Biocathodic Methanogenic Community in an Integrated Anaerobic Digestion and Microbial Electrolysis System for Enhancement of Methane Production from Waste Sludge

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Supporting Information

ABSTRACT: Understanding the microbial community structure relative to enhancement of methane production from digestion of waste-activated sludge (WAS) coupled with a bioelectrochemical system is a key scientific question for the potential application of bioelectrochemistry in biogas production. Little has been known about the influence of electrode on the structure and function of microbial communities, especially methanogens in a bioelectrochemical anaerobic digestion (AD) reactor. Here, a hybrid reactor, which coupled bioelectrolysis and AD, was developed to enhance methane recovery from WAS. The methane production rate reached up to 0.0564 m³ methane/(m³ reactor*d) in the hybrid reactor at room temperature, which was nearly double than that of the control anaerobic reactor (0.0259 m³ methane/(m³ reactor*d)) without bioelectrochemical device. Microbial community analysis revealed that hydrogenotrophic methanogen *Methanobacterium* dominated the cathode biofilm, which was the predominant contributor to accelerate the methane production rate from WAS. While acetoclastic methanogen *Methanoseta* was enriched in the sludge phase of all reactors, shifts of the microbial community structure of the biocathode was in significant correlation with the methane production. This study suggested a potential way to utilize a bioelectrochemical system with the regulated microbial community to enhance methane production from WAS.

KEYWORDS: Bioelectrochemical system (BES), Waste-activated sludge (WAS), Anaerobic digestion (AD), Methanogenic community

INTRODUCTION

Waste-activated sludge (WAS) is of major technical and economic concern during the treatment process of wastewaters in wastewater treatment plants (WWTPs), and WAS disposal represents up to 50% of current operating costs.¹ Anaerobic digestion (AD) is a common process for cost-effective sludge mass reduction due to energy recovery of methane from organics degradation. WAS digestion involves multiple stages including hydrolysis, fermentation, syntrophic acetogenesis, and methanogenesis. Although solubilization and hydrolysis of WAS are considerable limitation steps to eventual organic conversion and energy recovery, the activity of methanogens plays a vital role in the final step of methanogenesis.^{2,3} On the basis of whether CO₂ or methyl compounds are utilized as main carbon sources for the methane production, methanogens can be classified into two groups as hydrogenotrophs and methylotrophs.⁴ In general, acetate degradation is one of the rate-limiting steps in most AD reactors because the mixed functional methanogens (*Methanosarcina* spp.) prefer to use hydrogen, which has large half saturation coefficient for acetate

(>50–100 mg/L). Moreover, acetoclastic methanogens (*Methanoseta*) grow extremely slow (doubling time 5–7 days).⁵ Previous studies indicated that acetoclastic methanogens were generally more sensitive to inhibitory factors than hydrogenotrophic methanogens, such as ammonia concentration, liquid upward velocity, and hydraulic retention time.^{6–12} Hydrogenotrophic methanogens can grow faster (doubling time 4–8 h) than acetoclastic methanogens, which was testified with higher growth rates using different substrates (acetate, propionate, butyrate, long-chain fatty acids, glycerol, protein, glucose, and starch).¹³ However, acetoclastic methanogens were finally dominant in most conventional anaerobic conditions. The reason is that hydrogen is required at a low pressure to prevent inhibition of acetogenic bacteria, whereas acetoclastic methanogens take the advantage over hydrogenotrophic methanogens in the methane production. In

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addition, competition from sulfate-reducing bacteria (SRB),^{14,15} homoacetogens in hydrogen utilization, were considerable inhibitory factors for the growth of hydrogenotrophic methanogens.¹

