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Response of the microbial community structure of biofilms to ferric iron in microbial fuel cells

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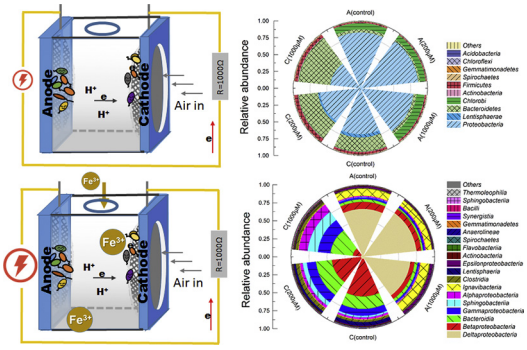
HIGHLIGHTS

- Ferric iron can facilitate the electricity generation of MFCs.
- Community structure of the biofilms in MFCs could be shaped by ferric iron.
- The predominant populations in the anode biofilms of MFCs were *Geobacter*.
- Response of the community of the cathode biofilms to Fe^{3+} was more obvious than of the anode biofilm.

GRAPHICAL ABSTRACT



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ABSTRACT

Ferric iron can affect the current generation of microbial electrochemical system (MES); however, how it influences microbial biofilm formation and metabolic activity has not been reported. Here, we describe the response of microbial electrode biofilm communities to insoluble ferric iron (Fe^{3+}) at different concentrations in microbial fuel cells (MFCs). Insoluble ferric iron (200 μM) improved electrochemical activity of the MFCs microbial biofilms during start-up and resulted in a higher maximum power density of 0.95 W/m^2, compared with the control (0.76 W/m^2), 500 μM Fe^{3+} (0.83 W/m^2), 1000 μM Fe^{3+} (0.73 W/m^2), and 2000 μM Fe^{3+} (0.59 W/m^2) treatments. Illumina Hiseq sequencing of 16S rRNA gene amplicons indicated that the predominant populations in the anode biofilms of the MFCs belonged to *Geobacter*, with relative abundance of 66–75%. Microbial cathode biofilm communities were more susceptible to Fe^{3+}, as an obvious shift in the cathode biofilm community structures occurred as Fe^{3+} concentration was increased. The most predominant populations in the MFC cathode biofilms without Fe^{3+} and with 200 μM Fe^{3+} were affiliated with *Thauera* (46% and 35%), whereas no absolutely predominant populations were present in the MFC cathode biofilm with 1000 μM Fe^{3+}. The results demonstrate that a low concentration of Fe^{3+} facilitated the power output of MFCs and shaped community structures of the electrode biofilm.

1. Introduction

Microbial electrochemical systems (MESs) are considered potential energy recovery technology based on direct conversion of organic matter into electricity (Rabaey et al., 2003). The current generation of MESs depends on exoelectrogenic community structure. Insight into the

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understanding of a microbial biofilm on an electrode surface is important to enhance the power output and reveal extracellular electron transfer (Wrighton et al., 2011). Ecological conditions, such as temperature, pH, light conditions, inoculum and carbon sources, influence the shape of microbial communities on biofilms in microbial fuel cells (MFCs) (Lu et al., 2011; Lu et al., 2012; Mei et al., 2015; Patil et al., 2011; Wu et al., 2013; Xing et al., 2009). Hence, optimizing the ecological conditions that facilitate exoelectrogen enrichment and the current output is essential for developing MFCs.

The effects of ferric or ferrous irons on anode biofilm formation and electrochemical performance of MES have been reported (Liu et al., 2017; Wei et al., 2013; Wu et al., 2013). Adding Fe^{3+} to MECs enhances both anaerobic digestion and anodic oxidation, resulting in effective mineralization of volatile fatty acids (Zhang et al., 2013). Dissimilatory metal-reducing bacteria are capable of utilizing extracellular insoluble metals for respiration (Kato et al., 2013). Modifying the anode with α-Fe_2O_3 nanorod and chitosan as well as rolling Fe_3O_4 into activated carbon enhances electrical output and increases maximum power density (Ji et al., 2011; Peng et al., 2012). Exogenous Fe (III) plays a critical role in biofilm formation and flavin secretion for *Shewanella oneidensis* MR-1 in MFCs and enhances the power output (Wu et al., 2013). Ferric iron supplements accelerate rapid enrichment of anodophilic consortium in MFCs (Wang et al., 2010). One investigation reported that the electrically conductive magnetite derived from Fe^{3+} facilitates direct interspecies electron transfer (Kato et al., 2012). The effect of Fe^{3+} on MFC performance needs to be investigated, and few studies have evaluated the response of the anode exoelectrogenic community to Fe^{3+}.

Some metal ions are involved in the cathodic process, and directly affect the performance of MESs (Huang et al., 2013). Fe^{3+} is reduced to Fe^{2+} at the cathode surface of MFCs (Ter Heijne et al., 2006), followed by Fe^{2+} reoxidation and precipitation as oxi(hydroxi)des (Lefebvre et al., 2012). Reoxidation of electron mediators (such as iron and manganese) by bacteria were investigated in cathodes with oxygen as the terminal electron acceptor (He and Angenent, 2006). The cycle of the Fe^{3+}/Fe^{2+} couple is also important for mediating degradation of persistent organic pollutants (Feng et al., 2011; Tao et al., 2010). As reported, adding $Fe(OH)_3$ might be a viable method to enhance current density in MECs, primarily by improving cathode performance, but the effect of Fe^{3+} on cathodic microbial community is poorly understood (Ren et al., 2012).

