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Characterization of methane production and microbial community shifts during waste activated sludge degradation in microbial electrolysis cells

Rui Sun, Aijuan Zhou, Jianna Jia, Qing Liang, Qian Liu, Defeng Xing, Nanqi Ren *

State Key Laboratory of Urban Water Resource and Environment, School of Municipal and Environmental Engineering, Harbin Institute of Technology, Harbin 150090, China

HIGHLIGHTS

- Methane production was enhanced by MECs fed with alkali-pretreated WAS.
- Efficient sludge reduction was achieved compared with open circuit controls.
- Pyrosequencing revealed the occurrence of hydrogenotrophic methanogenesis.
- The shift of microbial community resulted in methane increase in MECs.

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ABSTRACT

Microbial electrolysis cell (MECs) were investigated as a promising technology to manage waste activated sludge (WAS) reduction and bio-methane generation. The effect of WAS concentration on the MECs performance was discussed. At the optimal concentration of 15 g COD/L, maximum methane yield of MECs fed with alkaline pretreated WAS (A-WAS) were achieved with the value of 77.13 ± 2.52 L CH_4/kg-COD on Day 3, which had been improved by 1.5-fold compared with MECs fed with raw WAS (R-WAS), while that was negligible in open circuit controls. Efficient sludge reduction was also obtained in terms of TCOD, total protein, TSS and VSS removal. Pyrosequencing revealed the dominance of exoelectrogen *Geobacter* and hydrogen-producing bacteria *Petrimonas* in MECs fed with WAS. *Methanocorpusculum* with the capacity of methane generation using CO_2 and H_2 also showed overwhelming dominance (96.01%). The large proportions of *Petrimonas* and *Methanocorpusculum* indicated the occurrence of hydrogenotrophic methanogenesis in our methane-producing MECs.

1. Introduction

Due to the widely used biological wastewater treatment in municipal wastewater treatment plants (WWTPs), large volumes of waste activated sludge (WAS) were produced worldwide (Appels et al., 2008; Bougrier et al., 2007; Zhang et al., 2010). In China, the annual production of WAS reached 11.2 million tons, while that of the whole EU was only over 10 million tons (Chu et al., 2009). WAS disposal cost up to 50–60% of the total maintenance costs in WWTPs (Jin et al., 2009). The stabilization and reduction of WAS has caused global concerns. Harnessing the energy embedded in WAS through anaerobic digestion (AD) has been considered as one of the most attractive options (Feng et al., 2014; Athanasoulia et al., 2012). Though substantial methane production had been reported in literatures, the application of AD has faced with several drawbacks. The temperature of AD was generally maintained at or above 35 °C which was energy-intensive. Methane production rate of AD was limited due to the extensive retention time as long as over 20 days (Li et al., 2014). Protein as the main component of WAS can hardly utilized by fermentative bacteria during AD process.

Microbial electrolysis cell (MECs) has recently be increasingly researched as a versatile device which can obtain wastes treatment and biogas generation simultaneously. In MECs, a small voltage (0.2–0.8 V) is applied to drive the bioelectrochemical reactions. Exoelectrogenic bacteria on the anodes utilize organic substrate and release electrons. Through the closed circuit, electrons transfer to cathodes and are consumed by hydrogen production or methanogenesis (Logan et al., 2008). While high H_2 yield in MECs using various substrates such as acetate, glucose, wastewater, and sludge had been well documented (Heidrich et al., 2013; Xu et al., 2013; Lu et al., 2012a), one major phenomenon observed in MECs for H_2 production is associated methane production. Substantial

* Corresponding author at: School of Municipal and Environmental Engineering, Harbin Institute of Technology, P.O. Box 2650, 73 Huanghe Road, Nangang District, Harbin, Heilongjiang Province 150090, China. Tel.: +86 451 86289195.
E-mail address: rnq@hit.edu.cn (N. Ren).

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methane production had been widely reported in MECs studies aimed at H_2 recovery originally (Call and Logan, 2008; Hu et al., 2008). Despite that various methods had been developed to inhibit methane production, such as reactor air exposure (Call and Logan, 2008), low pH (Chae et al., 2010) or low temperature (Lu et al., 2012b), hydrogen production are always out-competed by methanogenesis after long-term operation of MECs. It was concluded that methane production might be more robust than H_2 production in MECs, especially for larger scale MECs systems (Cusick et al., 2011). Methane can be produced in MECs via two mechanisms: (i) hydrogenotrophic methanogenesis (Prathap et al., 2009) and (ii) electrons, protons and CO_2 directly converted to methane (Cheng et al., 2009). Rather than avoiding methane production in MECs, methane-producing MECs brings specific advantages (Villano et al., 2013). As an important energetic compound, methane have been widely used to produce electricity in wastewater treatment processes (Pham et al., 2006). By MECs, methane production rate and methane yield have be significantly enhanced compared to AD (Villano et al., 2013; Clauwaert and Verstraete, 2009). High-strength biomass is essential to obtain efficient methane production in AD, while MECs are suitable for diluted biomass substrate. More importantly, MECs are capable of methane generation at ambient temperature. In this sense, MECs would be one of the most promising fast and efficient methane-producing technologies.

Organic loading is a critical factor in methane-producing processes. Generally, in AD, increased substrate concentration would result in higher methane yield. Yet, optimal substrate concentration existed in MECs. The influence of substrate concentration on the performance of MECs were discussed in previous researches (Liu et al., 2012; Lu et al., 2010). To date, few researches had focused on the investigation of using WAS as the substrate in MEC for methane production and the optimal WAS concentration.