Bioelectrochemistry supplies a new pathway for hydrogen evolution from organic compounds, which depends on an electrolysis reaction and microbial catalysts in microbial electrolysis cells (MECs).^{16,17} Typically, an MEC oxidizes organic compounds at the anode in the presence of exoelectrogenic bacteria and transfers electrons and protons to the cathode where protons as electron acceptors boost hydrogen evolution.¹⁸ However, prior to hydrogen production at the cathode, methane was inevitable to accumulate in the single MEC due to the presence of methanogens at the cathode surface.¹⁹ The occurrences of CH₄ generation from hydrogen and CO₂ in the surface of a biocathode have been testified, and most of them were related to hydrogenotrophs.^{20–22} Hence, biocathodes in the MEC can potentially be employed to simultaneously catalyze methane production and stimulate growth of hydrogenotrophic methanogens. Recently, integrated reactors coupling bioelectrochemical system (BES) with AD were employed to successfully enhance methane production rate and operation stability with hydrogen evolution that finally induced the enrichment of hydrogenotrophic methanogens.^{23–26} Previous studies on methane-producing MECs concentrated on regulating strategy, feasibility, and stability; however, there is still a lack of better understanding of electron recovery evaluation to energy gas conversion in the combined system of BES and AD, which may change electron flow or electrode respiring communities in BES. Hu²⁷ showed the feasibility and inevitability of methane production in single MECs, providing the insight into the turning point from hydrogen to methane. Jansen²⁸ investigated long-term performance for carbon dioxide reduction in methane-producing MEC and showed strong stability for continuously converting CO₂ to methane and a promising energy efficiency achieved from current methane production rate. Our previous study also explored the strategy of external voltage optimization for inhibition and enhancement of methane production.²⁹ To date, studies showed few insights into the methanogenesis pathway through MEC in AD. The differences in methanogen metabolism activity between hydrogenotrophic and acetotrophic pathways determine the advantageous potential to increase the methane production rate in a hybrid reactor with hydrogenotrophic methanogens over the conventional AD process where acetotrophic methanogens are commonly dominant. The function and underlying mechanism of the biocathode in the hybrid system with complex substrates like WAS is still poorly understood.

The objective of this study was to evaluate the performance efficiency of a hybrid reactor that coupled MEC with AD in terms of methane production and organics degradation by employing WAS as the substrate. It was mainly considered the evaluation and calculation of increased methane production that contributed from the bioelectrochemical system rather than the anaerobic process. Therefore, typical batch cycles were chosen to calculate electron conversion to methane generation. During the experiment, the structure of microbial communities of cathode and anode biofilms and sludge were also examined to support the evaluated contribution from BES to AD.

METHODS AND MATERIALS

MEC-AD Reactor Construction. Two glass reactors were constructed for methane production from WAS. A cylinder (7 cm inner diameter × 18 cm height, total volume of 700 mL) was designed, and the WAS was mixed with a stirrer. The anode was a graphite brush (4 cm diameter × 8 cm length; 1.375 m²), and the cathode comprised carbon cloth (3 cm diameter, YW-50 YiBang; Taiwan) covered with a Pt catalyst layer (0.5 mg Pt/cm²) on one side. The anode brush was initially enriched in small single chamber MECs, and then a new Pt catalyst cathode was inserted into the glass reactor. The distance between the anode and cathode was 2 cm. External voltage was supplied between the anode and cathode by switching the power source (FDPS-150, Fudantianxin Inc., China) with a fixed voltage of 0.8 V. The effective volume of WAS was 550 mL (WAS of 500 mL + inoculation of 50 mL); thus, the headspace volume was 150 mL. Biogas was collected in a gas bag (0.5 L capacity; cali-5-bond, Calibrated Instruments, U.S.A.) which was attached on the top of the reactor. A control reactor without electrodes was operated under the same environmental conditions as the hybrid reactor. All reactor operations were conducted at room temperature of 20 ± 2 °C.

Anode Biofilm Enrichment. Two single chamber MECs (SMECs) were constructed with polycarbonate to domesticate the anode biofilm. Each reactor was a cube (8 cm × 8 cm) with an inner cylindrical chamber of 8 cm length and 5 cm diameter and an empty bed volume of 158 mL. A syringe (volume of 10 mL) was attached on top of each reactor for the gas collection.