In this study, the electrochemical performance of air-cathode MFCs supplemented with different concentrations of ferric iron was investigated to evaluate the effect of insoluble ferric iron on bacterial biofilms on electrode surfaces. The microbial community structures of the anode and cathode biofilm in MFCs were analyzed using Illumina Hiseq sequencing of 16S rRNA gene amplicons.

2. Materials and methods

2.1. MFC configuration

Single-chamber cuboid MFCs with a volume of 14 ml were constructed as described previously (Xing et al., 2008). The anode and cathode were placed on the opposite sides in a plexiglas cylindrical chamber (2 cm long by 3 cm in diameter). The anode was carbon paper with the areas of 7 cm^2 (Toray TGP-H-090, Japan). The air cathode consisted of stainless steel mesh rolled with activated carbon and polytetrafluoroethylene as catalyst layer (Dong et al., 2012). Titanium wire was used to connect the circuit. All MFC reactors were operated in fed-batch mode with external resistor of 1000 Ω in a temperature-controlled room at 35 °C. The effluent of a MFC that had been operating for 2 months in our lab and that had a steady power output was chosen as the source inoculum for the first two cycles. The nutrient solution (litter) consisted of 1 g sodium acetate, 5 ml vitamins, 12.5 ml minerals (Call and Logan, 2008), 100 mM PBS (KCl, 0.13 g/l; NH_4Cl, 0.31 g/l; $NaH_2PO_4 \cdot 2H_2O$, 5.54 g/l; $Na_2HPO_4 \cdot 12H_2O$, 23.11 g/l) and additional $FeCl_3$ of different concentrations (0, 200, 500, 1000, and 2000 μM). As the pH of the MFC solution was neutral, Fe^{3+} actually existed in the form of an insoluble precipitate. MFCs supplemented with Fe^{3+} were operated in triplicate. Voltages across the external resistance (1000 Ω) of the MFCs were measured using the Keithley 2700 multimeter/data acquisition system every 10 min.

2.2. Analytical methods

Cyclic voltammetry measurements of MFCs were performed during operations on an Autolab potentiostat (Metrohm, Netherlands) at a scan rate of 0.005 V/s. The maximum power density and polarization curves were obtained by varying the external resistors (50–2000 Ω). Conductivity of the influent and effluent in all MFCs was detected with a conductivity meter (DDSJ-308A, Leici). Chemical oxygen demand (COD) was measured according to standard methods of the American Public Health Association (Clesceri et al., 1998). COD of MFC influents and effluents during an entire cycle were measured after filtration through a 0.45 μm membrane filter.

2.3. DNA extraction and Hiseq sequencing of microbial biofilms

After three months of operation, the carbon paper of the anode was cut into pieces by sterilized scissor and microbial biofilm on the cathode was carefully scraped from the water facing side by using sterilized blade. The anode and cathode biofilms of MFCs (including that without additional Fe^{3+} and fed with 200 μM Fe^{3+} and 1000 μM Fe^{3+}) were sampled for DNA extraction using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, San Diego, CA, USA). DNA concentration and purity were confirmed by NanoPhotometer P-Class (Implen, GmbH). Prior to polymerase chain reaction (PCR) amplification, the DNA of the anode and cathode biofilms with parallel bioreactors were mixed seperately. The V4–V5 region of the bacterial 16S rRNA gene was amplified using a set of universal bacterial primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 907R (5′-CCGTCAATTCCTTTGAGTTT-3′). The PCR mixture (30 μl) consisted of 15 μl Phusion Master Mix (Phusion High-Fidelity PCR Master Mix with GC Buffer, 2×), 3 μl primer sets (2 μM), 10 μl DNA (1 ng/μl) and 2 μl H_2O. PCR amplification was implemented using the ABI GeneAmp 9700 PCR system under the following conditions: 98 °C 1 min; 30 cycles of 98 °C 10 s, 50 °C 30 s, 72 °C 30 s; 72 °C 5 min.

Sequencing was performed on the Illumina Hiseq 2500 platform according to standard protocols. Raw tags were overlapped (the minimum overlap of 10 bp and the maximum error rate of 0.1) using the Fast Length Adjustment of SHort reads (FL-ASH) software (V1.2.7, http://ccb.jhu.edu/software/FLASH/) (Magoc and Salzberg, 2011) and filtered (q value is less than or equal to 19) following the Quantitative Insights Into Microbial Ecology pipeline software (QIIME, V1.7.0, http://qiime.org/scripts/split_libraries_fastq.html) (Caporaso et al., 2010). Effective tags were obtained by removing the chimeric sequences (http://www.drive5.com/usearch/manual/chimera_formation.html) after alignment using the Gold database (http://drive5.com/uchime/uchime_download.html). Operational taxonomic units (OTUs) were determined based on the threshold of 97% similarity using UPARSE software (Uparse V7.0.1001). A representative sequence of each OTU was aligned for taxonomic identification using the GreenGene database (http://greengenes.lbl.gov/Download/) and Ribosomal Database Project (RDP) classifier (ver. 2.2 http://sourceforge.net/projects/rdp-classifier/) with a threshold of 80–100% (DeSantis et al., 2006; Wang et al., 2007). The principal coordinates analysis (PCoA) plots based on the relative abundance of OTUs were generated using weighted and unweighted UniFrac algorithms. Principal components analysis (PCA) plot based on the relative abundance of OTUs was generated by using ggplots2 package of R (Version 2.15.3). Diversity indices, Good's coverage, and species richness were generated in scikit-bio (http://scikit-bio.org/). The raw Illumina sequencing data were