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Composite electrode plate for enhancing methanogenesis and microbial community performance in microbial electrolytic cell#

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ABSTRACT

Anaerobic digestion coupled with microbial electrolytic cell (AD-MEC) is a promising method for waste energy conversion. However, the technology is currently stuck in a bottleneck. The optimization of the electrode structure is a prospective way to improve the methane production efficiency of AD-MEC. In this study, a new composite electrode structure applied in AD-MEC is developed, consisting of stainless steel wire mesh and carbon felt connection combined to form an electrode, and the two electrodes are connected by an insulating cloth to form a composite electrode plate. The composite electrode plate was applied to the AD-MEC reactor and the traditional AD reactor for comparative experiments. The results show that, the biogas production of the AD-MEC reactor increased by 76.6% compared to the control reactor at a voltage of 0.7V. In addition, the start-up time of the AD-MEC reactor was significantly reduced, the average daily methane content increased by 13.5%, and the cumulative methane production increased by 105.6%. Meanwhile, there are certain differences in microbial community richness and diversity between AD-MEC reactor and control reactor, which is considered to be one of the reasons for the improvement of methane production efficiency. The increase of biogas production and methane content demonstrates that the structural optimization of stainless steel wire mesh carbon felt composite electrode is an effective method to improve AD-MEC efficiency, waste treatment and energy recovery.

Keywords: Composite electrode plate; Stainless steel wire mesh; Carbon felt; Microbial electrolytic cell; Methanogenesis

1. INTRODUCTION

The extensive consumption of fossil fuels compels humanity to confront issues of energy shortages and environmental degradation[1,2]. In recent years, the AD-MEC technology, which coupled anaerobic digestion with microbial electrolysis cell for methane production, has been developed and has become a research hotspot[3,4]. Its advantages primarily include low microbial cost, abundant sources of electricity and organic matter (carbon sources), and well-established technologies for methane storage and transportation[5]. Based on the characteristics of MEC, researchers optimize the electrode materials and electrode structures to enhance the methane production efficiency of AD-MEC[6-9].

However, few studies have focused on the electrode surface area and the distance between the anode and cathode. To address these issues, a composite electrode plate is proposed by the author. Stainless steel mesh and carbon felt composite electrodes are used as the anode and cathode in the AD-MEC, and they are connected by a porous insulating fabric. The application of the composite electrode plate significantly shortens the distance between the cathode and anode, reduces the internal resistance of the AD-MEC, increases the surface area available for microbial attachment, and enhances the transfer of H^+ and microbial extracellular electron transfer (EET) processes, thereby improving methane production efficiency.

To validate the above hypothesis, a thermostatic AD-MEC experimental system is constructed by the author, and comparative experiments are conducted between the AD reactor and the AD-MEC reactor. Based on anaerobic experiments, 16S rRNA sequencing analysis of bacteria and archaea is performed on the water, sludge, and microorganisms attached to the anode and cathode of the composite electrode plates in each reactor. This analysis is used to further investigate the microbial community changes in the MEC reactor with the composite electrode plates compared to the AD reactor.

2. MATERIAL AND METHODS

2.1 Substrate and inoculum

The inoculum used in the anaerobic digestion experiment is active sludge obtained from a mesophilic anaerobic digester of an ecological company in Anhui