The anode mentioned above was soaked in acetone for 24 h and heated in a muffle furnace at 450 °C for 30 min before anode microbial community enrichment; a carbon cloth coated with Pt/C was used as the cathode. Activated sludge (Taiping wastewater treatment plant, Harbin, China) mixed with acetate solution at a ratio of 1:10 was inoculated to start up the reactor. External voltage was fixed at 0.8 V. The SMECs were fed with 158 mL of sodium acetate, which was 50:50 mixed with 50 mM PBS (Na₂HPO₄ 4.58 g/L, NaH₂PO₄·H₂O 2.45 g/L, pH 7.0 and nutrient solution, NH₄Cl 0.31 g/L, KCl 0.13 g/L). Once the reactor produced a voltage >0.10 V during a fed-batch cycle, the inoculum was replaced. To obtain uniform performance (current and gas production), reinoculation was conducted in some reactors. When all reactors showed similar performance and stabilization with a current of 4 mA, the brush was transferred into an MEC-AD reactor.

Pretreatment and Acidification of WAS. WAS was collected from a secondary sedimentation tank of a municipal wastewater treatment plant in Harbin, China. Sludge was concentrated by settling at 4 °C for 24 h and was filtered through a stainless steel sieve (50 eyes-screen). Alkali coupled with ultrasonic pretreatment showed high performance in our previous studies.^{30,31} Hence, this method was applied in this study. Ultrasonic pretreatment was performed with 28 + 44 kHz ultrasonicator (Ningbo Scientz Biotechnology Co., China).³¹ The ultrasonic energy density was 0.5 kW/L for 10 min. In the alkali pretreatment, the pH value of WAS was adjusted to 10 using 4 mol/L NaOH. For the bifrequency ultrasonic pretreatment, the highest acidification rate was obtained in 3 days for fermentation as described previously,³¹ hence, the alkali plus ultrasonic-pretreated WAS was hydrolyzed and acidified after inoculation (Taiping wastewater treatment plant, Harbin, China) in all reactors for 3 days, which was followed by the operation of the MEC-AD reactor.

Analytical Methods. The composition of volatile fatty acids (VFAs) was analyzed by using an Agilent 7890 gas chromatography system.³² Soluble chemical oxygen demand (SCOD), total chemical oxygen demand (TCOD), carbohydrate(saccharides), and protein were determined as described previously.³⁰ Voltage and current were recorded using a multimeter (model 2700; Keithley Instruments, U.S.A.) every 10 min. Gases (hydrogen, CO₂, and CH₄) were analyzed using a gas chromatography system (Fuli GC9790II, Zhejiang Analytical Instrument, Inc., Taizhou China).³⁰ The pH was measured using a pH meter (PHS-3C, Leici Instrument, Ltd., Shanghai, China).

Microbial Community Analysis. The samples for microbial community analysis were taken at six different time points and positions from both reactors. The sample of source sludge was the

sludge used as the inoculum of the reactor and feedstock for AD treatment. The anode startup sample was cut from carbon brush after acclimatization outside and before insertion into the hybrid reactor. Anode and cathode samples were collected at the end of operation in the hybrid reactor. Considering that the attachment of feedstock sludge on the surface of electrodes could affect the analysis for the microbial community in electrode biofilms, carbon brushes of the anode and carbon cloth of the cathode were softly soaked with distilled water for 1–2 min to remove the attached sludge on the surface prior to sampling for microbial community analysis. The MEC-AD reactor and AD reactor were continuously mixed by magnetic stirrer, and sludge samples were collected from the bottom of the reactors.

