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Microbial Electrolysis Facilitates Efficient Coal Bioconversion and CO_2-to-Methane Production

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Abstract: Methanogenic bacteria can convert exogenous CO_2 into biomethane, aiding carbon sequestration. Biological methods enhancing coal and CO_2 co-transformation during coalbed methane production are gaining attention, with microbial electrolysis cell (MEC) technology showing promise in improving anaerobic digestion (AD). This study compared AD and MEC-AD systems for biomethane co-production from coal and CO_2. The MEC-AD system produced 50.96 ml of methane, a 135.49% increase over AD alone (21.64 ml), and reduced CO_2 levels by 51.51% more than AD. 16S rRNA analysis showed MEC technology improved hydrolysis and interspecies electron transfer during coal digestion, boosting biomethane production. These findings highlight MEC's potential to enhance anaerobic digestion efficiency and support low-carbon or carbon-negative technologies for effective carbon sequestration.

Keywords: Biomethanation; Microbial Electrolysis Cell; Carbon Dioxide Reduction.

1. Introduction

Fossil fuels currently represent one of the most significant sources of energy for human society and are also the primary source of atmospheric carbon dioxide (CO_2). China, having contributed the most to global economic growth over the past decade, stands as the world's largest energy producer, consumer, and CO_2 emitter. With annual CO_2 emissions reaching approximately 10 billion tons[1], China accounts for roughly 30% of the global total, underscoring the urgency of developing CO_2 reduction and fixation technologies[2-4].

A microbial electrochemical system (MES) is an electrochemical device that uses electrically active microorganisms to convert organic matter's chemical energy into electrical energy or other forms. These microbes, attached to the anode, oxidize organic matter, capturing electrons and protons. Electrons move through cellular enzymes to the anode surface, while protons are released. The cell generates ATP during this process. Electrons then travel to the cathode via an external circuit, and protons migrate to the cathode. At the cathode, electrons, protons, and reductants react to form stable products, completing the electrochemical conversion[5]. A specific MES type, the microbial electrolysis cell (MEC), supplies external energy to drive non-spontaneous reactions[6]. In MECs, electroactive microbes at the anode degrade organic matter, releasing electrons that power methanogenic archaea at the cathode, enhancing energy and resource efficiency[7].

This study aimed to investigate the role of microbial electrolysis cell (MEC)-assisted anaerobic digestion (AD) in biomethane production from coal and CO_2 via anaerobic fermentation. To this end, both AD and MEC-AD reactors were constructed using high-volatile bituminous coal from Huainan as the substrate, to evaluate the impact of MEC on biomethane production. Furthermore, 16S rRNA high-throughput sequencing was employed to analyze the microbial community composition, with the goal of assessing alterations in functional microbial communities influenced by applied electric fields and predicting corresponding changes in microbial functions.

2. Materials and Methods

2.1. Sources of Coal Samples and Microbial Communities

Coal samples were collected from the Zhangji Coal Mine (116°29′E, 32°47′N) in Huainan Mining District, Anhui Province. The coal type is high-volatile bituminous coal (Vad 33.52%). Samples were collected using a fully automatic drilling rig (ZYWL-4000SYZYW-3200, Φ73 mm) from the 1313 (3) working face of the 13# coal seam, minimizing air exposure. Fresh coal was sealed in sterile bags and transported to the laboratory in an ice box. In the lab, the outer layer (~1–2 cm) was removed with a sterile blade, and the inner material was ground into powder using a sterile mortar and pestle. The coal powder was sieved through a 60-mesh sieve and stored in an airtight canister. All procedures were conducted in an anaerobic glove box. The coal's fundamental properties are listed in Table 1. Industrial analysis followed ISO 17246-2024, and elemental analysis followed ISO 17247-2013.

