Impact of Ferrous Iron on Microbial Community of the Biofilm in Microbial Fuel Cells

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The performance of microbial electrochemical cells depends upon microbial community structure and metabolic activity of the electrode biofilms. Iron as a signal affects biofilm development and enrichment of exoelectrogenic bacteria. In this study, the effect of ferrous iron on microbial communities of the electrode biofilms in microbial fuel cells (MFCs) was investigated. Voltage production showed that ferrous iron of 100 µM facilitated MFC start-up compared to 150 µM, 200 µM, and without supplement of ferrous iron. However, higher concentration of ferrous iron had an inhibitive influence on current generation after 30 days of operation. Illumina Hiseq sequencing of 16S rRNA gene amplicons indicated that ferrous iron substantially changed microbial community structures of both anode and cathode biofilms. Principal component analysis showed that the response of microbial communities of the anode biofilms to higher concentration of ferrous iron was more sensitive. The majority of predominant populations of the anode biofilms in MFCs belonged to Geobacter, which was different from the populations of the cathode biofilms. An obvious shift of community structures of the cathode biofilms occurred after ferrous iron addition. This study implied that ferrous iron influenced the power output and microbial community of MFCs.

Keywords: microbial fuel cell, ferrous iron, electricity generation, microbial community, high throughput sequencing

INTRODUCTION

Microbial electrochemical cell (MEC) has been admired as a versatile device that can be used for alternative energy generation, electrosynthesis, biosensor, and waste treatment (Hou et al., 2016; Liu et al., 2016a; Huang et al., 2017). However, practical implementation of microbial fuel cells (MFCs) remains restricted by reasons of low electron transfer efficiency and high material costs (Logan et al., 2006). For the past few years, researchers studied electrode materials, exoelectrogenic bacteria, reactor configuration and operational conditions of MFCs (Watson and Logan, 2010; Yong et al., 2011; Janicek, 2015), and pointed out that microbial biofilm was the most direct and key element that affect current generation (Mohan et al., 2008). However, microbial biofilm and its community structure of MFCs can be influenced by temperature, pH, carbon source, inoculum, and metal ion (Lu et al., 2011, 2012; Patil et al., 2011; Wu et al., 2013). The diverse populations developed in the biofilms in MECs have been widely analyzed (Mei et al., 2015). Geobacter as a typical dissimilatory metal-reducing bacterium (DMRB) is commonly identified in MFCs (Mohan et al., 2014; Zhu et al., 2014; Kumar et al., 2016). Hence, to understand and optimize ecological conditions that facilitate exoelectrogens enrichment and electron transfer are essential for MEC application.

Iron plays a central role in the development and maintenance of biofilm of Pseudomonas (Hunter et al., 2013). Although ferric iron has been identified as an important parameter
Due to its high redox activity, the Fe of microorganisms or the activity of enzymes (Lu et al., 2015) can affect the performance of MECs by influencing the metabolism of microorganisms or the activity of enzymes (Cvetkovic et al., 2010). The reactive metal ions may have the phenomenon of redox reaction, catalysis, or precipitation, etc. and thus directly affect the performance of MECs by influencing the metabolism of microorganisms or the activity of enzymes (Lu et al., 2015). A comparison of results with and without ferrous iron as a cathodic reactant also revealed that the addition of ferrous iron enhanced power generation in batch MFC (Wang et al., 2011). However, the knowledge related to the effects of ferrous iron on performances of MFCs and microbial communities of electrode biofilms is less known. To reveal the response of microbial community of the electrode biofilm to ferrous iron, in this study, electrochemical performances of MFCs supplemented with different concentrations of ferrous iron were investigated. Meanwhile, microbial community structures of the anodes and cathodes biofilms in MFCs were analyzed using Illumina Hiseq sequencing of 16S rRNA gene amplicons.

