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Effects of an applied voltage on direct interspecies electron transfer via conductive materials for methane production

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ABSTRACT

Direct interspecies electron transfer (DIET) between exoelectrogenic bacteria and methanogenic archaea via conductive materials is reported as an efficient method to produce methane in anaerobic organic waste digestion. A voltage can be applied to the conductive materials to accelerate the DIET between two groups of microorganisms to produce methane. To evaluate this hypothesis, two sets of anaerobic serum bottles with and without applied voltage were used with a pair of graphite rods as conductive materials to facilitate DIET. Initially, the methane production rate was similar between the two sets of serum bottles, and later the serum bottles with an applied voltage of 0.39 V showed a 168% higher methane production rate than serum bottles without an applied voltage. In cyclic voltammograms, the characteristic redox peaks for hydrogen and acetate oxidation were identified in the serum bottles with an applied voltage. In the microbial community analyses, hydrogenotrophic methanogens (e.g. *Methanobacterium*) were observed to be abundant in serum bottles with an applied voltage, while methanogens utilizing carbon dioxide (e.g., *Methanosaeta* and *Methanosarcina*) were dominant in serum bottles without an applied voltage. Taken together, the applied voltage on conductive materials might not be effective to promote DIET in methane production. Instead, it appeared to generate a condition for hydrogenotrophic methanogenesis.

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1. Introduction

Anaerobic digestion has been widely used to treat organic waste as well as to produce methane. In general, methane is formed via interspecies electron transfer (IET) between fermenting bacteria and methanogenic archaea. Fermenting bacteria produce diffusive electron carriers (e.g., hydrogen and formate), while methanogenic archaea utilizes diffusive electron carriers to produce methane (Giovannini et al., 2016). Recently, several studies revealed that adding conductive materials (e.g., granular activated carbon (GAC) and iron oxides) to methanogenic anaerobic reactors facilitates direct interspecies electron transfer (DIET) between the two groups of microorganisms (i.e., exoelectrogenic bacteria and methanogenic archaea) without mediating diffusive electron carriers (Kato et al., 2012; Zhao et al., 2016b). In DIET, conductive materials play a role as electrodes to accept electrons from

exoelectrogenic bacteria and donate the electrons to methanogenic archaea (Zhao et al., 2015). DIET via conductive materials is reported to be more efficient than that via IET in terms of rate and amount of methane production (Lee et al., 2016). This is due to the obviation of several steps involved in the production and consumption of diffusive electron carriers (Lovley, 2011). Also, electron transport through conductive materials is faster compared to that through molecular diffusion (Kato et al., 2012).

DIET via conductive materials for methane production can be achieved when specific groups of microorganisms attach on conductive materials. First, microorganisms capable of reducing conductive materials (i.e., exoelectrogens) should attach onto the conductive materials. Various bacteria have been reported to transport electrons extracellularly and to reduce conductive materials (Kiely et al., 2011). However, most were retrieved from microbial fuel cells instead of methanogenic reactors, promoting DIET via conductive materials. *Geobacter* species are the most well-known exoelectrogens observed in both microbial fuel cells (Oyiwona et al., 2016) and methanogenic reactors adopting DIET via conductive materials (Rotaru et al., 2014). Second, the methanogenic archaea that can accept electrons from the reduced conductive materials and can reduce carbon dioxide into methane, should be

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present on conductive materials. *Methanosaeta harundinacea* (Rotaru et al., 2014), *Methanosarcina barkeri* (Liu et al. 2012), and *M. acetivorans* (Salvador et al., 2017) are representative methanogens capable of this function.

The addition of a voltage is reported to produce methane from a biocathode containing methanogens in a microbial electrolysis cell, and the increased potential due to the addition of a voltage has been regarded to facilitate electron transfer to form methane from carbon dioxide. Likewise, imposing a voltage on conductive materials in methanogenic reactors may accelerate DIET and produce methane more efficiently. This speculation has been supported by the results of a study conducted by Zhao et al. (2016a), where applying a voltage on carbon resulted in an improvement in the methane production of a microbial electrolysis cell. However, the increase in methane production was not known to be a result of the increased DIET via conductive materials. This study therefore aimed to elucidate a mechanism for efficient methane production by imposing a voltage on conductive materials in the methanogenic reactors.

To this end, methanogenic serum bottles containing a pair of graphite rods as conductive materials were used. One set of duplicate serum bottles operated by applying a voltage to a pair of graphite rods, while the other set of the duplicate serum bottles operated without applying a voltage as negative controls. Various experiments were conducted, including temporal methane production, biomass production, potentiodynamic electrochemical measurement, and microbial community analysis. These would provide insight into an applied voltage on conductive materials to produce methane in association with DIET.

