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Response of microbial community structure to pre-acclimation strategies in microbial fuel cells for domestic wastewater treatment

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HIGHLIGHTS

- Three strategies were applied for substrate pre-acclimation of air-cathode MFCs.
- Serial pre-acclimation with acetate-glucose produced higher current from wastewater.
- Microbial communities were significantly varied by pre-acclimation strategies.
- *Anaerolinaceae* seemed to play important roles in MFCs using domestic wastewater.

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ABSTRACT

Microbial community structures and performance of air-cathode microbial fuel cells (MFCs) inoculated with activated sludge from domestic wastewater were investigated to evaluate the effects of three substrate pre-acclimation strategies: 1, serial pre-acclimation with acetate and glucose before supplying domestic wastewater; 2, one step pre-acclimation with acetate before supplying domestic wastewater; and 3, direct supply of domestic wastewater without any pre-acclimation. Strategy 1 showed much higher current generation (1.4 mA) and Coulombic efficiency (33.5%) than strategies 2 (0.7 mA and 9.4%) and 3 (0.9 mA and 10.3%). Pyrosequencing showed that microbial communities were significantly affected by pre-acclimation strategy. Although *Proteobacteria* was the dominant phylum with all strategies, *Actinobacteria* was abundant when MFCs were pre-acclimated with glucose after acetate. Not only anode-respiring bacteria (ARB) in the genus *Geobacter* but also non-ARB belonging to the family *Anaerolinaceae* seemed to play important roles in air-cathode MFCs to produce electricity from domestic wastewater.

1. Introduction

Microbial fuel cells (MFCs) have been widely studied as a device to produce electricity from organic compounds in domestic wastewater (Ahn and Logan, 2010; Min and Logan, 2004; Yu et al., 2012b; Zhang et al., 2013). One of the key factors of MFCs used to treat domestic wastewater is their microbial communities, which include anode respiring bacteria (ARB) that transfer electrons to electrodes and prefer simple organics (Kim et al., 2007; Logan, 2009).

The microbial community structure in an MFC is greatly affected by substrate type, which can be fermentable or non-fermentable (Chae et al., 2009; Chaudhuri and Lovely, 2003; Pant et al., 2010; Wang et al., 2012). Although fermentation metabolites such as butyrate, propionate, and acetate are all commonly used as substrates to enrich ARB on the anode surface, to date, acetate has been the most frequently used substrate. This is because ARB such as *Geobacter sulfurreducens* are known to readily produce electricity from acetate (Liu et al., 2005; Yi et al., 2009), and acetate can exclude or minimize the effect of fermentative bacteria that might be present in the anode chamber. However, domestic wastewater contains not only simple substrates but also highly complex organics that need to be hydrolyzed and fermented. Therefore, a pre-acclimation strategy employing fermentable substrates such as glucose is important to developing a microbial community that

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can effectively produce electricity from complex organics in wastewater.

To understand the precise structure of microbial communities, molecular methods are preferred over cultivation-based methods. Many previous studies that have analyzed microbial communities in MFCs applied denaturing gradient gel electrophoresis (Beecroft et al., 2012; Freguia et al., 2010; Jung and Regan, 2007; Rismani-Yazdi et al., 2011) and clone library analysis (Ki et al., 2008, 2010, 2011) based on 16S rRNA genes. However, only a limited portion of the microbial community could be analyzed using these tools. Since 454 pyrosequencing allows analysis of massive-scale 16S rRNA gene sequence libraries (Lee et al., 2010), this method provides more detail on the microbial community structure in MFCs.

This study aimed to evaluate the performance and microbial community in three groups of air-cathode MFCs with different pre-acclimation substrate strategies applied. Before using domestic wastewater as a substrate, the first and second groups were operated with a synthetic medium. The first group was serially pre-acclimated with acetate (a readily biodegradable substrate) and glucose (a fermentable substrate) to promote the growth of ARB, specifically, and then to ferment these bacteria. To remove the intentional growth of fermentative bacteria, the second group was pre-acclimated with acetate only. The third group used domestic wastewater without any pre-acclimation to show spontaneously developed microbial communities. To analyze the microbial communities, 16S rRNA gene-based 454 GS-FLX pyrosequencing was used.

2. Materials and methods

2.1. MFC construction and operation

Cubic-type air-cathode MFCs (anodic volume: 260 mL) were constructed as previously described (Yu et al., 2012a). Graphite felt (30 mm × 40 mm) was used for the anode, and a same-sized cathode was made from 30% wet-proof carbon cloth (E-Tek, BASF Fuel Cell Inc., USA), using a Pt/C catalyst (0.5 mg/cm^2) and a 5% Nafion solution (Cheng et al., 2006). The separator was made of a polypropylene non-woven fabric (Korea Non-Woven Tech. Co., Ltd., Korea) (Yu et al., 2014), and the anode and cathode electrode were connected with a copper wire.