Microbial community analysis was performed using the respective samples of raw sludge, anode startup, anode biofilm, cathode biofilm, sludge in the MEC-AD, and sludge in the AD reactor. Illumina MiSeq sequencing was constructed using bacterial primers 341F and 805R for the V3–V4 region of the 16S rRNA gene; moreover, we used 349F and 806R for archaea (Figure S15). The raw sequence data were collected in a MiSeq sequencing machine in fastq format. The forward and reverse directions and barcodes were produced into separated files. First, all sequences were assigned to samples according to the barcodes. The forward and reverse reads were merged into full length sequences. The average read length was more than 400 bp after merge. Chimera detection and removal was completed using U-Chime. All sequences were clustered using UCLUST software (ucclust v1.1.579) at 97% similarity level, and taxonomic assignment was through the Ribosomal Database Project classifier with minimal 50% confidence estimates.³³

Quantitative PCR (qPCR) was employed to analyze the abundances of methyl coenzyme M reductase (*mcrA*) gene for four samples collected from the anode and cathode and sludge in the MEC-AD and AD reactors. As shown in the Support Information (Figure S11), the primer used for every sample was described previously.³⁴

Calculation.

Input electric energy efficiency:

$$\eta_{\text{electricity}} = \frac{\frac{V_{\text{net}}}{V_{\text{m}}} \Delta H_{\text{methane}}}{\int_t^n UI dt}$$

where $\eta_{\text{electricity}}$ represents the input electric energy efficiency, V_{net} represents the volume of increased methane production from 1 to n day, calculated by using the accumulated value of MEC-AD minus AD value. Here, $V_{\text{m}} = 22.4$ L/mol represents the molar volume of gas. Also, U and I represent the external voltage and current value, and $\Delta H_{\text{methane}} = 891$ kJ/mol.³⁵ All parameters mentioned in the context were under at standard conditions.

Theoretical methane produced from electrons:

$$V_{\text{methane}} = UI t W_{\text{methane}} V_{\text{m}}$$

where $V_{\text{m}} = 22.4$ L/mol represents the molar volume of gas and V_{methane} means the volume of methane produced from electrons. Here, $T = 24$ h means 1 day, and $W_{\text{methane}} = 4.043$ mmol/wh indicates the conversion from electric heat into methane.³⁶

RESULTS

Accelerated Methane Production in MEC-AD Reactor.

The MEC-AD reactor was operated in batch mode with electrodes and exhibited considerable performance difference in methane production rate compared to the control reactor without electrodes. After the WAS was pretreated by alkali followed by ultrasonication, the methane yield was low under anaerobic conditions during the first 4 days of its hydrolysis and acidification process. The methane production rate kept increasing from the fifth day when electrons started to flow from anode to cathode; hydrogen was generally detected on the

fifth day (16.25 mL) and sixth day (8.66 mL). Meanwhile, the control reactor without electrodes exhibited the final stage of methanogenesis with little methane production during the AD process. Interestingly, the average methane yield in the hybrid reactor was 39.0 ± 14.0 mL/d, which was nearly double that of the AD reactor (18.1 ± 9.1 mL/d) until 18th day ($P = 0.034$). In phase 1 (from fourth to 18th day), the methane production rate of the MEC-AD (0.0564 m³ methane/(m³reactor*d)) reactor was higher than the AD reactor (0.0259 m³ methane/(m³reactor*d)).

As shown in Figure 1A, current value of the MEC-AD apparently dropped on 18th day and gradually declined in the

Figure 1. (A) Current change in MEC-AD reactor. (B) Accumulated methane production in MEC-AD and AD reactors. Electrons contribution was estimated based on current.

phase 2, and the reactor had simultaneously less methane production rate (0.0204 m³ methane/(m³reactor*d)). Moreover, the control reactor showed a similar decline trend in the methane production, which was caused by the consumption of organic compounds such as protein, saccharide, and VFAs. Electron contribution was considered to enhance methane yield (Figure 1B). Considering the contribution of electrons to methane yield, the sum of AD and electrons were calculated from methane produced in AD (control) and theoretical methane produced from electrons through MECs. The highest methane yield was 0.0387 m³ methane/(m³reactor*d). Besides the electrons contribution, the increase in methane production was slightly higher than that in the AD in phase 1.