Table 1. Proximate and ultimate analysis results of coal samples

Proximate (%)				Ultimate analysis (%)					*Ro* (%)
M_{ad}	A_{ad}	V_{ad}	FC_{ad}	C	H	O	N	S	
1.66	9.67	33.52	55.15	84.89	1.41	11.57	1.75	0.38	1.24

2.2. Experimental Setup and Operation

A 250-mL single-chamber, membrane-free AD and MEC-AD reactor was used (Fig. 1). The anode was carbon felt (0.3 cm × 0.3 cm × 0.1 mm), and the cathode was titanium mesh (0.3 cm × 0.3 cm × 0.1 mm). The anode was treated with 1 mol/L NaOH and HCl for 24 hours each, rinsed to neutral, ultrasonically cleaned for 15 minutes, and stored after furnace treatment at 350°C for 60 minutes. The reactor was loaded with coal powder, bacterial solution, and MSM (1:1:10 ratio, 15 g:15 mL:150 mL) plus 20 mL PBS buffer to maintain a neutral pH. The headspace gas was 60% CO_2 and 40% N_2. The reactors were connected to an electrochemical workstation with a 10 Ω resistor, applying 0.8 V and recording data. Maintained at 30 °C, the system deemed started when current exceeded 1 mA. A control group without voltage was included. Gas composition was analyzed every 3 days via gas chromatography, and gas exchange occurred at biomethane peak production in the MEC group. Samples for 16S rRNA sequencing were collected at the start and end of the reaction.

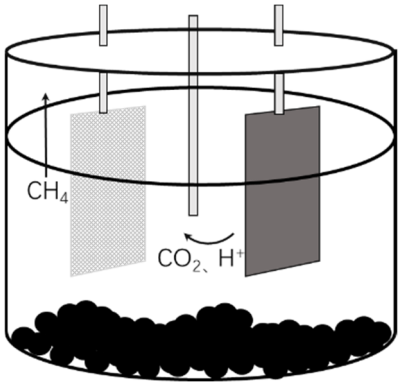


Fig 1. Diagram of the experimental setup of the electrolytic cell system

2.3. Scanning Electron Microscope

A Thermo Fisher Scientific Apreo 2S field emission scanning electron microscope was utilized to observe electroactive biofilms (EABs) and microorganisms adhered to the surfaces of the cathode and anode at the conclusion of the reaction.

2.4. Microbial Community Analysis

Culture samples were collected on days 10, 25, and 45 and stored at -80°C. Total DNA was extracted using the FastDNA® SPIN Kit for Soil (MP Biomedicals). The V4-V5 region of the bacterial 16S rRNA gene was amplified with 515F/907R primers, and the V8-V9 region of the archaeal 16S rRNA gene was amplified with 1106F/1378R primers. Amplification and sequencing were performed by Hangzhou Lianchuan Biotechnology Co., Ltd. For 16S rRNA analysis, Amplicon Sequence Variants (ASVs) were generated using the DADA2 algorithm in QIIME. Species annotation was conducted using the SILVA database (Release 138, https://www.arb silva.de/documentation/release138/) in the NT-16S database with a confidence threshold of 0.7.

3. Results and Discussion

3.1. Determination of Gas Components

The biomethane and CO_2 content in the headspace of MEC and AD groups were analyzed via gas chromatography, with gas exchanges on days 9 and 18. Methane production in the AD group increased gradually, while the MEC group showed a significant rise during the first two cycles, peaking at 28.12 ml in the first cycle. After the day 18 gas exchange, methane production in the MEC group slightly decelerated (Fig. 2a). Overall, the MEC group's cumulative methane production was 135.49% higher than the AD group's. For CO_2, the AD group showed a gradual decline, while the MEC group had a more rapid reduction, especially in the first two cycles. After day 18, the CO_2 reduction rate in the MEC group slightly decreased (Fig. 2b). Cumulatively, CO_2 conversion was 46.93 ml in the MEC group and 9.105 ml in the AD group, with conversion rates of 52.13% and 10.12%, respectively. These results show that MEC technology effectively enhances CO_2 fixation and biomethane production.

