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

Qi Yin, Xiaoyu Zhu⁎, Guoqiang Zhan, Tao Bo, Yanfei Yang, Yong Tao, Xiaohong He, Daping Li⁎, Zhiying Yan

Key Laboratory of Environmental and Applied Microbiology, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China

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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/m²) 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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In recent years, Geobacter has been observed to be able to directly transfer electrons to methanogens, such as Methanoaeta 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 of 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 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 (GP-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 \( R_{ex} = 2 \Omega \) 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 (H₂, CH₄ and CO₂) 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 (CH₃COOH → CH₄ + CO₂). 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
AD–G, compared with AD, implied carbon dioxide may be reduced to additional methane. Hydrogen is an efficient electron donor for carbon dioxide reduction, but the hydrogen content was always below the detection limit in the AD and AD–G reactors (Fig. 1c). As DIET has been proved between Geobacter and Methanosarcina in a methane production environment (Rotaru et al., 2014a), it was hypothesized that the reducing energy may derived from the electrons transferred from Geobacter, leading to part of the carbon dioxide being reduced to methane. Moreover, Fig. 1d shows that hydrogen gas was detected in the AD–MEC–G reactor, which has been proved to drive carbon dioxide to methane conversion in AD–MEC systems (Bo et al., 2014). In addition, a recent study showed that with the presence of Geobacter, DIET becomes an important way for methane production in bioelectrochemical systems (Zhao et al., 2015). Since Methanosarcina can utilize both electrons and hydrogen for methane production, it is therefore suggested that carbon dioxide could be more efficiently reduced to methane via two pathways in the AD–MEC–G reactor: hydrogen interspecies transfer (HIT) and DIET.

2.2. Effect of Geobacter sp. on biofilm conductivity

The AD–MEC system with the inoculation of Geobacter showed better electrochemical performance. The highest current reached up to 75.0 mA (Fig. 1d), resulting in a current density of 304.3 A/m², which was 1.8-fold that of the AD–MEC reactor without Geobacter (166.7 A/m²) (Bo et al., 2014). With the current increase, overall energy efficiency was increased from 66.7% (AD–MEC reactor) (Bo et al., 2014) to 74.6% (AD–MEC–G reactor). The internal resistance of the AD–MEC–G system was only 12.3 Ω, which was decreased more than a factor of 3 compared with the AD–MEC system (38.0 Ω) (Bo et al., 2014). As we know, the resistance can limit the current output of a bioelectrochemical system, for which the components can be defined as (Malvankar et al., 2012; Manohar et al., 2008; Rabaey et al., 2009):

\[ R_{\text{int}} = R_{\text{anode}} + R_{\text{cathode}} + R_{\text{anolyte}} + R_{\text{catholyte}} + R_{\text{membrane}} + R_{\text{biofilm}} \]

where, \( R_{\text{int}} \) refers to the internal resistance of bioelectrochemical system; \( R_{\text{anode}}/R_{\text{cathode}} \) refers to the charge transfer resistance and \( R_{\text{anolyte}}/R_{\text{catholyte}} \) refers to electrolyte resistances for anode and cathode; \( R_{\text{membrane}} \) is the resistance of proton exchange membrane; and \( R_{\text{biofilm}} \) is the resistance associated with formed microbial film. Because the reactor AD–MEC–G has the same structure as the AD–MEC–G reactor used in our previous study (Bo et al., 2014), only \( R_{\text{biofilm}} \) was different. Thus, it was demonstrated that the \( R_{\text{biofilm}} \) decreased significantly in the presence of Geobacter. Malvankar et al. (2012) had pointed out that \( R_{\text{biofilm}} \) plays a key role in achieving high current density for a MFC. Based on our results, higher current density may be associated with the presence of Geobacter. Therefore, Geobacter may play a key role in stimulating electron transfer from organic matters to the anode, evidenced as higher current. The higher yield of methane (360.2 mL/g-COD) produced from the AD–MEC–G system means that more electrons were recovered as methane.
2.3. Microbiological compositions and relevant features

In reactor AD–MEC–G, 24.7% of total reads were affiliated with Geobacter sp. and 19.7% of total reads were assigned to Methanosarcina sp. (Fig. 2). These two groups of genera comprised the dominant microbiota in the methane producing system. In the AD–G reactor, the dominant microbiota was also composed of these two genera, Geobacter sp. (25.3%) and Methanosarcina sp. (18.8%). These results confirmed that Geobacter and Methanosarcina can coexist together in a biofilm. However, there were a variety of methanogens in the AD reactor, which were dominated by Methanoregula sp., Methanollia sp. and Methanoseta sp. Although Methanoseta has been reported to use the DIET pathway for methane formation (Rotaru et al., 2014b), Geobacter in the AD reactor was less than 0.1%, implying that methane generation in the AD system was probably via the traditional HIT pathway.

Desulfuromonas sp. presented 32.9% of total sequences in the AD–MEC–G reactor. Since Desulfuromonas sp. was reported to exchange electrons in MFC (Zhang et al., 2014), it might have participated in the improvement of AD–MEC–G system performance.

2.4. Mechanism of methane production enhancement by co-cultivation of Geobacter and Methanosarcina

Cyclic voltammetry was employed to investigate the bioelectrochemical behaviors of microorganisms in the AD–MEC–G reactor. The anodic biofilm of reactor AD–MEC–Gs showed a pair of clear oxidation and reduction peaks at −0.3 V (p1 and p2), whereas no electrochemical activities were observed in the AD and AD–G reactors (Fig. 3a). There was a previous report that observed a similar peak at −0.3 V representing the electrochemical activity of methanogens (Bo et al., 2014). Thus, Methanosarcina probably possesses electrochemical activity to accept electrons from the anode or extracellular electron transfer components, e.g. Geobacter (Zhu et al., 2012). As Fig. 3b shows, there was a peak (p3) that increased from −0.15 V and then reached a stable potential of 0.02 V. This was similar to the reported peak for Geobacter (Zhu et al., 2012). When the methanogen inhibitor (MI) 2-bromoethanesulfonate was applied to reactor AD–MEC–G, p1 and p3 decreased significantly, while p2 almost disappeared from the voltammograms (Fig. 3b). It is well known that 2-bromoethanesulfonate acts specifically to inhibit the methyl transfer reaction during

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Fig. 2 – The relative abundance of prokaryotic community at genus levels.

Fig. 3 – (a) Cyclic voltammograms comparison of biofilms in reactor AD, AD–G and AD–MEC–G. (b) Cyclic voltammograms comparison of biofilm of AD–MEC–G reactor before and after methanogens inhibitor (MI) addition. AD: anaerobic digestion; MEC: microbial electrolysis cell.
methane production (Bouwer and McCarty, 1983). The inhibition of activity of Geobacter thus implied that DIET was a methane syntrophic interaction between Geobacter and Methanosarcina in the AD–MEC-G system.

3. Conclusions

Our study clarified that Geobacter and Methanosarcina could coexist together in the AD–MEC coupled system, resulting in an enhanced methane yield of 360.2 mL/g-COD, which is comparable to the theoretical methane yield of an anaerobic digester. Geobacter was found to increase the current density and reduce system resistance of the MEC significantly, leading to more electrons being recovered as methane by Methanosarcina via HIT as well as DIET pathways.

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