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Enhanced methane production in an anaerobic digestion and microbial electrolysis cell coupled system with co-cultivation of *Geobacter* and *Methanosarcina*

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ABSTRACT

The anaerobic digestion (AD) and microbial electrolysis cell (MEC) coupled system has been proved to be a promising process for biomethane production. In this paper, it was found that by co-cultivating *Geobacter* with *Methanosarcina* in an AD–MEC coupled system, methane yield was further increased by 24.1%, achieving to 360.2 mL/g-COD, which was comparable to the theoretical methane yield of an anaerobic digester. With the presence of *Geobacter*, the maximum chemical oxygen demand (COD) removal rate (216.8 mg COD/(L·hr)) and current density (304.3 A/m^3) were both increased by 1.3 and 1.8 fold compared to the previous study without *Geobacter*, resulting in overall energy efficiency reaching up to 74.6%. Community analysis demonstrated that *Geobacter* and *Methanosarcina* could coexist together in the biofilm, and the electrochemical activities of both were confirmed by cyclic voltammetry. Our study observed that the carbon dioxide content in total gas generated from the AD reactor with *Geobacter* was only half of that generated from the same reactor without *Geobacter*, suggesting that *Methanosarcina* may obtain the electron transferred from *Geobacter* for the reduction of carbon dioxide to methane. Taken together, *Geobacter* not only can improve the performance of the MEC system, but also can enhance methane production.
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Introduction

Biogas, an abundant renewable energy source, is the most successful biofuel product derived from bio-waste, but its low production and impurities, mainly carbon dioxide, have hampered its value and application potential (Persson, 2003). A new technology which couples an anaerobic digester (AD) with a microbial electrolysis cell (MEC) has been developed to increase the production and purity of biogas simultaneously (Bo et al., 2014; Cheng et al., 2009; Logan and Rabaey, 2012). Our previous study demonstrated that redundant carbon dioxide produced from AD can be *in situ* converted to additional methane by electromethanogens utilizing hydrogen formed from MEC as an electron donor, generating high quality biogas (Bo et al., 2014).

Various *Geobacter* species have been found to reduce system resistance, lower the activation energy barrier and increase current density in microbial fuel cells (MFCs) because *Geobacter* can directly transfer electrons to the anode or other bacteria (Malvankar et al., 2011, 2012). Efficient electron transformation and high current are equally important for MEC (Lovley et al., 2011; Malvankar et al., 2012; Morita et al.,

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2011). In recent years, *Geobacter* has been observed to be able to directly transfer electrons to methanogens, such as *Methanosaeta* and *Methanosarcina*, to reduce carbon dioxide to methane (Malvankar et al., 2011; Reguera et al., 2005; Rotaru et al., 2014a,b; Zhao et al., 2015). Generally, carbon dioxide reduction to methane is processed *via* sequential pathways: electron to proton transfer, hydrogen formation and carbon dioxide reduction (Bo et al., 2014). The new pathway (direct interspecies electron transfer, DIET) without the step of hydrogen formation is apparently more efficient than the traditional pathway.

In order to further increase the methane production in the AD–MEC process, a new method, *i.e.*, co-cultivating *Geobacter* and *Methanosarcina* to improve the performance of MEC and regulate the carbon dioxide to methane conversion pathway, is reported. First, the AD and MEC coupling system with co-cultivation of *Geobacter* and *Methanosarcina* was developed to increase the production of methane. The syntrophic interactions of *Geobacter* and *Methanosarcina* during methane production in the anaerobic digester reactor were then studied. The mechanisms for remarkably high methane being produced by co-cultivating *Geobacter* and *Methanosarcina* in the coupling system were finally explored.

1. Material and methods

1.1. Inoculum

The *Geobacter*-containing inoculum was obtained from the solution from the anode chamber of an existing two-chamber MEC reactor (W.T. Su et al., 2012). Pure *Methanosarcina* sp. was purchased from the German Collection of Microorganisms and Cell Cultures (DSM 804).

