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A novel method to characterize bacterial communities affected by carbon source and electricity generation in microbial fuel cells using stable isotope probing and Illumina sequencing

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ABSTRACT

Stable isotope probing and high throughput sequencing were used to characterize the microbial communities involved in carbon uptake in microbial fuel cells at two levels of electricity generation. With acetate, the dominant phylotypes involved in carbon uptake included *Geobacter* and *Rhodocyclaceae*. With glucose, both *Enterobacteriaceae* and *Geobacter* were dominant.

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1. Introduction

There has been increasing attention towards microbial fuel cells (MFCs) due to their dual functionality for organic waste degradation as well as energy production (Li et al., 2014). Power generation involves the oxidation of organic substrates and electron transfer to an anodic electrode (Logan, 2008). Although many factors are thought to influence the efficiency of energy generation, the organic substrate type and the microbial community present are particularly important variables to consider. In most cases, acetate is preferred as the anode electron donor due to its high Coulombic efficiency (CE). Glucose usually has high power density (PD) when used as an electron donor, while it generates much less CE than acetate due to its fermentable characteristic (Chae et al., 2009). Researchers have also tested various inocula to MFCs for improving energy generation efficiency. For example, *Clostridium cellulolyticum*, *Geobacter sulfurreducens* (Ren et al., 2008) and *Shewanella* (Debabov, 2008) have been added to MFCs to evaluate the corresponding current density generation.

MFC performance is also affected by electricity generation, which demands for electrons from microbial oxidation of organic matters. Electrical output can be adjusted by varying the external resistance, and it has been found that different levels of electricity generation could significantly affect organic degradation (Zhang et al., 2010). Thus, it is hypothesized that microbial community on the anodic electrode will also change with different levels of electricity generation. Revealing this community change will be important to the understanding of the roles of different microbial species in electricity generation and provide the information that may guide the future operation of an MFC system.

Recent advances in molecular microbiology have shown great potential for improving our understanding of microbial communities present in MFCs (Zhi et al., 2014). For example, high throughput sequencing technologies have provided a much greater depth of information on MFC microbial ecology (Jia et al., 2013; Lesnik and Liu, 2014). Other methods also have potential for determining which microorganisms are dominant and active. Recently, stable isotope probing (SIP) was combined with denaturing gradient gel electrophoresis (DGGE) to investigate the syntrophic interactions in an MFC (Kimura and Okabe, 2013). SIP is advantageous as it allows the identification of metabolically active microorganisms from diverse microbial communities through tracking the flow of isotopically labeled atoms incorporated into biomass. This technique broadens the scope for linking function with identification due to its independence from cultivation. SIP involves the exposure of the microbial community to a labeled substrate. Microorganisms assimilate the isotope into biomass including their nucleic acids and their identity is determined from 16S rRNA analysis.

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In the current study, a novel molecular approach was applied to investigate the microbial community involved in carbon uptake under different MFC operating conditions. Specifically, we combined SIP with high throughput sequencing (MiSeq Illumina) to determine which microorganisms were responsible for carbon assimilation from glucose and acetate. Although both approaches have been used separately to examine MFC communities, this is the first study to unite these methods enabling a better understanding of the dominant microorganisms involved in carbon flow. The study also investigated the effect of two levels of current generation (controlled by different external resistors, 10 Ω and 1000 Ω) on the active microbial community. In all, the microorganisms involved in carbon uptake from eight MFC anodes were investigated.

2. Materials and methods

2.1. Chemicals

Reagents were purchased from one or more of the following vendors: Fisher Bioreagent (Thermo Fisher Scientific, NJ, USA), Integrated DNA Technologies (Coralville, IA, USA) and Sigma-Aldrich (St. Louis, MO, USA).

2.2. MFC setup and operation

Multiple MFCs were set up in two-chamber configuration using glass bottles, similar to those in the prior study (Fig. 1) (Xiao et al., 2012). The anode was inoculated with anaerobic sludge from a local municipal wastewater treatment plant. The anode material was carbon cloth (3 cm × 4 cm, PANEX®30PW03, Zoltek Corporation, St. Louis, MO, USA) with surface area of 12 cm^2 and the cathode material was carbon brush (5 cm, Gordon Brush Mfg. Co., Inc., Commerce, CA). Both electrodes were soaked in a 100 mL solution in the anode and cathode chambers. The solution in anode chamber contained 0.3 g/L NH_4Cl, 1 g/L NaCl, 0.03 g/L $MgSO_4$, 0.04 g/L $CaCl_2$, 0.2 g/L $NaHCO_3$, 5.3 g/L KH_2PO_4, 10.7 g/L K_2HPO_4 and 1 mL/L trace solution. Trace solution contained 10,000 mg/L $FeCl_2$–$4H_2O$; 2000 mg/L $CoCl_2$–$6H_2O$; 1000 mg/L EDTA; 500 mg/L $MnCl_2$–$4H_2O$; 142 mg/L $NiCl_2$–$6H_2O$; 123 mg/L Na_2SeO_3; 90 mg/L $AlCl_3$–$6H_2O$; 69 mg/L Na_2MoO_4–$2H_2O$; 50 mg/L $ZnCl_2$; 50 mg/L H_3BO_3; 38 mg/L $CuCl_2$–$2H_2O$; and 1 mL/L HCl (37.7% solution). The solution in the cathode chamber contained potassium ferricyanide at a concentration of 500 mM. All water was deionized water. The anode and cathode chambers were separated by a cation exchange membrane (Ultex CMI 7000, Membranes International, lnc., Glen Rock, NJ, USA).

