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Enrichment of specific electro-active microorganisms and enhancement of methane production by adding granular activated carbon in anaerobic reactors

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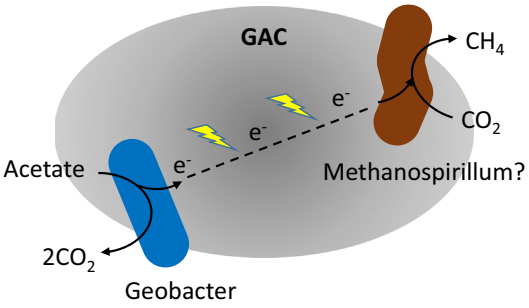
HIGHLIGHTS

- GAC supplementation significantly increased methane production.
- Increased methane production was mainly due to biomass attached to GAC.
- *Geobacter*, *Methanospirillum*, and *Methanolinea* were enriched on GAC.
- Direct interspecies electron transfer via GAC was generated.

GRAPHICAL ABSTRACT



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ABSTRACT

Direct interspecies electron transfer (DIET) via conductive materials can provide significant benefits to anaerobic methane formation in terms of production amount and rate. Although granular activated carbon (GAC) demonstrated its applicability in facilitating DIET in methanogenesis, DIET in continuous flow anaerobic reactors has not been verified. Here, evidences of DIET via GAC were explored. The reactor supplemented with GAC showed 1.8-fold higher methane production rate than that without GAC (35.7 versus 20.1 ± 7.1 mL-CH_4/d). Around 34% of methane formation was attributed to the biomass attached to GAC. Pyrosequencing of 16S rRNA gene demonstrated the enrichment of exoelectrogens (e.g. *Geobacter*) and hydrogenotrophic methanogens (e.g. *Methanospirillum* and *Methanolinea*) from the biomass attached to GAC. Furthermore, anodic and cathodic currents generation was observed in an electrochemical cell containing GAC biomass. Taken together, GAC supplementation created an environment for enriching the microorganisms involved in DIET, which increased the methane production rate.

1. Introduction

Anaerobic digestion is an efficient method for obtaining bioenergy in the form of methane from organic wastes (McCarty et al., 2011). In general, anaerobic methanogenesis is carried out by several groups of microorganisms involved in disintegration, hydrolysis, fermentation, and methanogenesis (Lee et al., 2012). Among them, interspecies electron transfer (IET) between secondary fermenting bacteria producing diffusive electron carriers (e.g. hydrogen and formate) and methanogenic archaea play an important role in determining the performance of anaerobic methanogenesis (Stams and Plugge, 2009). However, disruptions often occur in the syntrophic associations between the two groups of microorganisms in anaerobic digesters, which eventually leads to instabilities in performances. Granular activated carbon (GAC)

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can be supplemented into methanogenic reactors to overcome this disruption and increase the stability in digester start-up. Aktaş and Çeçen (2007) claimed that GAC supplementation is beneficial for increasing the performance of anaerobic digestion by adsorbing toxic organic compounds inhibiting the activity of methanogens as well as by providing a high surface area for microbial growth.

GAC is a conductive material as well as an efficient adsorbent. The electric conductivity of GAC is reported to be greater than 1000 S/m (Kastening et al., 1997). Supplementation of GAC in an anaerobic digester may facilitate a condition where direct interspecies electron transfer (DIET) between secondary fermenting bacteria and methanogenic archaea can occur (Liu et al., 2012; Zhi et al., 2014). In this case, GAC can be an electrode that accepts electrons from anode-reducing microorganisms (secondary fermenting bacteria), and in turn, the reduced GAC can donate electrons to cathode-oxidizing microorganisms (methanogenic archaea). Considering the experiments conducted by Kato et al. (2012), this scenario (i.e. DIET via GAC) could be possible. They traced methanogenic activities in vials supplemented with conductive iron-oxide materials and rice paddy field soil, and found a syntrophic association between anode-reducing bacteria (*Geobacter*) and methanogenic archaea via iron-oxide materials (i.e. DIET). Furthermore, Liu et al. (2012) demonstrated the applicability of GAC to DIET in methanogenesis in the co-culture of *Geobacter metallireducens* (anode-reducing bacteria) and *Methanosarcina barkeri* (cathode-oxidizing archaea). They also demonstrated that GAC could enhance methane production in the methanogenic granules of up-flow anaerobic sludge blanket (UASB) reactors that had shown DIET between *Geobacter* and *Methanosaeta* via microbial nanowire (Morita et al., 2011). Similar observations were recently reported in UASB reactors (Xu et al., 2015; Zhao et al., 2015). Nevertheless, it is not certain whether or not supplementation of GAC in conventional anaerobic digesters based on suspended biomass can facilitate a condition where anode-reducing and cathode-oxidizing microorganisms for DIET outgrow typical fermenting and methanogenic microorganisms.