2. Materials and methods

2.1. Seed culture

Seed culture for the following batch experiments was obtained from a fed-batch anaerobic reactor (0.5 L working volume). The reactor was initially filled with 0.4 L culture medium, 0.1 L inoculum, and 5 g coal-based GAC. Because GAC is a conductive material, GAC was expected to facilitate DIET in the methanogenic serum bottles. The culture medium was composed of 5.5 g CH_3COONa , 5 g NaHCO_3 , 0.23 g NH_4Cl , 0.05 g KH_2PO_4 , and 0.05 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ per 1 L N_2 -purged deionized (DI) water. Acetate in the culture medium was used as the carbon source for methane production, and inoculum was obtained from an anaerobic digester treating waste activated sludge in the Jungnang wastewater treatment plant (Seoul, South Korea). GAC was purchased from Samcully (Seoul, South Korea) and had 0.43–0.48 g/mL of bulk density and 950 m^2/g of specific surface area. The reactor operated continuously by feeding the culture medium once a day to meet 25 days of hydraulic retention time at 35 °C for a year. Although such a long retention time might result in endogenous breathing, the reactor operating condition was set up because an efficient DIET between acetate utilizing bacteria and methane forming archaea via GAC was empirically observed (Lee et al., 2016). The seed culture showed efficient DIET between acetate utilizing bacteria and methane forming archaea via GAC (Lee et al., 2016). *Geobacter* was the predominant acetate-utilizing exoelectrogen in the seed culture (Lee et al., 2016).

2.2. Batch experiments

To investigate the effects of applying voltage on the methanogenic performance, batch experiments based on two duplicates were set up using 0.1 L serum bottles for 15 days. Although the methane production potential of the seed culture without carbon addition was not measured, the contribution of methane produced

from the seed culture appears to have been minimal because the seed biomass was very low (16.7 mg COD), and the incubation period was not too long (15 days). The bulk solution of the seed culture (30 mL, concentration of the seed culture was 555 ± 10 mg COD/L) and 20 mL of the culture medium were filled in each serum bottle, which was equipped with a pair of graphite rods (15 mm in length and 6 mm in diameter) and an Ag/AgCl reference electrode. Graphite was frequently used in the microbial fuel cell research due to its higher mechanical strength, good electrical conductivity, chemical stability, and lower cost than that of other materials (Wei et al., 2011). A voltage (+0.39 V) was applied on a pair of the graphite rods using DY2100B potentiostat (Digi-Ivy, Austin, TX, USA) while open circuit potential (i.e., no voltage supply) was maintained for the other two serum bottles as negative controls. Fig. 1 shows a schematic diagram of the two types of serum bottles. The initial pH of the serum bottles was 7.0, and the serum bottles were covered with rubber caps and placed in an incubator without agitation at 35 °C, after purging the head space using nitrogen gas for 5 min. The biogas production from the serum bottles was measured daily using a 50 mL syringe during incubation.

2.3. DNA extraction, PCR, and pyrosequencing

After the end of batch runs, 1 mL bulk solution was transferred in a 2 mL microcentrifuge tube and centrifuged at 11,000 rpm for 3 min. The supernatant was discarded, and the remaining solids were used to extract DNA. In parallel, the biomass attached on the graphite rod was collected, and the graphite rod was gently washed with DI water three times to remove the suspended biomass and was transferred in a 15 mL conical tube containing 5 mL of DI water. The conical tube was vibrated to beat the attached biomass off using a vortex mixer at 11,000 rpm for 3 min. The detached biomass from the graphite rod was used to extract the DNA following the protocol for a suspended biomass. The MoBio PowerSoil DNA extraction kit (Solana Beach, Ca, USA) was used to extract the community DNA from the suspended or attached biomass by following the manufacturer's instructions. PCR amplification of bacterial 16S rRNA gene fragments was performed using a universal primer set: 27 F (5'-AGAGTTT GATCMTGGCTCAG-3') (Massol-Deya et al. 1995) and 518 R (5'-AT TACCGCGGCTGCTGG-3') (Goodfellow and Stackebrandt 1991). On the other hand, PCR amplification of archaeal 16S rRNA gene fragments was performed using a universal primer set: 571R primer (5'-GCYTAAGSRNCCGTAGC-3') (Kan et al., 2011) and 1048 ArcR-M (5'-CGRCGGCCATGCACCWC-3') (Huber et al., 2007). Pyrosequencing of the PCR-amplified gene fragments was conducted at Macrogen (Seoul, South Korea) following the manufacturer's instructions (454 Life Science, Branford, USA). Detailed protocols for the PCR conditions and pyrosequencing followed that from previous studies (Kim et al., 2013; Lee et al., 2012). The potential chimeric sequences were removed using the chimera uchime algorithm in the Mothur program (Schloss et al., 2009). The taxonomic classification of the sequences was performed using the RDP's Bayesian Classifier (Cole et al., 2005) with an 80% threshold level. A neighbor joining tree was constructed using the MEGA program (Huber et al., 2007) to analyze the phylogenetic relationship of the representative phylotypes.

2.4. Current measurement and cyclic voltammetry experiment

Electric currents generated from the biomass on the graphite rods were measured using the DY2100B potentiostat (Digi-Ivy, Austin, TX, USA) which was linked to two graphite rods (one for the working electrode and the other for the counter electrode) and a reference electrode (Ag/AgCl). Cyclic voltammograms for the attached biomass in the serum bottles were generated by

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