The major components of WAS, saccharide, protein, and VFAs were released after pretreatment. In batch operation, concentration of saccharide and protein increased to 98 and

Table 1. Measured Main Characteristics of WAS during Pretreatment, Acidification, and Methanogenesis Stage^a

		fraction of waste activated sludge								
	fraction	saccharide	Protein	total VFAs	ammonium nitrogen	phosphate	VSS(g/L)	TCOD	VSS RE%	TCOD RE%
pretreatment	origin	8	30	378	144	90	14	15650	—	—
	U+A	98	417	396	225	170	—	—	—	—
MEC-AD	Ac	102	250	1433	343	281	—	—	—	—
	end	44	87	175	505	186	7.2	9201	48.5	41.2
AD	Ac	151	253	1640	735	330	—	—	—	—
	end	49	93	232	631	198	8.7	10025	37.9	35.9

^aAll units are mg/L. RE% represents removal efficiency. U+A represents ultrasonic plus alkali pretreatment. Ac represents acidification process. Symbol “—” represents no data.

Figure 2. Changes of VFAs including acetic (HAc), propionic (HPr), n-butyric (n-HBu), iso-butyric (i-HBu), n-valeric (n-HVa), and iso-valeric (i-HVa) acids.

417 mg/L, respectively, after pretreatment (Table 1 and Figure S12). The final concentration of saccharide and protein in the MEC-AD was slight lower than that of the AD reactor (Table 1). In batch operation, the conversion of saccharides and proteins to VFAs were detected, resulting in considerable accumulation of acetate, propionate, n-butyrate, iso-butyrate, n-valerate, and iso-valerate in either MEC-AD or AD (Figure 2 and Figure S13). At the end of the AD process, butyrate and

valerate were barely depleted in all reactors. The final concentrations of acetate and propionate in MEC-AD were lower than that of the AD reactor. In all reactors, there was an obvious increase in the concentration of ammonium nitrogen as the cell wall of microbes was broken during the AD process. Interestingly, phosphate concentration increased during the whole process and subsequently decreased at the end. Removal efficiency (RE) of VSS in the MEC-AD reached 48.5%, which

Figure 3. Taxonomic classification of 16S rRNA gene sequences from microbial communities of original sludge, electrode surfaces, and sludge in both reactors at phylum level (A). The distribution of the most dominant class (B). Hierarchical clustering analysis at genus level representing all OTUs present at a relative abundance $\geq 1\%$ in at least one of samples (C). Distribution of dominant genus (relative abundance $\geq 1\%$) at cathode biofilm and two pathways of methane formation in the surface of electrode was proposed (D).

was obviously higher than that of the AD (37.9%). According to the removal of VSS, the methane yield of MEC-AD was 111.19 mL/g VSS that was higher than that of AD (103.09 mL/g VSS). Overall TCOD removal efficiencies were 41.2% in the MEC-AD, whereas only 35.9% were obtained in the AD process (Table 1). Considering the consumption of additional electric energy, we calculated the input electric energy efficiency. As shown in Figure SI4. More than 100% input electric energy efficiency showed that the additional electric energy was totally covered by the increased methane yield, which implied that a huge potential is required to apply this modification to conventional reactors. Although the input electric energy efficiency always exceeded 100% during the whole batch operation, the turning point suggested that the strategy of external voltage should be optimized in the future.