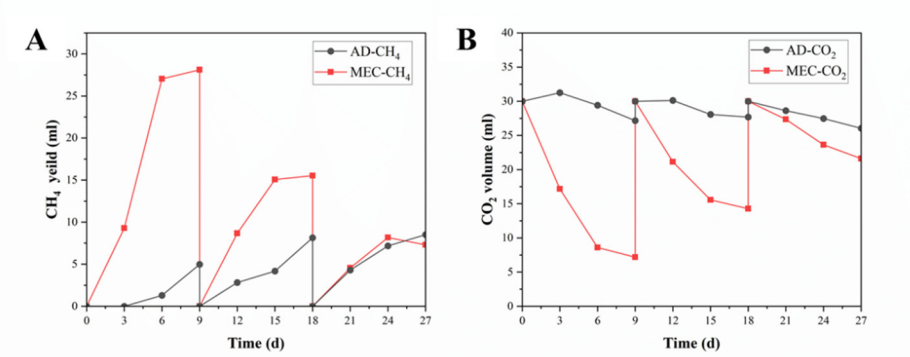


Fig 2. Plot of volume change of (a) biomethane and (b) carbon dioxide in the air volume fraction in the headspace of the reactor

3.2. Scanning Electron Microscope Analysis

SEM images revealed a thick and dense biofilm on the anode surface (Fig. 3a), potentially comprising conductive proteins such as heme cytochromes, which facilitate electron transfer between electroactive microorganisms and the anode. In contrast, minimal biomass was observed on the cathode (Fig. 3b), indicating that microbial adhesion to the cathode is challenging. Observations of the morphological structure of cathode-associated microorganisms revealed a relatively uniform population of methanogenic bacteria, predominantly spherical in shape and partially forming cell clusters.

3.3. Cyclic Voltammetry Analysis

Cyclic voltammetry (CV) was used to assess the redox processes and capacitance characteristics of the cathode at the start and end of the reaction. The CV curves showed no distinct redox peaks, indicating energy storage primarily through electrode bilayer adsorption (Fig. 4). The area under the Current 2 curve was larger than that of Current 1,

suggesting enhanced capacitive properties during the reaction, likely due to biofilm formation, which increases surface complexity and charge storage capacity. Additionally, the Current 2 curve showed higher currents across the same potential range, indicating that the cathode biofilm facilitates electron transfer and accelerates the CO_2 reduction reaction by providing additional active sites. This enhances the electrode's electrochemical performance.

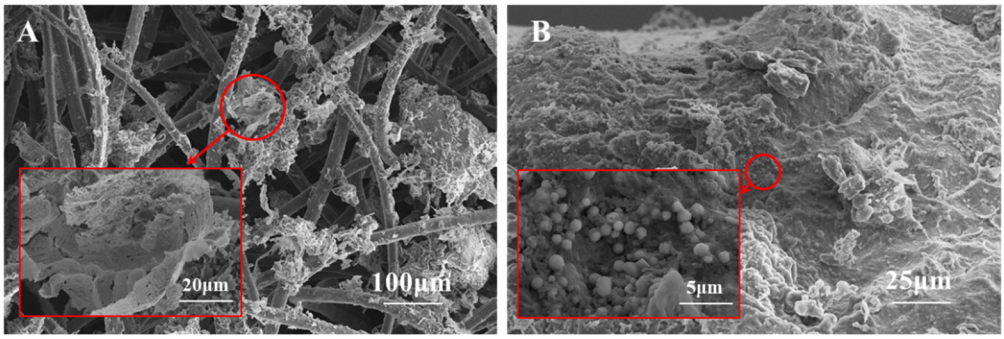


Fig 3. Scanning electron microscopy (SEM) observation of (a) anodic biofilm; (b) cathodic biofilm as well as cathodic microorganisms

