

Development of a moving-bed carrier for stimulating direct interspecies electron transfer for improving anaerobic digestion

Hyun-Jin Kang¹, Jeong-Hoon Park¹, Hee-Deung Park^{1*}

¹School of Civil, Environmental and Architectural Engineering, Korea University, Seoul 02841, South Korea

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Presenting author email: heedeung@korea.ac.kr

Introduction

Interspecies electron transport (IET) is an essential process for a stable biogas production in anaerobic digestion. Electrons via the electron carriers (e.g. hydrogen and formate) generated by fermenting bacteria are transferred to methanogens during the methane fermentation (Fennell and Gossett, 1998). However, the diffusional limitation of the electron carriers from the fermenting bacteria to methanogenic archaea is a bottleneck in the methane fermentation processes. Many studies have suggested that supplementing conductive materials into a digester, electron transfer is facilitated without the use of the electron carriers, resulting in faster reduction of carbon dioxide to methane (Liu et al., 2012). This is direct interspecies electron transfer (DIET) via conductive materials (Figure 1).

Most DIET experiments have been performed in batch reactors, but there are no cases reported for full-scale application. When conductive materials are supplemented to a continuous-flow anaerobic digester, conductive materials should be recycled to maintain DIET in the digester. Furthermore, complex organic matters are known to be suitable substrates for DIET for methane production. These two are the main causes for limiting full-scale application of DIET via conductive materials in anaerobic digestion.

In this study, to overcome practical issue of DIET via conductive materials for full-scale applications, we fabricated a moving-bed carrier packed with conductive materials and incorporated a fermenting reactor. The moving-bed carrier was designed to be maintained in a digester with recycling of conductive materials, while the fermenting reactor could provide substrates (e.g. alcohols and fatty acids) for exoelectrogens. Feasibility of the carrier and fermenting reactors was tested using two continuous-flow bench-scale anaerobic reactors.

Material and Methods

Conductive carbon cloth (Zoroflex, Crawford, NE, USA) was cut into strips of 2 cm x 5 cm. As a non-conductive control, cotton cloth was treated in a similar manner. Carbon cloth or cotton cloth strips were wet-sterilized by autoclaving before packing. Carbon cloth or cotton cloth strips were packed into plastic structures named Bio-balls (Xinyou, Foshan, China) to fabricate moving-bed carriers.

Two continuous-flow glass-jacketed reactors with 50 moving-bed carriers packed with carbon cloth or cotton cloth (Figure 2) were operated for a period of 65 days. The working volume of each reactor was 700 ml, and temperature of the reactors was maintained at 35°C. Nutrients were supplied based on Endo medium (Endo et al., 1982) as follows; KH_2PO_4 (0.5 g/L), NH_4Cl (0.53 g/L), $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (0.1 g/L), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.75 g/L), and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (0.02 g/L), trace elements $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.025 g/L), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.005 g/L), $\text{CoCl}_2 \cdot 5\text{H}_2\text{O}$ (0.001 g/L) and $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ (0.015 g/L). After adding all substances, the reactors were purged with nitrogen gas for 20 min. pH in the two reactors was maintained 7 - 7.5 by NaHCO_3 as a pH buffer.

Methane content in the produced biogas was analyzed using gas chromatography (GC, Shimadzu, Japan) using a thermal conductivity detector (TCD) and a 1.8 m x 3.2 mm stainless-steel column packed with Porapak Q (80/100 mesh) with high purity helium (>99.999 %) as a carrier gas. The temperatures of the injector, detector, and column were 90, 80, and 90 °C, respectively, and the current of detector was 78.125 mV. Acetic acid and ethanol were analyzed using high performance liquid chromatography (HPLC, Waters, USA) using a refractive index (RI) detector and an ultraviolet (UV) detector (210 nm), and a 300 mm x 7.8 mm Aminex HPX-87H (Bio-Rad, USA) ion exclusion column with H_2SO_4 of 5 mM as the mobile phase. The RI and UV detector temperature was 65 and 35 °C, respectively. All liquid samples were pretreated using 0.45 μm of PTFE membrane filter before injection to HPLC. TS, VS and others were measured by standard method of APHA (APHA, 1998).