MATERIALS AND METHODS

MFC Configuration and Operation

Single-chamber MFCs with volume of 14 mL were constructed as previously described (Xing et al., 2008). Anodes were made of carbon paper (Toray TGP-H-090, Japan), while cathodes were stainless steel mesh by rolling activated carbon and polytetrafluoroethylene (PTFE) (Dong et al., 2012) (the area of anode and cathode were both 7 cm²). Domestic wastewater was used as inoculum in the first 5 days. Nutrient solutions were consisted of 1 g/L sodium acetate, 5 mL/L vitamins, 12.5 mL/L minerals, 100 mM phosphate buffer saline (PBS, pH of 6) and FeSO₄ with different concentrations. The final pH value of nutrient solution was 6.2 ± 0.1. The final concentrations of FeSO₄ in MFCs were 32 (control), 100, 150, and 200 µM.

Voltages across the external resistor (1000 Ω) of MFCs were measured using Keithley 2700 multimeter/data acquisition system. All MFCs were operated at 35°C and each Fe²⁺ concentration have three replicates. Cyclic voltammetry (CV) measurements of MFCs at the 15th day were performed on Autolab potentiostat (Metrohm, Netherlands) with scan rate of 0.01 V/s.

RESULTS AND DISCUSSION

Electricity Generation and Electrochemical Activity of MFCs

Cyclic voltammetry curves showed that MFCs supplemented with 100 µM ferrous ion (Fe²⁺) obtained the highest current peak on the 15th day (Figure 1). The results suggested that low concentration of Fe²⁺ could obviously improve electrochemical activity of MFCs in the start-up period. During another 15 days of operation, MFCs with 100 µM ferrous ion showed the best electrochemical characteristics compared to MFCs with 150 and 200 µM Fe²⁺, and MFCs without additional Fe²⁺ supplement (Figure 2). The maximum voltage of 0.55 V was monitored in MFCs fed with 100 µM Fe²⁺, and then following the order control (0.54 V), 150 µM Fe²⁺ (0.52 V) and 200 µM Fe²⁺ (0.47 V). After all MFCs were operated for 30 days, MFCs of
with an average of 710 OTUs (Table 1). The anode biofilms in MFCs supplemented with ferrous iron showed slightly lower population diversity than that in control MFCs without ferrous iron supplement. Shannon indices were 3.72, 4.71, and 5.21 for the anodes biofilms with 100, 200 µM Fe²⁺, and without Fe²⁺, respectively. By contrast, Fe²⁺ increased the population diversities of the cathode biofilms, Shannon indices increased from 4.3 (control) to 5.02 (100 µM Fe²⁺) and 5.54 (200 µM Fe²⁺), suggesting that Fe²⁺ affected microbial community structure of the electrode biofilms in MFCs. Principal component analysis based on OTUs showed three clusters, the anode biofilms of MFC without Fe²⁺ was separated from the anode biofilms of MFC supplemented with Fe²⁺ of 100 and 200 µM Fe²⁺ and the cathode biofilms (control, 100, and 200 µM Fe²⁺; Figure 3).

**Bacterial Composition of the Anode and Cathode Biofilms**

The bacterial communities of the anode biofilms were substantially shifted when additional Fe²⁺ was supplemented in MFCs. *Proteobacteria* were the most dominant phylum observed both in the anode (71–75%, relative abundance) and cathode biofilms (41–78%) (Figure 4A). *Chlorobi* (11–14%) and *Bacteroidetes* (4–8%) were also predominant phyla in the anode biofilms. The relative abundances of *Lentisphaerae* in the cathode biofilms, were much higher than that in the anode biofilms, reached to 31% (100 µM Fe²⁺), 22% (200 µM Fe²⁺), and 4% (control). *Deltaproteobacteria*, *Ignavibacteria*, and *Betaproteobacteria* were the most predominant classes in the anode biofilms and accounted for 75% more or less, of which, the abundance of *Deltaproteobacteria* in the anode of MFCs with 100 µM reached to 50%, speculating that *Deltaproteobacteria* were the dominant class since MFC start-up period (Figure 4B). By contrast, microbial community structures of cathodes were different from anodes. *Alphaproteobacteria*, *Gammaproteobacteria*, *Bacteroidia*, and *Lentisphaeria* were the predominant classes on the cathodes. Cathodes of MFCs with additional Fe²⁺ had similar communities that were much different with control group.