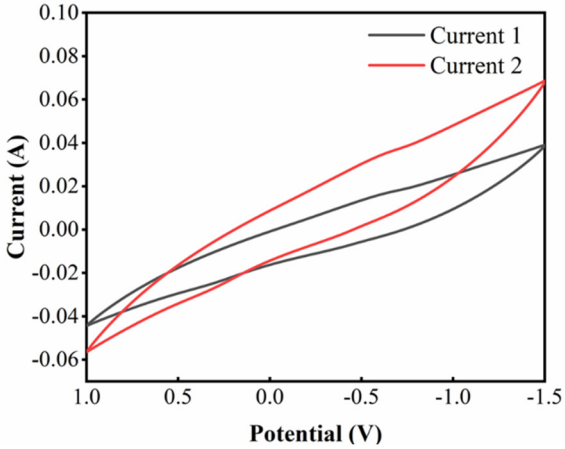


Fig 4. CV test curves at the initial cathode and at the end of the reaction

were analyzed at the end of the reaction. The application of an external voltage significantly increased the Shannon diversity of the fermentation broth from 4.82 ± 0.09 to 7.01 ± 0.08 (Fig. 5a). NMDS analysis based on Bray-Curtis distances revealed significant differences in microbial community structure among treatments (Fig. 5b). In the AD group, the phylum Firmicutes dominated with over 90% abundance, while in the MEC group, the phylum Proteobacteria showed a marked increase. At the genus level, *Bacillus* was highly enriched in the AD group (over 50%), whereas the MEC group exhibited a more balanced distribution of *Clostridium*, *Bacillus*, *Sedimentibacter*, *Acetoanaerobium*, and *Providencia* (Fig. 5d). These results indicate that external voltage application enhances microbial diversity and alters community structure, potentially improving anaerobic fermentation and CO_2 reduction efficiency.

3.4. Microbial Community Composition and Diversity

The microbial composition and diversity in the reactor

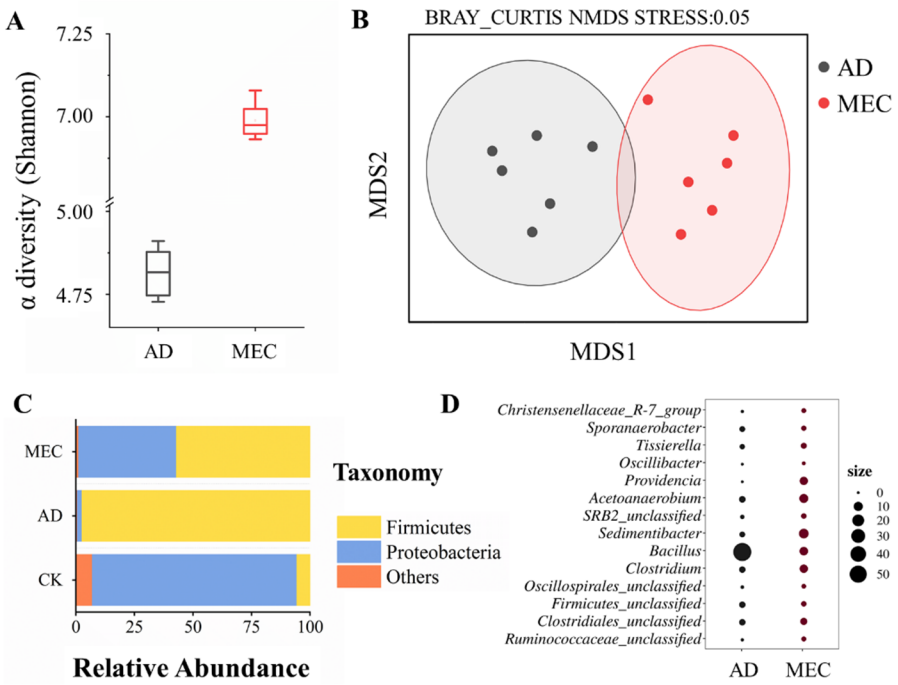


Fig 5. AD and MEC bacterial diversity and composition. (a) α-diversity; (b) β-diversity; (c) phylum-level stacked plot; (d) genus-level bubble plot

At the reaction's end, archaeal species composition was analyzed. In the MEC group, Euryarchaeota dominated with over 99% abundance, compared to 75.65% in the AD group, showing voltage-driven enrichment of specific Euryarchaeota (Fig. 6a). Methanosarcina, a hydrogenotrophic methanogen, was the main genus in both groups, capable of methane production via multiple pathways. However, Methanoculleus and Methanomassiliicoccus were more abundant in the MEC group (Fig. 6b). These genera convert inorganic carbon to methane, with Methanoculleus using CO_2 and hydrogen or diols. The enrichment of these CO_2-to-methane converting archaeal genera, facilitated by an external power source, likely enhances CO_2 methanation.