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Province, China. After the inoculated sludge is obtained, it is incubated in a biochemical incubator set at 35°C for later use. The total solids (TS) of the inoculated sludge are measured at 30.84±0.34%, and the volatile solids (VS) are measured at 10.48±0.14%. The water used in the anaerobic experiment is the wastewater diluted from the mesophilic anaerobic digester. Additionally, 12.5 mL of nutrient solution for trace elements were added per 1 L waste water. The nutrient solution (per L): nitrilotriacetic acid: 1.5g, $MgSO_4 \cdot 7H_2O$: 3.0g; $MnSO_4 \cdot H_2O$: 0.5g; NaCl: 1.0g; $FeSO_4 \cdot 7H_2O$: 0.1g; $CoCl_2 \cdot 6H_2O$: 0.1g; $CaCl_2$: 0.1g; $ZnSO_4 \cdot 7H_2O$:0.1g; $CuSO_4 \cdot 5H_2O$: 0.01g; $AlK(SO_4)_2 \cdot 12H_2O$: 0.01g; H_3BO_3: 0.01g; and $Na_2MoO_4 \cdot 2H_2O$:0.01g[10]. To minimize errors, the substrates in each reactor are standardized to cellulose, hemicellulose, lignin, and soluble starch produced by China National Pharmaceutical Group. A total of 10 grams of substrate is added to each reactor, with the proportions based on the composition of rice straw, which are 39.9% cellulose, 23.18% hemicellulose, 5.70% lignin, and the remaining amount as soluble starch.

2.2 Experiment bench setup

The thermophilic MEC anaerobic digestion test bench is composed of a microbial anaerobic reaction module, a current detection module, and a gas collection module. The anaerobic digestion module consists of an anaerobic reactor, a water bath, and a custom-designed synergistic stirring device. The anaerobic reactor is a cylindrical vessel made of high-density polyethylene, with dimensions of 570 mm in length, 340 mm in width, and 700 mm in height. The total volume and working volume of the reactor are 1 liter and 0.7 liters, respectively. The reactor lid is equipped with an integrated channel that extends below the liquid surface, through which a stainless steel stirrer passes. The stirrer's submerged portion is coated with a silicone material. The water bath operates on either 120V or 230V AC voltage, and the working temperature is set to 35°C before the experiment begins. All reactors are stirred at the same speed using stainless steel/silicone blades driven by a gearbox. The stirrer motor is powered by a 24V DC voltage.

The gas collection module consists of gas sampling bags and a gas flow meter produced by Anaero Technology. The gas flow meter measures the volume of gas collected by counting the number of drum rotations. Before the experiment, multiple calibrations are performed, and the average gas volume per drum rotation is recorded. The gas produced by the reactor is conveyed through a silicone tube connected to the bottom of the reactor. A certain amount of gas enters the reactor, causing the drum inside to rotate. The number of rotations is recorded in a microcontroller, and the gas volume is calculated by multiplying the drum rotations by the volume per rotation, which is then read by a computer terminal.

The current detection module is composed of a DC power supply, an ammeter, and a data logger (JM342 Modbus). The DC power supply is connected to both ends of the electrode plates, providing a stable voltage. The ammeter continuously monitors the current passing through the electrode plates during the anaerobic reaction, and the data logger records the current data in real-time.

2.3 Experimental methods

The total solids (TS), volatile solids (VS), and mixed liquor suspended solids (MLSS) are measured according to the APHA standard methods[11]. Gas production is collected using a gas flow meter (Anaero Technology, UK). The biogas composition (CH4, CO2) is analyzed using a gas chromatograph (Agilent, GC8860, USA). The pH value is measured using a pH meter (REX, PHSJ-4F, China). After the experiment begins, water samples from the reactor and gas samples from the gas bags are collected at fixed times daily. These measurements are repeated three times using the methods described above.

2.4 *Microbial community analysis*

In order to analyze the changes in microbial communities in the AD-MEC reactors with composite electrode plates and AD reactors, supernatant and sludge samples from each reactor, as well as sludge attached to the anode and cathode of the composite electrode plates in the AD-MEC reactors, are collected at the end of the experiment. These samples are sent to a third-party testing platform for microbial diversity analysis. The testing items include 16S rRNA standard bacterial detection and standard archaeal detection. The V3-V4 region of the bacterial 16S rRNA gene, with a variable length of approximately 468 bp, is amplified using primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The V4-V5 region of the archaeal 16S rRNA gene, with a variable length of approximately 434 bp, is amplified using primers Arch524F (5'-TGYCAGCCGCCGCGGTAA-3') and Arch958R (5'-YCCGGCGTTGAVTCCAATT-3'). After microbial diversity testing is completed, the impact of the composite electrode plates in the AD-MEC reactors on microbial communities is analyzed based on the

microbial community composition. Sequencing results are analyzed on the Majorbio Cloud Platform, a third-party platform.