DIET removes several steps associated with hydrogen production and consumption, which lead to greater energy efficiency than with IET via a diffusive electron carrier (Lovley, 2011). Furthermore, electron transport via conductive materials is much faster than molecular transport of electron carriers (Kato et al., 2012). Thus, the primary advantage of DIET via GAC instead of via diffusive electron carriers would be the enhanced methane production (Liu et al., 2012). If methanogenic activity is accelerated by supplementing conductive GAC in conventional anaerobic digesters, the anaerobic digesters will be more compact and stable than those utilizing IET via diffusive reducing equivalents (Liu et al., 2012).

The present study investigated methanogenic performance and microbial community changes in laboratory-scale anaerobic reactors supplemented with and without GAC. The main objective of this investigation was to test whether or not DIET between anode-reducing bacteria and methanogenic archaea via electron conductive GAC can be achieved in a continuous flow reactor. To identify the putative bacteria and archaea involved in the DIET, an anaerobic reactor supplemented with GAC was operated, and 454 pyrosequencing of 16S rRNA genes was conducted for the attached biomass on GAC. To further explore a DIET via GAC, current generations by the biomass attached on GAC were also measured.

2. Methods

2.1. Reactors, seed sludge, and synthetic wastewater

Two continuous flow glass jacketed reactors were operated for a period of 45 days. The working volume of each reactor was 500 mL, and the temperature of the reactors was maintained at 35 °C. GAC (5 g) was supplemented into one of the reactors. The reactor without GAC was used as a control. Magnetic bars were used to stir the biomass in the reactors. Coal-based GAC was purchased from Samchully (Seoul, South Korea). The bulk density, specific surface area, and particle diameter of GAC were 0.43–0.48 g/mL, 950 m^2/g, and 1.18–1.41 mm, respectively. Seed biomass was obtained from a conventional anaerobic digester that treats waste activated sludge in the Jungnang Wastewater Treatment Plant (Seoul, South Korea). The digester consisted of two reactors in series to promote anaerobic digestion, and operated at a mesophilic condition (~38 °C) (Lee et al., 2012). 100 mL seed biomass and 400 mL of synthetic wastewater were poured into the reactors and head space of the reactors was purged for 20 min using nitrogen gas. Synthetic wastewater per liter was prepared by adding or dissolving 4 mL CH_3COOH, 5 g $NaHCO_3$, 0.23 g NH_4Cl, 0.05 g KH_2PO_4, and 0.05 g $MgSO_4 \cdot 7H_2O$ to the remaining deionized (DI) water, and the pH of the synthetic wastewater was 7.2 ± 0.2. The acclimation period (10 days) was provided without the addition of feed. Afterward, the synthetic wastewater was continuously fed into the two reactors with 25 mL/d to adjust the hydraulic retention time to 20 days. During the reactor operation, the biogas generated from the reactors was collected daily using 1-L Tedlar gas sampling bags (Sigma–Aldrich, Saint Louis, MI, USA), and suspensions were collected daily for analyzing the chemical oxygen demand (COD).

2.2. Methanogenesis in batch reactors

Three sets of duplicate anaerobic vials were set up using serum bottles (100 mL working volume) containing suspension + GAC, suspension, and GAC, respectively. 80 mL of suspension (2.5 g VSS mg/L) with GAC (0.1 g) was added to the first set of vials, while only 80 mL of suspension was added to the second set of vials. For the third set of vials, GAC (0.1 g) and the filtrate of suspension (80 mL) obtained using 1-μm filter paper were added. Then, 20 mL of the synthetic wastewater used in the continuous flow reactors was poured into the vials. After purging the head space using N_2 gas for 20 min, the bottles were sealed using rubber caps and were placed in an incubator at 35 °C without stirring. Biogas production was measured daily using 50 mL gas syringes.

2.3. Analytical methods

Methane and carbon dioxide in the biogas were analyzed using a gas chromatograph (GC, Gow Mac series 580, USA) equipped with 1.8 m × 3.2 mm stainless-steel column packed with Porapak Q and a thermal conductivity detector. The temperatures of the oven, injector, and detector were operated at 40 °C. Helium (99.999%) was used as the carrier gas. COD was analyzed based on a reactor digestion method using the HACH COD measurement kit for low range (3–150 mg/L) (Loveland, CO, USA) following the manufacturer's protocol. For soluble COD, samples were filtered using a 0.45 μm membrane filter prior to COD analysis. Biomass concentrations in suspension and on GAC were also measured based on COD due to the availability of small biomasses, and the unit of biomass concentration was converted into volatile suspended solids (VSS) by multiplying by 1.42 (Rittmann and McCarty, 2012).

2.4. DNA extraction, PCR, and pyrosequencing

Suspensions (2 mL) from the reactors with or without GAC were centrifuged at 14,000 rpm for 5 min to harvest the suspended biomass. For the biomass attached to GAC, the GAC (0.1 g) was sampled from the reactor using sterilized tweezers and washed three times using DI water to remove the remaining suspended biomass. The attached biomass was then harvested from GAC by vigorously vortexing for 5 min. The harvested suspended and attached biomass was used to extract DNA using the MoBio PowerSoil DNA