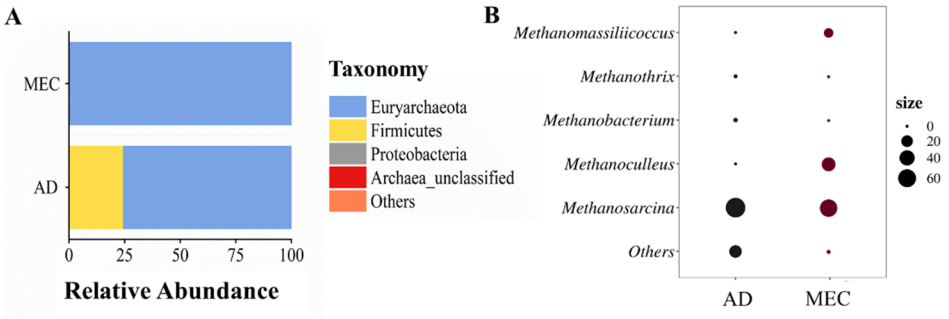


Fig 6. Composition of AD and MEC archaea. (a) phylum-level stacked diagram; (b) genus-level bubble diagram

3.5. Analysis of Related Functional Genes

Differences in functional genes related to extracellular electron transport, EAB formation, and cytochrome C between MEC-AD and AD were analyzed using PICRUSt 2, with functional genes screened based on a p-value (t-test) of <0.05 (Figure 7). The results showed that, except for pilD, the relevant functional genes were significantly enriched in MEC-AD. This suggests that the applied voltage significantly enhanced microbial extracellular electron transfer, EAB formation, and cytochrome C, thereby facilitating the conversion of coal and exogenous CO_2 into biomethane.

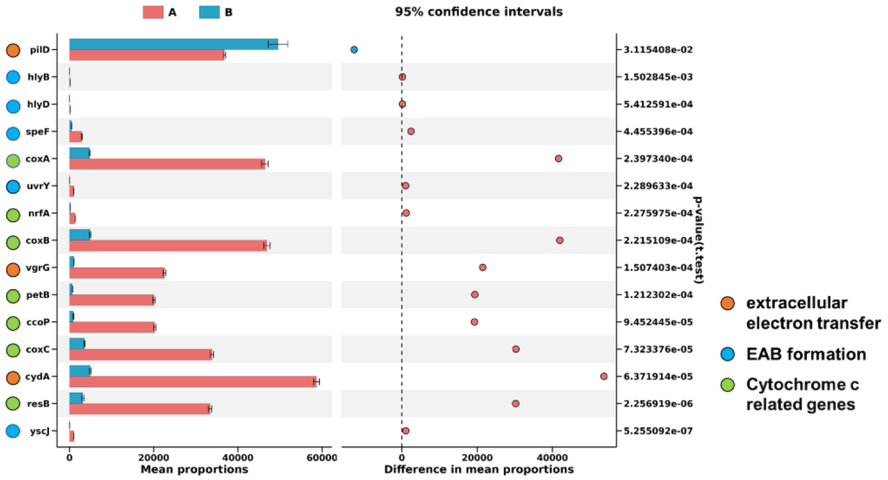


Fig 7. Differences between functional genes

4. Conclusion

This study investigated MEC-AD and AD reactors using coal as the substrate. The MEC-AD system, leveraging external voltage, enhanced biomethane production efficiency, yielding 50.96 ml of methane, 135.49% higher than the AD system's 21.64 ml. It also demonstrated significant potential for converting exogenous CO_2. Bacterial diversity in the MEC-AD system increased markedly, with balanced development of Clostridium, Bacillus, Sedimentibacter, Acetoanaerobium, and Providencia. Among archaea, Methanoculleus and Methanomassiliicoccus, which convert inorganic carbon to methane, were notably enriched. Functional genes for extracellular electron transfer, EAB formation, and cytochrome C were significantly upregulated. Thus, applying an external power source effectively promotes microbial conversion of coal and CO_2 to methane, offering a novel approach to boost coalbed methane production and reduce CO_2 emissions.

Acknowledgments

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