MICROBIAL COMMUNITY ANALYSIS

Microbial Composition. The microbial communities were characterized at the terminal points to compare community structure, diversity, and function in different reactors. Sludge in the MEC-AD showed the highest diversity (Shannon index of 6.17) among six different communities, and the Shannon index of the cathode biofilm was 4.93, which was lower than others (Figures SI5 and SI6). The relative bacterial community abundances were identified on the phylum level, and the main difference among six bacterial communities was attributed to the different distribution of phylum *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Actinobacteria*. *Proteobacteria* dominated in the

original sludge (50.3%), which accounted for 13.5% and 16.7% in the AD sludge and MEC-AD sludge, respectively (Figure 3A). At the class level, the main composition of *Deltaproteobacteria* was *Geobacter*, which led to higher relative abundance in the anode biofilm (Figure 3B).

To further compare the difference between microbial communities, the distribution of bacteria was identified at genus level. *Parabacteroides*, *Petrimonas*, and *Proteiniclasticum* accounted for 15.0%, 10.1%, and 5.1%, respectively, in the AD sludge that was close to 11.4%, 7.3%, and 7.1% in the MEC-AD sludge (SI7). *Petrimonas* belonged to fermentative acidic bacteria, and *Proteiniclasticum* contributed to protein hydrolysis.³¹ The methanogenic community was dominated by *Methanosaeta* in the sludge with and without electrodes. Visualization of variability in the community composition in both MEC-AD and AD sludge using principal coordinates analysis (PCoA) indicated that there was no significant difference (Figure SI8). However, the microbial community in the cathode biofilm exhibited a significant difference in the sludge in both reactors. Biofilms of the anode and cathode were highly similar but different from sludge in the AD and MEC-AD reactor (Figure 3C). The number of classified OTUs shared by biofilms on electrodes and sludge in all reactors was 858, and more than half of the shared OTUs belonged to *Proteobacteria* (39%), *Firmicutes* (24%), and *Bacteroidetes* (11%) (Figure SI9).

The acetogens, *Acetobacterium* and *Acetoanaerobium*, were enriched at the cathode (relative abundance of up to 23.1% and

Figure 4. (a) Relative abundance of methanogens. (b) Taxonomy of methanogens and its relative abundance.

6.3%, relatively) (Figure 3D), and they could play a vital role in hydrogen scavenging and acetic acid synthesizing.³⁷ Moreover, *Methanosaeta* was also detected in the cathode biofilm, where acetate produced by homoacetogens and electrons supplied by electrode supported the growth of *Methanosaeta*.³⁸ The portion of *Longilinea* and *Bellilinea* reached 6.82% and 1.15%, respectively, which ascribed to the enrichment of hydrogenotrophic methanogens.³⁹

Electrochemically active *Geobacter* increased from 0.11% at the anode during startup to 21.86% on the fifth day, which was in accordance with the increase in *Deltaproteobacteria*. The relative abundance of *Methanosaeta* went up to 0.49% in the anode biofilm compared to ~0.03% in the startup stage. *Acetoanaerobium* and *Acetobacterium* accounted for 4.34% and 2.50% in the anode startup, respectively, while the values decreased to 0.33% and 0.19% in the anode biofilm.

Methanogens Taxonomy and qPCR Analysis. Among methanogens, *Methanobacterium*, *Methanobrevibacter*, *Methanoculleus*, *Methanosphaera*, and *Methanospirillum* were classified into the hydrogenotrophic methanogens, while *Methanomethylovorans* (facultative), *Methanosaeta* (obligate), and *Methanosarcina* (facultative) were acetotrophic methanogens.⁴⁰ Bacteria sequencing results revealed that the cathode biofilm had the highest portion of methanogens, with a relative abundance of 6.99% for hydrogenotrophic and 0.012% for acetotrophic. According to the sequencing analysis of archaea separately (Figure 4a and b), *Methanobacterium* was predominant at the cathode and accounted for 91.08%. *Methanosaeta*, which was obligate to utilize acetate as substrate for methane production, was enriched at the anode significantly (61.77%). Furthermore, acetotrophic methanogens (facultative) accounted for 16.01% at the anode. Hence, the lower relative abundance (20.49%) of hydrogenotrophic methanogens was shown. Hydrogenotrophic methanogens accounted for 64.41% in the MEC-AD sludge, which was slightly higher than that of AD sludge (49.92%). Apparently, the ratio of acetoclastic methanogens and hydrogenotrophic methanogens was nearly 1:1 in the sludge phase in both reactors, which revealed similar methanogen distribution in the sludge phase in both reactors. This justified that the introduction of the electrode influenced the methanogenic community slightly. Figure 5 revealed the significant difference in function gene copies of the *mcrA* gene that encodes the enzyme to catalyze the terminal step in methanogenesis. The number of *mcrA* in the cathode was obviously higher than in the anode or sludge of MEC-AD or AD, indicating methanogens enriched at the cathodic biofilm.