In this study, raw WAS (R-WAS) and alkaline-pretreated WAS (A-WAS) were utilized as the substrate of single-room MECs to obtain coupling WAS reduction and methane production. The influence of three different sludge concentrations on MECs performance were discussed. 454-pyrosequencing was applied to analyze the structure and syntrophic interactions of microbial communities and *Archaeal* communities of the anode biofilms of MECs, which might provide an insight on the mechanism of methane-producing process.

2. Methods

2.1. Raw and alkaline-pretreated WAS

WAS used in our research was collected from the secondary sedimentation tank of Wenchang Wastewater Treatment Plant of Harbin, China. The sludge supernatant was removed after absolute-rest precipitation for 24 h and the remaining sludge was stored at 4 °C, which was used as R-WAS. The pH of R-WAS was adjusted to ~12 by 4 M NaOH followed by another 24 h precipitation to obtain A-WAS. The specific alkaline dosage was 1.16 ± 0.02 g NaOH per gram of volatile suspended solids (VSS). The pH of A-WAS remained at 9.49 ± 0.22. Characteristics of R-WAS and A-WAS were given in Table 1.

2.2. MECs set-up and operation

Eight single-chamber MECs in parallel with an effective volume of 25 mL were assembled and used in our study. Materials of cathodes and anodes were carbon cloth coated with Pt/C catalyst and graphite fiber brushes, respectively. To collect biogas produced, anaerobic tubes linked with gas bags were glued to the top of MECs. A fixed voltage of 0.6 V was applied to MECs using a programmable power source. The voltages produced by MECs across the resistance (10 Ω) were recorded by the Keithley 2700 data system. All reactors were running in fed-batch mode at room temperature (~20 °C). MECs were inoculated with WAS and the substrates were R-WAS and A-WAS. Substrates were diluted to three different concentrations by 100 mM PBS (Liu and Logan, 2004), which were 10 g/L, 15 g/L and 20 g/L, respectively. Influent and effluent of MECs were sampled for further analysis after stable biogas production was achieved.

Table 1
Characteristics of buffered R-WAS and A-WAS.

	R-WAS	A-WAS
Total suspended solids (TSS, g/L)	22.52 ± 2.06	23.06 ± 2.63
Volatile suspended solids (VSS, g/L)	14.58 ± 0.23	16.49 ± 0.78
Total chemical oxygen demand (TCOD, mg/L)	26,151 ± 50	27,108 ± 1404
Soluble chemical oxygen demand (SCOD, mg/L)	431 ± 40	3601 ± 343
Soluble carbohydrate (mg COD/L)	44 ± 2	467 ± 16
Soluble protein (mg COD/L)	7292 ± 86	8357 ± 252
Total protein (mg COD/L)	5337 ± 87	6651 ± 408
Volatile fatty acids (VFAs, mg COD/L)	1047 ± 58	1571 ± 113
pH	6.91 ± 0.07	9.68 ± 0.10
Moisture content (%)	98.08 ± 1.62	99.90 ± 1.50
Conductivity (mS/cm)	3.67 ± 0.19	4.92 ± 0.19

2.3. Analysis methods

Sludge samples were centrifuged, and the obtained supernatant were then filtered by 0.45 μm filter membrane and finally stored at 4 °C prior to analysis (Sun et al., 2014). TCOD, SCOD, TS, VS, TSS, VSS were analyzed according to the standard methods (American Public Health Association (APHA, 1998)). For the measurement of SCOD, soluble carbohydrate and soluble protein, the sludge samples were centrifuged, and the obtained supernatant were then filtered by 0.45 μm filter membrane. The filtrate was analyzed for SCOD by potassium dichromate method. As for most carbohydrates in the samples were polysaccharide, phenol sulfuric acid colorimetric method was chosen to analyze carbohydrates concentration (Herbert et al., 1971). The protein concentration was determined using Bicinchoninic Acid Protein Assay Kit (Sigma–Aldrich) (Smith et al., 1985). The pH was measured by Shang Hai Lei Ci PHS-2F type pH meter. The volume of biogas produced by MECs was measured by a glass syringe and the biogas composition was obtained using FULI 9790II gas chromatograph. VFAs were analyzed by gas chromatograph (GC4890, Agilent, America) (Lu et al., 2009). Methane production rate (m^3/m^3 d) and methane yield were calculated to evaluate the performance of MECs on methane production (Lu et al., 2009).

2.4. DNA extraction, PCR amplification and 454 pyrosequencing

After stable operation, small amounts of the graphite fiber brush of MECs were cut using sterile scissors for DNA extraction. PowerSoil DNA Isolation Kit (MoBio, America) was used for total genomic DNA extraction according to instructions. The following PCR amplification procedure was detailed described in previous research (Jia et al., 2013). Universal primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 533R (5′-TTACCGCGGCTGCTGGCAC-3′) were used to amplify V1–V3 region (length of ~455 bp) of the bacterial 16S rRNA gene. *Archaeal* primers were 344F (5′-ACGGGGYGCAGCAGGCGCGA-3′) and 915R (5′-GTGCTCCCCCGCCAATTCCT-3′). Pyrosequencing was conducted by 454 GS-FLX pyrosequencing system (Roche, America). Setting the similarity at 97%, the