2.5 Section of results

Each result was reported as the mean value of measurements from three replicate samples. Student's t test was performed to evaluate significance with a threshold of 0.05.

3. RESULTS

3.1 Cumulative biogas production and daily biogas production

The anaerobic experiment is divided into three groups. Group A consists of reactors with only activated anaerobic sludge. Group B consists of reactors with anaerobic sludge and substrate. Group C consists of reactors with anaerobic sludge, substrate, and an applied voltage. Since the effect of voltage on AD-MEC is not the focus of this study, the applied voltage is set at a constant 0.7V, based on literature references[12]. The anaerobic experiment runs for a total of 34 days, after which the reactors almost stop producing biogas. The cumulative gas volume produced by each reactor is measured by a gas flow meter, and the data is input into a computer terminal.

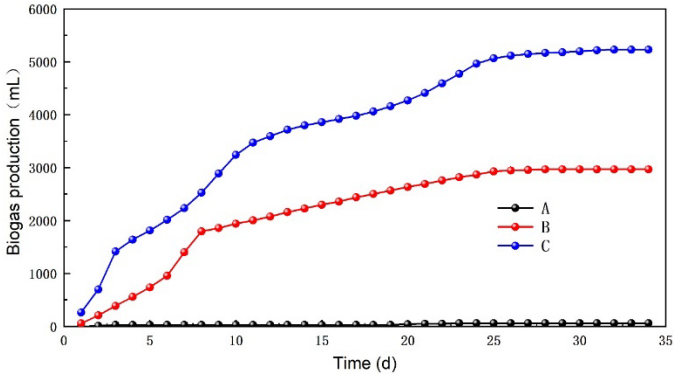


Fig. 1 Cumulative biogas production of each reactor

Figure 1 presents the cumulative biogas production of each reactor. As shown in Figure 1, reactor A, which only contains activated anaerobic sludge without any substrate, has limited anaerobic digestion potential, producing a cumulative 61.17 mL of gas over the 34-day experimental period. The cumulative biogas production trends of reactors B and C are generally similar. These trends can be divided into four stages: the initiation stage, the substantial gas production stage, the stable gas production stage, and the gas production bottleneck stage.In Figure 1, it can be seen that reactor B experiences an initiation stage for the first 5 days, followed by a substantial gas production stage from day 5 to day 9. Starting on day 10, reactor B enters a stable gas production stage until gas production stops after day 29. Reactor C, which includes a composite electrode plate and an applied voltage, initiates faster than reactor B. reactor C goes through the initiation stage in 3 days, quickly entering the substantial gas production stage and maintaining it for a longer period (from day 3 to day 13). Additionally, from day 19 to day 25, there is a noticeable increase in gas production, suggesting that some substrates undergo further hydrolysis enhanced by MEC, thus increasing the biogas production potential. By the end of the 34-day experiment, the cumulative biogas production of Reactors B and C is 3038.45 mL and 5320.39 mL, respectively. To reduce errors, the biogas production of Reactor A is subtracted from the production of Reactors B and C. Ultimately, Reactor C produces 2281.94 mL more biogas than Reactor B, resulting in an overall efficiency improvement of 76.6%.

3.2 Daily biogas production

Figure 2 shows the daily biogas production of Reactors B and C. Since reactor A does not contain any substrate and lacks sufficient carbon sources, its gas production potential is limited, producing only 61.17 mL of biogas over the 34-day experimental period. Therefore, reactor A is not discussed in Figure 2. As seen in Figure 2, reactor C clearly starts up faster than reactor B, with biogas production of 263.86 mL, 435.48 mL, and 716.82 mL on the first three days, respectively. This indicates that the MEC accelerates the production of biogas from easily hydrolyzed substrates.