Figure 5. qPCR analysis of the *mcrA* abundances in four tested microbial communities.

DISCUSSION

Bioelectrochemical Contributions to Methane Enhancement in the Hybrid Reactor. Saccharides and proteins directly released from microbial cells after pretreatment resulted in obvious accumulation of VFAs via acidogenesis, which was in accordance to our previous study.³¹ The low accumulation of saccharides and proteins could be ascribed to the utilization of WAS in MEC.^{20,41} Moreover, lower TCOD and VSS removal efficiency of the AD reactor also represented the positive effect of MEC for substrate degradation.⁴² In addition to effective degradation of complex organics, a nearly 2-fold increase in the methane production rate with the hybrid reactor was achieved over the AD reactor. In a previous study, though all electrons contributed to methane, a higher amount of methane was produced from electrochemical regulation,²⁰ which was agreeable with the results of this study. Obviously, all methane should be originated from substrate. During the operation period, although fermentation progressed at all times, the VFAs concentration showed a difference in the MEC-AD reactor to AD. Acetate decreased sharply in MEC-AD in the initial days compared to AD, indicating a higher degradation rate caused by the anodic microbial community that competitively consumed acetate for anaerobic respiration.³⁷ The initial change in acetate was according to methane generation in MEC-AD. The value of acetate concentration in MEC-AD was significantly lower than that in AD (single tail, P-value 0.067), implying a higher degradation rate of acetate in

hybrid reactor. The n-butyrate, n-valerate, iso-valerate, and total VFAs value of MEC-AD were significantly lower than that of AD ($P = 0.030, 0.007, 0.004, \text{ and } 0.057$, respectively). The rest of the VFAs showed a slight difference in the P -value of propionate (0.269) and iso-butyrate (0.370). An obvious accumulation of propionate, n-butyrate, iso-butyrate, and n-valerate occurred at around 20 days in AD reactor, which slightly happened in MEC-AD. This could be caused by the rapid acetate consumption in MEC-AD;⁴³ hence, the negative effect of VFAs accumulation for methane production was relieved in the addition of MEC into AD process. Furthermore, more than 100% input electric energy efficiency showed a huge potential to apply this modification to conventional reactor. The additional electric energy was due to the increased methane yield. Hybrid reactor coupling BES and AD showed remediating capacity and stable energy output in the studies carried out by researchers.^{23,44}

Microbial Community Analysis. According to the heat map and PCoA analysis (Figure S7), there was no obvious difference in original communities between AD and MEC-AD. The main reason for a variety of methane production was caused by the shift in biofilms on the electrodes. The relative higher abundance of *Clostridia* and *Bacteroidia* suggested acidogenesis and hydrolysis in the fermentative process. At the anode, the primary role of *Geobacter* focused on extracellular electron transfer from organics to electrode. With respect to previous studies on the competition between *Geobacter* and *Methanosaeta* for the same substrate, acetate would be the most primarily oxidized into electrons and protons by exoelectrogenic bacteria rather than methanogens.^{45,46} Recent finding of direct interspecies electron transfer (DIET) for methane production implied that *Methanosaeta* were more likely to accept electrons from *Geobacter* rather than surviving through acetate metabolism.^{38,47} However, methane production from interspecies electron transport did not result in obvious change in methane production rate because of the slow grow rate of *Methanosaeta*.⁵ Therefore, the shift of microbial community in the cathodic biofilm was of great importance to contribute to the methane production rate increase.