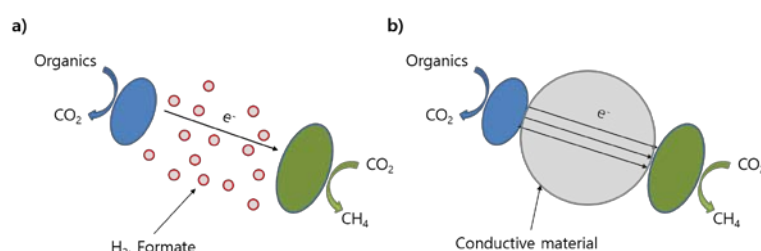


Figure 1. Comparison between IET (a) and DIET via conductive material.

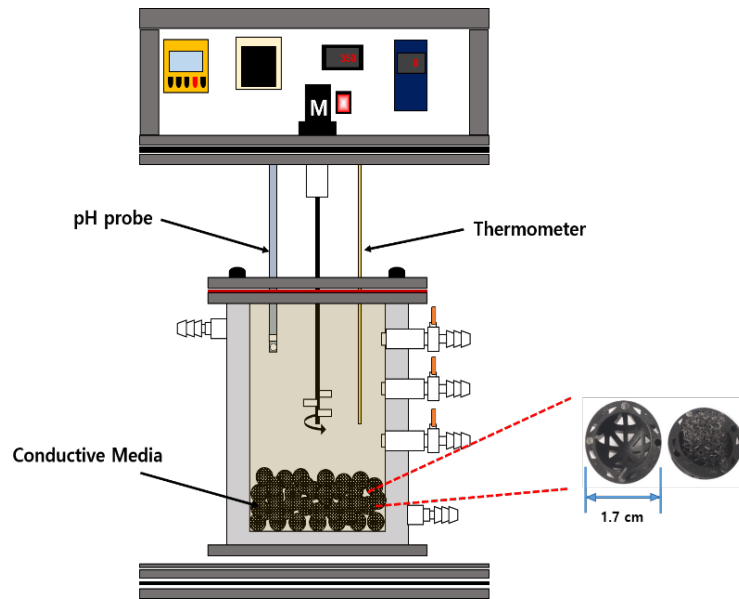


Figure 2. Schematic diagram of anaerobic digestion reactor and media for conductive material filling.

Results and Discussion

Two reactors were continuously fed with acetate (20 days hydraulic retention time). The reactor with moving-bed carriers packed with carbon cloth showed ~ 30% higher methane production volumes and ~ 40% methane production rates compared with that with cotton cloth. Metagenomic analyses for the suspended and attached biomass samples identified specific microorganisms for each sample. Specifically, exoelectrogens (e.g. *Geobacter* species) and *Methanosaeta* were dominant in the attached biomass samples. These results indicate that enhanced methane production performance was due to microorganisms involved in DIET, and highlight utility of the moving-bed carriers packed with carbon cloth for stimulating DIET via conductive materials.

For the application of the reactors for complex organics, fermentation substrates from a batch fermenting reactor and glucose was fed to the reactors with moving-bed carriers packed with carbon cloth, respectively. Although the reactor fed with glucose could not show high performance, the reactor fed with fermentation substrates for exoelectrogens demonstrated methane production performance similar to that of the operation with acetate feed. This result suggests that a fermenter for producing simple organic compounds was essential to facilitate DIET via conductive materials.

Conclusions

Moving-bed carriers packed with carbon cloth was efficient to increase both methane production volume and methane production rate without recycling the carriers. Metagenomic analysis demonstrated that the improvement in methane production performance was due to microorganisms involved in DIET. Furthermore, the reactor operated with fermentation substrates could stimulate DIET, indicating necessity of a fermenting reactor for treating complex organics. Taken together, this study can provide practical solutions for applying DIET via conductive materials in full-scale digesters.

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