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Bacterial community shift and improved performance induced by *in situ* preparing dual graphene modified bioelectrode in microbial fuel cell

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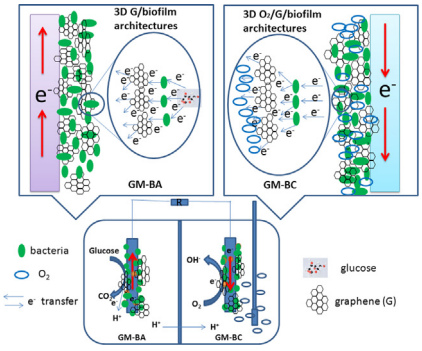
HIGHLIGHTS

- D-GM-BE was prepared by microbial-induced reduction of GO and polarity reversion.
- *Proteobacteria* and *Firmicutes* were the dominant bacteria in GM-BE.
- Typical exoelectrogens in GM-BE shared much higher proportion.
- The maximum power density obtained by D-GM-BE MFC was 2.34 times than C-BE-MFC.

GRAPHICAL ABSTRACT



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ABSTRACT

Dual graphene modified bioelectrode (D-GM-BE) was prepared by *in situ* microbial-induced reduction of graphene oxide (GO) and polarity reversion in microbial fuel cell (MFC). Next Generation Sequencing technology was used to elucidate bacterial community shift in response to improved performance in D-GM-BE MFC. The results indicated an increase in the relative ratio of *Proteobacteria*, but a decrease of *Firmicutes* was observed in graphene modified bioanode (GM-BA); increase of *Proteobacteria* and *Firmicutes* were observed in graphene modified biocathode (GM-BC). Genus analysis demonstrated that GM-BE was beneficial to enrich electrogens. Typical exoelectrogens were accounted for 13.02% and 8.83% in GM-BA and GM-BC. Morphology showed that both GM-BA and GM-BC formed 3D-like graphene/biofilm architectures and revealed that the biofilm viability and thickness would decrease to some extent when GM-BE was formed. D-GM-BE MFC obtained the maximum power density by 124.58 ± 6.32 mW m^{-2}, which was 2.34 times over C-BE MFC.

1. Introduction

Bacteria is regarded as catalysts to convert chemical energy into electricity in microbial fuel cell (MFC), which plays a huge role in the processes of extracellular electron transfer (EET) and energy transfer (Logan and Regan, 2006; Zhao et al., 2017). Obtaining the microbial structure and community in bioelectrode MFC is one of the significant elements to improve the MFC performance, which is beneficial for the development and application of MFC (Xu et al., 2017).

With the development of metagenomics, the Next Generation Sequencing technology has been applied to characterize microbial communities in MFCs in recent studies (Daghio et al., 2015). Luo

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et al. (2016) discussed the microbial communities in anode biofilm with high concentration of sodium acetate, which indicated that *Proteobacteria*, *Bacteroidetes*, and *Synergistetes* were the dominant phylum, and identified *Geobacter* as the dominant genus. To explain the high performance of aerobic biocathode MFC, Milner et al. (2016) studied the microbial community of aerobic biocathode with high oxygen reduction reaction (ORR) activity, which demonstrated that uncultured *Gammaproteobacteria* dominated the high performance and high ORR activity of aerobic biocathode MFC. These studies stated that metagenomics has been served in the field of MFC successfully, and metagenomics would provide much more microbial information to drive the development of MFC.

Graphene is a promising material with excellent physical/chemical characteristics (Cai et al., 2016), which has been applied in MFCs via different modes. In MFCs, graphene modifying anode is generally realized by coating graphene on electrode (Mehdinia et al., 2014), electrodepositing polymer on graphene nanosheets (Shaari and Kamarudin, 2017), etc.; graphene modifying cathode is usually completed by doping N (Hou et al., 2016), Fe (Tang et al., 2017) or Mn (Khilari et al., 2013) to graphene on electrode. These modifying methods are relative complicated and a large amount of chemicals are required. However, there existed an interesting phenomenon: graphene oxide (GO) could be *in situ* reduced by *Shewanella* (Wang et al., 2011), *Escherichia coli* (Gurunathan et al., 2013), etc., which were significant composition of exoelectrogens in MFCs. Owing to its easy operation and environmental friendliness, this approach has been developed in MFCs. Yong et al. (2014) fabricated 3D macroporous rGO/bacteria hybrid biofilm in MFC anode by self-assembly of GO via *Shewanella oneidensis* MR-1, which improved power density and EET process. Yuan et al. (2012) constructed graphene scaffolds anode by *in situ* microbially reduced GO via mixed bacteria, which increased power density and coulombic efficiency (CE) in MFCs. This method was relatively easy to be achieved in MFC anode, but there existed development space for *in situ* modifying cathode. Zhuang et al. (2012) implanted the microbially reduced graphene (completed in anode) into cathode, which formed 3D graphene/biofilm biocathode and enhanced catalytic activity to oxygen reduction reaction (ORR). In our previous studies, *in situ* graphene modified biocathode (GM-BC) was built by microbial-induced reduction of GO in anode and conducting polarity reversion to graphene modified bioanode (GM-BA), which proved to be an effective method to *in situ* prepare GM-BC. Based on *in situ* microbial-induced reduction of GO and polarity reversion, a three-step method to prepare *in situ* dual graphene modified bioelectrode (D-GM-BE) in a MFC was proposed: GM-BA was initially prepared via microbial-induced reduction of GO in anode; then GM-BC was prepared based on the polarity reversion of GM-BA; GM-BA was needed to be prepared once again.