Methane Formation Mechanism within Biocathode Communities. Hydrogen was the main product from end-fermentative acids at a high applied voltage²⁹ and even fermentative liquid from WAS.⁴⁸ In addition, the electrochemical reduction from CO_2 to CH_4 (-0.240 V vs SHE) with lower potential was easier to occur than the hydrogen evolution (-0.414 V).²⁸ Hydrogen was detected during the initial days (5th and 6th day) in closed circuit. However, hydrogenotrophic methanogens and acetogenic bacteria were substantially enriched at the surface of a carbon cloth, resulting in minor detection of hydrogen. Recently, the cathode turned out to be selective for microbial enrichment due to its diverse redox potential.^{49–51} Some new strains at the cathode via microbial electroreduction were isolated, which indicated the selective function of the cathode.⁵² Previous results showed that controlling the redox potential could activate the hydrogenotrophic methanogen *M. thermautotrophicus* at a cathode potential of -0.850 V .⁵³ In this study, potential at the cathode reached $\leftarrow 1.0 \text{ V}$, which was favorable for hydrogen evolution and methanogens growth in the presence of Pt catalyst.

On the basis of the microbial community analysis of the cathode biofilm, *Methanobacterium* was enriched significantly on the electrode where hydrogen was generated. *Methanosaeta*

accounted for a higher portion at the cathode, and it was related with the enrichment of homoacetogens, which perform the synthesis of acetate from hydrogen and bicarbonate or electrons. As shown in Figure 3D, the following two pathways contributed to the methane production at the cathode of the MEC-AD: (i) Hydrogen that permeated from surface of cathode was utilized by hydrogenotrophic methanogens rapidly. (ii) Acetoclastic methanogens generated methane from acetate that was synthesized from hydrogen or electrons. It was interesting that acetoclastic methanogens were enriched at the cathode, which indicated that the hydrogen evolution not only stimulated the methane formation from hydrogen and carbon dioxide but also acetate synthesis.⁵⁴ The enrichment of hydrogenotrophic methanogens on the cathode resulted in a rapid conversion rate from hydrogen/electrons into methane, indicating a potential strategy for improving methane production rate in AD process with endogenous hydrogen. Compared with exogenous hydrogen injection, it was advantageous in gas–liquid mass transfer because atomic or molecular hydrogen were favorable for hydrogenotrophic methanogens.^{55,56} In total, the introduction of a microbial electrolysis system in the AD process showed tremendous potential to regulate the methanogenic community toward a higher methane production rate with a fast growth rate of hydrogenotrophs.

CONCLUSION

The results of this study indicated that AD of WAS coupled with the microbial electrolysis process enhanced the methane production rate and process stability, compared with the conventional AD reactor as the control. According to the results of Illumina MiSeq sequencing, methanogens, especially hydrogenotrophic methanogens *Methanobacterium* and acetoclastic *Methanosaeta*, were enriched in the cathode biofilm, and two major pathways of methane production occurred on the cathode surface. The introduction of MEC into the AD reactor formed an independent bioelectrochemical section which departs from the AD section to produce methane effectively. This study suggested a possible approach to reduce WAS digestion time with an increased methane production rate by using microbial electrolysis regulation.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.6b01221.

Figures S1–S10: S1 (QPCR), S2 (components conversion in MEC-AD and AD reactor), S3 (VFAs change in both reactors), S4 (input electric energy efficiency), S5 (alpha diversity), S6 (Shannon index of all samples), S7 (distribution of genus in different samples), S8 (PCoA analysis), S9 (venn diagram), and S10 (accumulative methane production from semicontinuous mode). (PDF)

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Notes

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