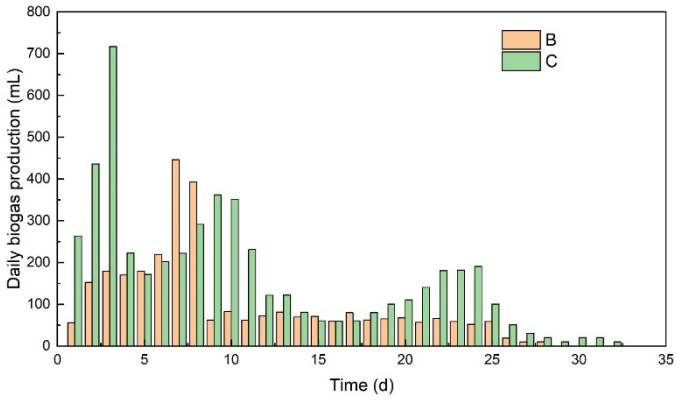


Fig. 2 Daily biogas production from reactors B and C

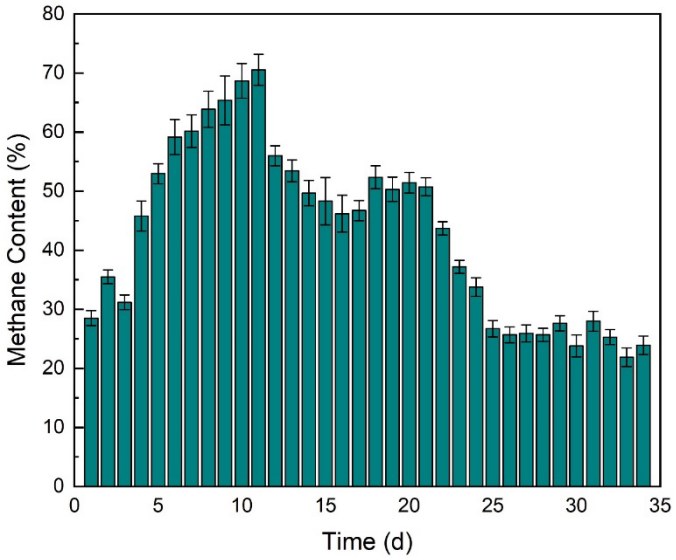
Reactor B remains in the initiation stage for the first 4 days, reaching its peak gas production of 446.35 mL on day 7. After day 10, reactor B enters a stable gas production stage. It is evident that reactor C has significant advantages over reactor B in both startup speed and the duration of the peak gas production phase.

The improved biogas production in reactor C may be due to the applied voltage, which provides a potential difference for the redox reactions of methanogenic microorganisms, thereby enhancing the hydrolysis of substrates by bacteria and the anaerobic digestion by methanogens. Another possible reason is the use of composite electrode plates in reactor C, which increases the surface area for microbial attachment and enhances microbial reaction efficiency. The shorter distance between the cathode and anode of the composite electrode plate reduces the proton transfer distance between the cathode and anode chambers, allowing protons to move directionally before the electrode plates while electrons move through the external circuit to complete the cycle, thereby improving the overall microbial redox reaction efficiency of the system. The reasons for the increased biogas production are further explored from the perspective of microbial communities and methanogenic archaea, with analysis results provided in section 3.4.

