine in Inner Mongolia, China), bituminous coal D (the Qianqiu mine in Henan Province, China), and bituminous coal C (the Quanling mine in Shanxi Province, China) were used in the biological gas production experiments, while corn straw was collected from Yongxing village, Jiaozuo City in Henan Province. The proximate and ultimate analyses of samples are shown in Table 1. The ultimate and proximal analyses were carried out according to the Standards ISO 17247-2013 and ISO 17246-2010, respectively. The coal samples were crushed to 0.1–0.15 mm, and corn straw was ground through a 0.613 mm screen. The prepared lignite B, bituminous coal D, and bituminous coal C were separately mixed with the corn straw in proportions of 2:1, 3:1 and 3:1, respectively, and used as the fermentation materials.

2.2. Configuration of enrichment culture of methanogens

Fresh mine water (experimental bacterial source) was collected in a sterilized plastic drum, stored in the laboratory at 4 ˚C, and was used in medium to enrich for methanogens at 35 ˚C. The initial pH of the prepared methanogen medium is 7.43.

Enrichment culture of methanogens: 1000-mL mine water was added to 0.4 g K_2HPO_4, 2.0 g $MgCl_2$, 0.4 g KH_2PO_4, 1.0 g yeast, 1.0 g NH_4Cl, 0.001 g resazurin, 0.5 g cysteine, 0.2 g Na_2S, 0.2 g $NaHCO_3$, 2.0 g sodium acetate, 0.2 g KCl, 2.0 g NaCl and 10 mL of trace element solution (Guo et al., 2018).

Trace element solution: 1000-mL distilled water was added to 1.5 g triglycolamic acid, 0.5 g $MnSO_4{\cdot}2H_2O$, 3.0 g $MgSO_4{\cdot}7H_2O$, 0.1 g $FeSO_4{\cdot}7H_2O$, 1 g NaCl, 0.1 g $CoCl_2{\cdot}6H_2O$, 0.1 g $CaCl_2{\cdot}2H_2O$, 0.01 g $CuSO_4{\cdot}5H_2O$, 0.1 g $ZnSO_4{\cdot}7H_2O$, 0.01 g H_3BO_3, 0.01 g $AlK(SO_4)_2$, 0.02 g $NiCl_2{\cdot}6H_2O$ and 0.01 g Na_2MoO_4 (Guo et al., 2018).

All of the above formula of methanogen medium belong to analytical reagents.

Table 1
Proximate and ultimate analyses of experimental samples/%

Sample	M_{ad}	A_{ad}	V_{ad}	$R_{o,ran}$	C_{daf}	H_{daf}	N_{daf}	S_{daf}	O_{daf}
lignite B	7.46	10.71	44.58	0.43	67.14	5.52	1.83	0.88	24.63
bituminous coal D	5.22	11.46	40.52	0.56	71.89	4.71	1.02	1.66	20.72
bituminous coal C	22.31	2.09	8.32	1.51	91.20	4.51	0.46	0.53	3.3
corn straw	10.88	10.92	60.78	/	47.05	6.05	0.10	0.20	46.6

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

2.3. Experimental methods

(1) Biological biogas production experiment

Fermentation substrates were prepared according to Table 2. Corresponding samples and 200 mL of culture medium enriched for 4 days were added to the 250-mL experimental bottle that was sterilized under high pressure and filled with N_2 for 2–3 min. These experimental bottles were sealed, connected to a water collection bottle, and placed in incubator at 35 ˚C. The biogas production experiment included three parallel samples, and the biogas production was recorded every three days by the drainage method. The experimental duration was 18 days. The biomethane concentration was quantified at the end of the biogas production experiment using gas chromatography instrument (Agilent 7890GC; Agilent Technologies Inc, Santa Clara, CA, USA). The methane concentration, methane production and the percentage of carbon in coal or carbon in corn straw converted to biomethane for each experiment are shown in Table 2. The fermentation solution was analyzed every 3 days by GC–MS, and 16S rRNA gene sequencing was performed at the peak of gas production.

(2) Intermediate liquid product analysis

For the GC–MS analysis, a gas chromatograph/mass spectrometer (Agilent GC–MS 7890-5977A, USA) equipped with the free fatty acid phase (FFAP) chromatographic column (30 m × 0.25 μm × 0.5 mm) was used to analyse the intermediate liquid products during the fermentation processes.

(3) Determination of cellulosic substances

Corn straw was extracted by soxhlet extraction with ethanol to remove resins and pigments and hydrolyzed with 72% concentrated sulfuric acid and 4% dilute sulfuric acid in two steps to hydrolyze the organic components of lignin into easy-to-quantify substances. Cellulose and hemicellulose were hydrolyzed to form monosaccharides, which were quantified by high performance liquid chromatography, and lignin were quantified by burning method.

(4) Microbial structure analyses

Samples were collected at different fermentation stages to investigate the microbial dynamics. Genomic DNA was extracted from the fermentation solution using an E.Z.N.A. Soil DNA Kit (Omega, USA). The DNA concentration was evaluated using a Qubit 2.0 instrument (life, USA) to ensure that the requirements for sequencing were met. Prior to sequencing, the PCR products from each fermentation solution were normalized in equimolar amounts, which were used to generate the PCR amplicon libraries. Then, the fermentation solution was delivered to Sangon BioTech (Shanghai Biotech Biotechnology Co., Ltd.) for library construction and sequencing using an Illumina MiSeq system (2 × 300 bp reads; Illumina, USA) according to the instructions.

(5) Bioinformatic and statistical analyses

Following MiSeq sequencing, the obtained sequences were automatically annotated to different classification levels (including the