1.2. Reactor construction

The barrel-shaped, single-chamber reactors were made of stainless steel (SUS304, 250.0 mL, 10.0 × 7.6 cm). The reactor AD was inoculated with waste activated sludge (2 mL). The reactor AD–G was inoculated with waste activated sludge (2 mL) and *Geobacter*-containing inoculum (2 mL). The reactor AD–MEC–G was inoculated with waste activated sludge (2 mL), *Geobacter*-containing inoculum (2 mL) and *Methanosarcina* sp. culture (2 mL). Reactor AD–MEC–G contained a 5.0 × 5.0 cm carbon felt anode pretreated according to a previous description (W. Su et al., 2012). Titanium wires were used to connect the anode to the barrel-shaped reactor wall, which served as cathode. An Ag/AgCl electrode (sat. KCl. 0.197 V *vs.* standard hydrogen electrode) was used as the reference electrode. A voltage of 1.0 V was applied to the reactor AD–MEC–G by a DC Power supply (GPD-4303S, GWINSTEK, Taiwan), and a 16-channel voltage collection instrument (AD8223, RBH Co., Ltd., China) was used to monitor the voltage across an external resistor (R_{ex} = 2 Ω) for current calculation.

1.3. Experiments

All reactors were operated for three months, feeding with sodium acetate (10.0 g/L) in a buffered nutrient medium (Liu and Logan, 2004). After acclimation, batch tests were conducted with 230.0 mL of the medium described above. All reactors were sealed with rubber stoppers and gas was collected in a 2.0 L gas bag. Samples were withdrawn every 12 hr, centrifuged for 5 min at 10,000 r/min, diluted 10 times with distilled water and then filtered by a 0.22 μm filter. All experiments were conducted in triplicate at a temperature of 25 ± 2°C with initial pH of 6.8.

1.4. Analysis and calculation

Gases (H_2, CH_4 and CO_2) were detected according to our previous procedure (Jiang et al., 2013). Short chain fatty acids were analyzed on an HPLC 1260 (Agilent Technologies, Inc., USA) equipped with an Agilent Hi-Plex H column (300.0 × 6.5 mm) and a refractive index detector (45°C). Microbial samples were scraped from three different sites of the anodic biofilm, and mixed together for DNA extraction and high-throughput sequencing (Caporaso et al., 2011, 2012). Cyclic voltammetry was conducted in the potential range from −0.5 to 0.4 V at a low scan rate of 5 mV/sec.

Carbon recovery was based on the total moles of methane carbon recovered compared to the initial moles of carbon of the substrate. Overall energy efficiency relative to both the energy of the substrate and electrical input was evaluated as per a previous description (Call and Logan, 2008).

2. Results and discussion

2.1. Biogas production rate

As shown in Fig. 1a, the cumulative methane volume in the AD–MEC–G system achieved 642.9 mL in 72 hr, showing a methane yield of 360.2 mL/g-COD, which was increased by 59.7% and 32.4% compared to the AD (225.5 mL/g-COD) and AD–G (272.7 mL/g-COD) reactors, respectively. The result is also higher than that obtained in an AD–MEC reactor (289.6 mL/g-COD) (Bo et al., 2014). We obtained a 24.1% increment by co-cultivating *Geobacter* and *Methanosarcina*. It is well known that the maximum possible methane yield is 350.0 mL/g-COD in an anaerobic digester at standard temperature and pressure, which is equal to 370.0 mL/g-COD at 25°C and standard pressure (Zhang et al., 2010). The methane yields from anaerobic digester processes are usually very far from the theoretical upper limit. Nevertheless, the theoretical value was almost achieved in the AD–MEC–G system. The carbon recovery based on total moles of carbon for AD–MEC–G, AD–G and AD was 46.6%, 36.6% and 30.0%, respectively. Meanwhile, the COD removal efficiency increased from 55.6% for AD to 100.0% for the AD–MEC–G reactor in 72 hr (Fig. 1b). The maximum COD removal rate in the AD–MEC–G reactor (216.8 mg COD/(L·hr)) was enhanced by 29.6% compared to our previous study (AD–MEC system) of 167.3 mg COD/(L·hr) (Bo et al., 2014).

Generally, more COD degradation should result in more carbon dioxide emission ($CH_3COOH \rightarrow CH_4 + CO_2$). However, the carbon dioxide content in the total gas decreased gradually from 34.8% for reactor AD to 15.0% for reactor AD–G and 6.9% for reactor AD–MEC–G (Fig. 1c). The increase of methane yield as well as decrease of carbon dioxide content in

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