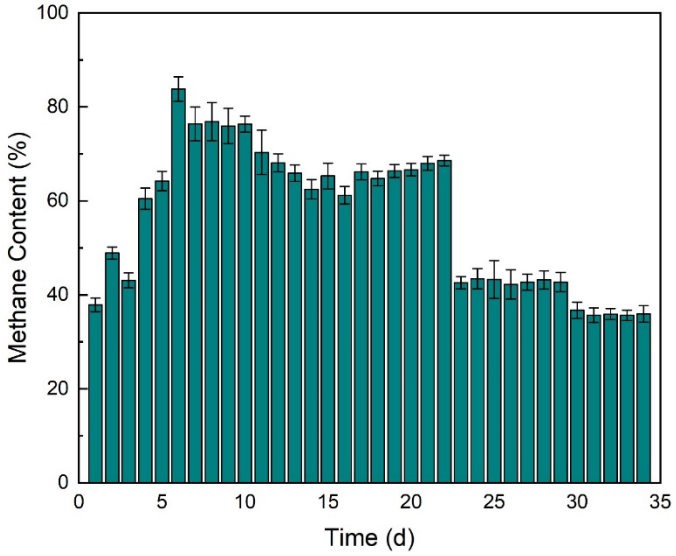
3.3 Daily methane content

During the 34-day anaerobic experiment period, samples were taken daily at fixed times from the gas collection bags of Reactor B and Reactor C, and methane content was measured using a gas chromatograph GC8860. The specific data are shown in Figure 3. Figure 3 illustrates the daily variation in methane content for Reactor B and Reactor C. As shown in Figure 3(a), Reactor B was in the startup phase during the first 4 days, with methane content increasing from 28.5% to 45.7%. Starting from Day 5, the methane content continued to rise, reaching a peak of 70.5% on Day 11. After Day 11, the methane content slowly decreased, likely because the easily hydrolyzed substrates had mostly completed their hydrolysis and gas production, leaving behind substrates that are more difficult to hydrolyze and have lower gas production potential.

As shown in Figure 3(b), Reactor C exhibits methane contents of 37.8%, 48.8%, and 43.1% during the first three days of the initiation stage. Starting from day 4, the methane content steadily increases, reaching a peak of 83.8% on day 6. This rapid increase may be attributed to the MEC reactor's ability to enhance substrate hydrolysis, accelerating the rate of biogas production. Additionally, Reactor C maintains a higher methane content peak for a longer duration compared to Reactor B. From day 5 to day 22, the methane content in Reactor C consistently exceeds 60%. The likely reasons for this include the applied voltage and the use of composite electrodes, which not only enhance the activity of methanogenic microorganisms but also promote the degradation of recalcitrant organic matter by the microbial community. As a result, Reactor C shows not only an increase in biogas production but also a higher methane content.



(a)



(b)

Fig. 3 Daily methane content in reactors B (a) and C (b)

3.4 Microbial community

To investigate the changes in microbial communities in reactors B and C, the supernatant and sludge samples from each reactor, as well as the sludge attached to the anode and cathode of the composite electrode plate in reactor C, are collected at the end of the experiment. These samples are sent to a third-party testing platform for microbial diversity analysis. For ease of subsequent analysis, the supernatant and sludge samples from

reactor A are labeled as A1 and A2, respectively. Similarly, the supernatant and sludge samples from reactor B are labeled as B1 and B2, while the supernatant, sludge, anode, and cathode samples from reactor C are labeled as C1, C2, C3, and C4, respectively. The results of the microbial diversity analysis are as follows.