The predominant genera varied significantly among all anodes and cathodes biofilms (Figure 5). The majority of predominant populations in the control MFCs were affiliated with *Geobacter* spp. (30.7%) and *Legionella* spp. (50.3%). *Geobacter* was also the predominant genus in the anode of MFC supplemented with 100 and 200 µM Fe²⁺, the relative abundance of which population reached up to 49.3 and 24.4%. Another predominant genus in the anode biofilms of MFC (200 µM Fe²⁺) was affiliated to *Rhodanobacter* (19%). In the cathode biofilms of MFCs with 100 and 200 µM Fe²⁺, higher relative abundance of predominant genera belonged to *Legionella* spp. (2 and 6%), and no absolutely predominant populations were present. Hierarchical cluster analysis of microbial communities based on genus taxonomy revealed that the relative abundance of *Sphaerochaeta*, *Dechloromonas*, *Paracoccus*, *Thermomonas*, and *Rhodanobacter* increased in the anode biofilms of MFCs supplemented with 200 µM Fe²⁺ (Figure 6). Meanwhile, the
only with the biofilm, demonstrating that microbial biofilms increasing redox potential at the biofilm electrode was associated that the pH is not always a limiting factor in a biofilm. Meanwhile, biofilms, pH decreased through different growth phases, showing electrochemical systems (BESs) (Lu et al., 2015). In mature anode transfer and redox reaction to affect the performance of bio-

redox active sites in the enzymes which catalyze the electron biofilm formation at the early stage. The metal ions may act as negative effect (Wei et al., 2013), presumably the Fe

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stimulated electrochemical activity of MFCs during the start-up period, but Fe

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M) was lower than control and 100 µM Fe

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during MFC steady operation. Rhodanobacter accounted for a large proportion (19%) in MFCs with Fe

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concentration of 200 µM. To date, the function of Rhodanobacter was mostly investigated on denitrifying (Green et al., 2012) and thiosulfate-oxidizing (Lee et al., 2007), but little is reported about Fe

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oxidation especially mediated by C-type cytochromes (Croal et al., 2007; Bird et al., 2011). Whether it participates in interspecies interaction with Geobacter should be further proved. Other exoelectrogenic bacteria also formed a certain proportion in different anode biofilms, such as Pseudomonas (1–6%) and Arcobacter (3–7%) (Fedorovich et al., 2009; Yong et al., 2011). Pseudomonas has a positive role to benefit other exoelectrogens in anode biofilm under a high concentration of salt addition (Liu et al., 2016b). Arcobacter can be selectively enriched in an acetate-fed MFC and rapidly generates a strong electronegative potential (Fedorovich et al., 2009). It indicated that additional ions, like Fe

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, will take part in biofilm metabolism or microbial communication, which resulted in community structure changes.

The microbial communities on the cathodes clearly differed from the anodes biofilms in all MFCs. The most predominant genera in the cathode biofilms of MFCs without additional ferrous iron came from Legionella spp. (50.3% of relative abundance). However, the relative abundance of Legionella on the cathode biofilms declined to 2–6% with Fe

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addition, suggesting that Legionella was inhibited by high concentration of Fe

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. The abundance of Fe(II)-oxidizing bacteria, Janthinobacterium (Geissler et al., 2011), in the cathode biofilms of MFC with 200 µM Fe

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were relatively higher than other groups

| Sample name        | Effective tags | OTUs | Shannon | Chao1   | Simpson | ACE     | Good’s coverage |
|--------------------|----------------|------|---------|---------|---------|---------|-----------------|
| Anode (control)    | 53,807         | 824  | 5.21    | 908.307 | 0.884   | 900.018 | 0.997           |
| Anode (100 µM)     | 53,136         | 630  | 3.716   | 691.84  | 0.733   | 703.657 | 0.998           |
| Anode (200 µM)     | 54,932         | 679  | 4.706   | 786.135 | 0.886   | 796.327 | 0.997           |
| Cathode (control)  | 51,054         | 692  | 4.3     | 755.5   | 0.748   | 773.924 | 0.997           |
| Cathode (100 µM)   | 54,592         | 679  | 5.021   | 757.026 | 0.879   | 771.527 | 0.998           |
| Cathode (200 µM)   | 50,373         | 741  | 5.542   | 810.327 | 0.927   | 813.045 | 0.997           |