The anode compartments were inoculated with activated sludge obtained from a domestic wastewater treatment plant (Busan, Korea). The synthetic medium consisted of (apart from the carbon source) K_2HPO_4, 4.35 g/L; KH_2PO_4, 3.38 g/L; NH_4Cl, 0.115 g/L; NaCl, 0.04 g/L; $MgSO_4{\cdot}7H_2O$, 0.01 g/L; $CaCl_2{\cdot}2H_2O$, 0.02 g/L; KCl, 0.02 g/L; and yeast extract, 0.005 g/L. Domestic wastewater, which contained soluble chemical oxygen demand (COD) of 146 ± 27 mg/L, was collected from an effluent line of the primary clarifier at a wastewater treatment plant (Busan, Korea). Before using domestic wastewater as a substrate, it was filtered through a GF/C filter (Cat No 1822-110, 110 mm ∅, Whatman, UK).

Three groups of air-cathode MFCs (each in triplicate) were operated with different pre-acclimation substrate strategies. Two groups were pre-acclimated with acetate (150 mg-COD/L) until the MFCs performed stably. Then, the first group further acclimated with glucose (150 mg-COD/L) as a fermentable substrate (strategy 1), and the second group received domestic wastewater (strategy 2). After the glucose-fed MFCs began to stably generate current, the glucose was replaced by domestic wastewater. The third group was operated with domestic wastewater directly, without any pre-acclimation stage (strategy 3). To increase current generation, external resistance initially equipped for 1000 Ω was reduced to 91 Ω after generation of a stable voltage. All MFCs were operated at ambient temperature in batch mode.

2.2. Chemical and electrochemical analyses

Voltage (E, V) was measured with a data acquisition system (Model 7700, Keithley Instruments Inc., Cleveland, OH, USA) and recorded on a personal computer. Current (I, A) was calculated based on Ohm's law ($I = E/R_{ext}$), where R_{ext} (Ω) is the external resistance.

COD was measured using a COD_{Cr} analysis kit (HS-COD-LR, Humas Co. Ltd., Korea) with a spectrophotometer (HS 2000, Humas Co. Ltd., Korea). Coulombic efficiency (CE) was computed using a previously reported equation (Logan et al., 2006). COD removal efficiencies and CEs were calculated at every single-batch cycle and the average data of each substrate condition were evaluated.

2.3. Microbial community analysis

To investigate the effect of substrate pre-acclimation strategies on microbial communities in anodic biofilms, MFCs were dismantled when they showed stable current production at each acclimation step with substrates such as acetate, glucose, and domestic wastewater. Then, some pieces of graphite felt from anodes were sampled for DNA extraction. DNA was extracted using a PowerSoil DNA extraction kit (Mo Bio Labs, Carlsbad, CA, USA) following the manufacturer's instructions. The bacterial 16S rRNA genes within the variable V1–V3 region were amplified from the extracted DNA using barcoded fusion primers that contained the 454 adapter (CCATCTCATCCCTGCGTGTCTCCGAC in the reverse primer and CCTATCCCCTGTGTGCCTTGGCAGTC in the forward primer), a 454 key sequence (TCAG), a barcode sequence (7–11 bases in the reverse primer), a linker (2 bases), and the 27F (GAGTTTGATCMTGGCTCAG) and 518R (WTTACCGCGGCTGCTGG) universal primer sequences. The polymer chain reaction (PCR) mixture was the following: 1 μL of 100 ng template DNA, 35.5 μL of molecular biology grade water, 5 μL of 10× buffer, 4 μL of dNTPs (2.5 mM), 2 μL of 10 pmol each primer, and 0.5 μL of Taq polymerase (5 U/μL). PCR amplification was performed using a PTC-200 Peltier thermal cycler (MJ Research, Waltham, MA, USA) with the following conditions: an initial denaturation at 95 °C for 5 min; 30 cycles of denaturation (30 s at 95 °C), annealing (30 s at 55 °C), extension (30 s at 72 °C); and a final extension at 72 °C for 7 min. PCR products were purified with a QIAquick PCR Purification Kit (QIAGEN, Valencia, CA, USA). Pyrosequencing was performed by ChunLab Inc. (Seoul, Korea) using a 454 GS-FLX Titanium Sequencing System (Roche, Branford, CT, USA). Low quality sequences (read length <300 bp and average quality score <25) were trimmed. Finally, multiple sequence alignment and complete linkage clustering were utilized to cluster the sequences from 0–3% dissimilarity using the ExTaxon-e database (Kim et al., 2012). Operational taxonomic units (OTUs) were defined via the CD-HIT program (Li and Godzik, 2006). Alpha diversity (e.g., Shannon and Chao 1 indices), beta diversity based on Fast Unifrac analysis (Hamady et al., 2010), and a gradient heat map analysis were carried out using CLcommunity software (ChunLab Inc., Seoul, Korea).

All sequences were submitted to the Sequence Read Archive (SRA) of the National Centre for Biotechnology Information (NCBI) database (accession number: SRR5124915).

3. Results and discussion

3.1. Current generation

Current generation in MFCs differed by pre-acclimation strategy, as shown in Fig. 1. While MFCs with strategies 1 and 2 applied showed stable current generation (maximum 0.6 mA) during the period of acetate pre-acclimation, MFCs with domestic wastewater