In situ D-GM-BE is an important development direction for electrode modification. Whereas bacteria act the key role in the process of microbial-induced reduction and polarity reversion, therefore, it is necessary to master the inner regularity between graphene and bacteria. And this is quite critical to understand the complexity and assembly rule of *in situ* GM-BE, therefore, it is extremely urgent to study the bacterial community shift induced by *in situ* preparing D-GM-BE in MFC.

In this study, Next Generation Sequencing technology was used to investigate the bacterial community shift in response to *in situ* microbial-induced reduction and polarity reversion in D-GM-BE MFC. Understanding bioinformatics was of great significance for mastering mechanisms of EET process and ORR in D-GM-BE. Electrochemical analysis was performed to assess the performance of D-GM-BE MFC. Field emission scanning electron microscopy (FESEM) and confocal scanning laser microscopy (CSLM) were conducted to observe the changes of bacterial biofilms caused by *in situ* preparing D-GM-BE. This study aimed to provide bacterial information induced by *in situ* preparing D-GM-BE, and contribute to the development and application of D-GM-BE MFC.

2. Materials and methods

2.1. MFC running conditions

A two-chamber MFC was adopted in this study, the volume of each chamber was 240 mL. Each MFC reactor was equipped with carbon felt (6 cm × 5 cm) as basic electrode. Anode and cathode chamber were separated by a cation exchange membrane (CEM) as our previous studies (Li et al., 2014; Sun et al., 2015), and active sludge was originally acquired from an urban sewage treatment plant in Guangzhou City. GO solution was synthesized by improved Hummers method (Chen et al., 2015), and GO solution (1 mg L^{-1}) was injected into anode chamber along with carbon source, PBS buffer, trace mineral and vitamin solution. The operation cycles of MFC system and schematic operation were shown in Supplementary material, GO solution was injected in the original three cycles, GM-BA was prepared by *in situ* microbial-induced reduction of GO in anode; GM-BC was obtained by operating polarity reversion on GM-BA; then GM-BA was prepared again in the new anode chamber. After another three cycles, *in situ* D-GM-BE MFC was completed. In the meantime, there existed simultaneous operation of control bioelectrode (C-BE) MFCs. All MFC reactors were maintained in a constant temperature room (30 ± 1 °C), all tests were conducted in duplicate at least and the mean value was recorded for all data.

2.2. Sample collection and DNA extraction

After steady operation of D-GM-BE MFC, the biofilm samples of GM-BA and GM-BC were collected. Meanwhile, the biofilm samples of C-BA and C-BC were also collected. The samples were named number 1–4, which represented GM-BA, GM-BC, C-BA and C-BC. These samples (~10 cm^2) were cut down from electrodes, and these cutting carbon felts were put in a plastic 50 mL centrifuge tube with 20 mL of 50 mM PBS buffer and 5 mL of 2 mm sterile glass beads. These biofilm samples were extracted from different bioelectrodes via vortex shaking for 5–10 min and were concentrated by centrifugation (Huang et al., 2014). DNA extraction was manipulated with E.Z.N.A soil DNA kit (Omega, USA). The quality of DNA extraction was checked on an agarose gel which was further verified by spectrophotometer.

2.3. PCR amplification and pyrosequencing

The 16S universal primers 341F (CCCTACACGACGCTCTTCCGATCTG CCTACGGGNGGCWGCAG) and 534R (ACTGGAGTTCCTTGG CACCCGAGAATTCCAGATTACCGCGGCTGCTGG) were used to amplify the V3 region of the 16S rRNA gene (Miao et al., 2016). The polymerase chain reaction (PCR) reaction mixture (30 μL volume) contained: 15 μL of 2× Taq master mix, 1 μL of each primer (10 μM), 10 ng of the genomic DNA, and ddH_2O to make up the volume. The first PCR thermal cycling scheme was set as follows: initial denaturation at 94 °C for 3 min, 5 cycles of denaturation at 94 °C for 30 s, annealing at 45 °C for 20 s, and extension at 65 °C for 30 s, 20 cycles of denaturation at 94 °C for 20 s, annealing at 55 °C for 20 s, and extension at 72 °C for 30 s, followed by a final extension period at 72 °C for 5 min. PCR amplification was conducted on a 1000-Series Thermal Cycling Platform (Bio-Rad, USA). A second PCR process was aimed to add the compatible primer of Illumina, and the PCR thermal cycling scheme was set as follows: initial denaturation at 95 °C for 30 s, 5 cycles of