Effect of Fe

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on Predominant Populations in the Electrode Biofilms
ferrous iron with appropriate concentration (100 µM) stimulated electrochemical activity of MFCs during the start-up period, but Fe

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cannot enhance power output after 30 days of operation and higher concentration of Fe

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had the negative effect (Wei et al., 2013), presumably the Fe

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facilitated biofilm formation at the early stage. The metal ions may act as redox active sites in the enzymes which catalyze the electron transfer and redox reaction to affect the performance of biocatalyst (Croal et al., 2004). Recent studies effects on the performance of BESs by inhibiting the activity of microorganisms (Jiang et al., 2011). The relative abundance of Geobacter increased from 30.7 to 49.3% in MFCs with 100 µM Fe

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but decreased to 24.4% in MFCs with 200 µM Fe

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, implying higher Fe

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concentration could not further enrich Geobacter. As a result, the power output of MFC with higher Fe

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concentration (200 µM) was lower than control and 100 µM Fe

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during MFC steady operation. Rhodanobacter accounted for a large proportion (19%) in MFCs with Fe

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concentration of 200 µM. To date, the function of Rhodanobacter was mostly investigated on denitrifying (Green et al., 2012) and thiosulfate-oxidizing (Lee et al., 2007), but little is reported about Fe

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oxidation especially mediated by C-type cytochromes (Croal et al., 2007; Bird et al., 2011). Whether it participates in interspecies interaction with Geobacter should be further proved. Other exoelectrogenic bacteria also formed a certain proportion in different anode biofilms, such as Pseudomonas (1–6%) and Arcobacter (3–7%) (Fedorovich et al., 2009; Yong et al., 2011). Pseudomonas has a positive role to benefit other exoelectrogens in anode biofilm under a high concentration of salt addition (Liu et al., 2016b). Arcobacter can be selectively enriched in an acetate-fed MFC and rapidly generates a strong electronegative potential (Fedorovich et al., 2009). It indicated that additional ions, like Fe

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were relatively higher than other groups
FIGURE 4 | Microbial community taxonomic wind-rose plots based on relative abundance of 16S rRNA sequences of the anode and cathode biofilms in MFCs at the phylum (A) and class levels (B).
FIGURE 5 | Relative abundance of predominant genera in the anode and cathode biofilms in MFCs supplemented with different concentrations of ferrous iron.
Effect of Environmental Factors on MFC Performances

Some environmental factors, such as nutrients, pH, and temperature, influence the performances of MFCs by changing microbial activity and community structure. Our study indicated...
that ferrous iron changed microbial community structures of electrode biofilms of MFCs. Other metals (e.g., Ca, Mg, Pt, Au, Pd, Fe, V, Mn) and metal-nanomaterials affected current generation of MECS by changing the metabolism and enzyme activity of microorganisms (Lu et al., 2015). These studies have analyzed effect of single metal on electricity generation by MFCs, however, the effect of combined metals on microbial community structure and performance of MFCs should be further investigated.

Neutral pH is considered as the optimal condition for current generation by MFCs (Gil et al., 2003; Jadhav and Ghangrekar, 2009). However, a higher pH has been demonstrated to enhance the electrochemical activity of riboflavin which is a metabolite responsible for extracellular electron transfer in some species (Yuan et al., 2011; Yong et al., 2013). By contrast, MFCs have been operated at pH less than 4.0 and produced high current densities by acidophilic bacterium (Malki et al., 2008; Liu et al., 2016). Previous studies proved that temperate substantially affected the performances of MECS or MFCs by shaping microbial community (Lu et al., 2011, 2012). Synergistic effect of metals, pH and temperature on performances of MECS and correlation analysis of these environmental factors should be further investigated in the future.

**AUTHOR CONTRIBUTIONS**

DX designed the experiment. QL performed specific experiments. QL, BL, and DX contributed to analyze the experiment data. QL, WL, WZ, XZ, and DX wrote the manuscript. All authors were involved in revision of the manuscript and approved its final version.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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