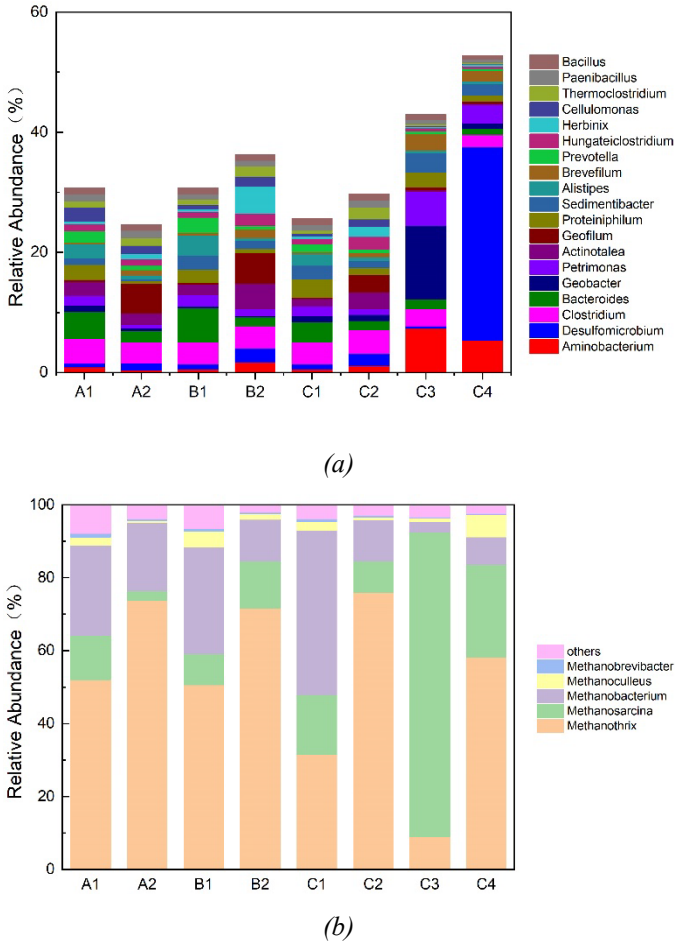


Fig. 4 Microbial information of genera level in each reactor ,(a) Bacterial genera; (b) Archaeal genera (note: both bacterial and archaeal genera with relative abundance higher than 1 % were shown here)

Desulfomicrobium is a type of sulfate-reducing bacterium that can directly obtain electrons from the cathode to produce hydrogen[13]. As shown in Figure 4(a), *Desulfomicrobium* is enriched in the cathode sample C4 (32%), significantly exceeding other samples. This indicates that the applied voltage enhances the activity of *Desulfomicrobium*, thereby promoting the interspecific hydrogen transport methane production (IHT) process and increasing methane production efficiency. The extracellular electroactive bacterium Geobacter is found in higher abundance in the anode sample C3 of reactor C (12%) compared to other groups. *Geobacter* can cooperate with the aceticlastic methanogen *Methanothrix*, accelerating the syntrophic metabolism to produce methane. As shown in Figure 4(b), under the experimental conditions of this study, *Methanothrix* is widely present in all reactors, with a higher abundance in the sludge samples than in the supernatant samples of each reactor. *Methanothrix* is a methanogenic archaeon that produces methane through direct interspecies electron transfer (DIET). Studies have shown that *Methanothrix* cooperates with the extracellular electroactive bacterium *Geobacter*, accelerating the syntrophic metabolism to degrade propionate and butyrate, leading to methane production[14]. Additionally, *Methanosarcina* and *Methanobacterium* also show relatively high abundance in all reactors. *Methanosarcina* is an aceticlastic methanogen that rapidly converts acetate, hydrogen protons, and CO_2 into methane, preventing pH drops and maintaining high methanogen activity, thereby increasing the methane production rate. *Methanobacterium* is present in all reactors, but its abundance decreases sharply in the cathode and anode of reactor C (3%), whereas *Methanosarcina* shows the highest abundance in the anode sludge of reactor C (84%). This suggests that the applied voltage stimulation alters the community structure, increasing the relative abundance of *Methanosarcina* while inhibiting the activity of *Methanobacterium*. Similar findings have been reported in the literature[15]. The changes in the bacterial and archaeal communities indicate that the MEC anaerobic reactor with the composite electrode plate can enhance the activity of methanogens, promote the DIET process, and thereby increase the methane production efficiency of the anaerobic reactor

4. CONCLUSIONS

The biogas production and methane content in reactor C, equipped with the composite electrode, are significantly increased compared to the control reactor B. Reactor C produces 226.1 mL more biogas per gram of substrate than reactor B. The total methane production in reactor C and the control reactor B is 3148.7 mL and 1531.5 mL, respectively, representing a 105.6% increase compared to the control. Additionally, the activity of aceticlastic methanogens on the composite electrode plate in reactor C is significantly enhanced. The composite electrode, with its small electrode spacing and large microbial attachment area, is applied in the AD-MEC reactor to promote the extracellular electron transfer (EET) process, thereby increasing methane

production and demonstrating its potential for use in AD-MEC systems.

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DECLARATION OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. All authors read and approved the final manuscript.

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