The substrates for MFCs startup were unlabeled sodium acetate or glucose with an initial concentration of 1 g/L. After ~30 days of operation, labeled (^{13}C) or unlabeled substrates were added (1 g/L). Overall, eight sets of MFCs were investigated including the addition of labeled (^{13}C) acetate, labeled (^{13}C) glucose, unlabeled acetate, and unlabeled glucose and the external resistance was manipulated to two levels (10 and 1000 Ω). All MFCs were operated at room temperature. To ensure adequate label uptake from acetate or glucose into biomass, anode electrodes were collected following 14 days of operation. The anode electrodes were then stored at −20 °C.

2.3. DNA extraction

Total genomic DNA was extracted using the Power Soil DNA extraction kit, following the manufacturer's instruction (MO BIO Laboratories, Inc. Carlsbad, CA). Eight samples were investigated, including materials obtained from an MFC amended with i) unlabeled acetate operated at 10 or 1000 Ω, ii) labeled acetate operated at 10 or 1000 Ω, iii) unlabeled glucose operated at 10 or 1000 Ω, and iv) labeled glucose operated at 10 or 1000 Ω. The extracted DNA samples were quantified with the Nanodrop-1000 (Thermo Fisher Scientific lnc.).

2.4. Isopycnic centrifugation

The extracted DNA was ultracentrifuged in cesium chloride gradients separately to obtain density-resolved gradients and fractions, as previously described (Luo et al., 2009; Sun and Cupples, 2012; Sun et al., 2010, 2012, 2014a,b; Xie et al., 2010, 2011). For each MFC treatment, replicate DNA samples were subject to ultracentrifugation. In all, sixteen DNA samples (eight treatments in replicate) were ultracentrifuged. For each sample, approximately 10 μg of total genomic DNA was mixed with a Tris–EDTA (pH 8.0) buffer and CsCl solution. This mixture was added to a 5.1 mL Quick-Seal polyallomer tubes (1.3 × 5.1 cm, Beckman Coulter) the buoyant density (BD) of this mixture was adjusted to around 1.72 g/mL using a model AR200 digital refractometer (Leica Microsystems Inc.) and then sealed using a tube topper (Cordless quick-seal tube topper, Beckman). The tubes were then centrifuged at 178,000 ×g for 46 h at 20 °C in a Wx Sorvall Ultra 80 ultracentrifuge fitted with a Stepsaver 70 V6 Vertical Titanium Rotor (Thermo Fisher Scientific lnc.).

Each of the 16 ultracentrifuged samples were separated into 20 fractions (250 μL) by displacing the samples with molecular grade water. A syringe pump attached to a needle (BD, 23G and 1 inch) was used to displace samples from the top of the tube. This resulted in fractions being collected from higher to lower BD values. The BD of each fraction was calculated from the refractive index obtained using a refractometer. DNA from each of the fraction was recovered using a glycogen and ethanol precipitation. Precipitated DNA was then re-suspended in 30 μL PCR grade water and stored at −20 °C for further analysis. The

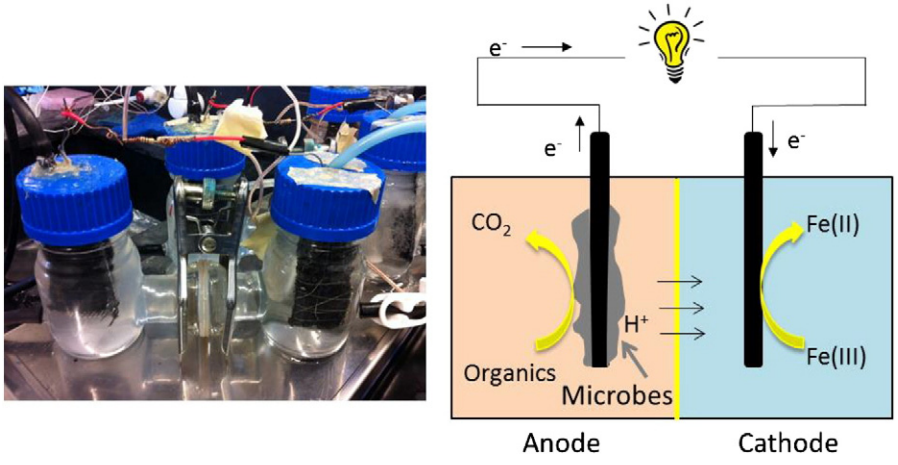

Fig. 1. Picture (left) and schematic (right) of the two chamber MFCs used in this study.

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