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Enhanced denitrification efficiency with immobilized bacteria in microbial fuel cell

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ABSTRACT

In this paper, the influence of immobilized bacteria on the denitrification performance of microbial fuel cells (MFC) was studied, and a novel MFC with immobilized bacteria on electrodes was developed. The anode of the MFC was immobilized with electroactive microorganisms, while the cathode was immobilized with denitrifying bacteria, utilizing water-based polyurethane as a carrier. The results showed that the electroactive microorganisms immobilized on the anode improved the electron utilization efficiency, while the denitrifying bacteria immobilized on the cathode improved the denitrification performance. In addition, to ensure the efficiency of MFC, the number of electrodes can be reasonably determined according to the number of immobilized bacteria. High-throughput sequencing analysis showed that the immobilization of bacteria on MFC electrodes was beneficial to maintain the stability of the functional bacterial community structure. Therefore, the biochemical approach of utilizing immobilized bacteria to remove nitrate in MFC is a promising technology.

Keywords: Microbial fuel cell (MFC); Denitrification; Immobilized bacteria; Electroactive microorganisms; Community structure

1. Introduction

Microbial fuel cell (MFC) is currently recognized as a promising biological treatment technology for the removal of nutrient-rich substances in wastewater [1–3]. A typical MFC consists of a cathode compartment and an anode compartment separated by a proton exchange membrane (PEM) [4,5]. Anaerobic electroactive microorganisms in the anode chamber can directly convert chemical energy stored in organic matter into electrical energy [6]. The electrons flow to the cathode through an external electric circuit and react with electrons acceptor, such as oxygen or nitrate [7]. Recently, nitrate removal has received more attention during the development of MFC for wastewater treatment [8,9].

However, the electroactive microorganisms in the anode were easily affected by fluctuations in the influent water quality, which cause fluctuations in the anode potential. In conventional MFCs, electroactive microorganisms were uniformly distributed in the anode compartment. Electrons were distributed everywhere in the entire space of the anode chamber, which will cause electrons cannot be completely transported to the cathode, and the utilization rate was low which affects energy recovery [10,11]. Denitrifying bacteria on the cathode may be lost when drainage and affect the

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nitrate removal of MFC. Therefore, MFC is an open system with unstable voltage [12] and fluctuating denitrification performance. Moreover, with the long-term operation of MFC, the microbial community is constantly evolving and the microbial community may change, which will lead to poor wastewater treatment effect [13–15].

Microbial immobilization technology [16] uses physical or chemical methods to immobilize free microbial cells in a specific spatial region while retaining the catalytic properties of bacteria for continuous or repeated use [17,18]. Immobilization free microorganisms could reduce the loss of bacteria, increase biomass and protect the cells, it has been introduced into the wastewater treatment industry in recent years [19,20]. Herein, in the present work, the bacteria on electrode in MFC were immobilized and the denitrification performance of MFC was investigated. A novel electrode flexibly movable MFC with immobilized bacteria was developed in this study. High-throughput sequencing was used to analyze the community structure of bacteria on electrode. Utilizing immobilized bacterial electrodes in MFC as a biochemical method for nitrate removal is a promising technology, and this study is expected to promote the application of MFC in wastewater treatment.

2. Materials and methods

2.1. Immobilization of bacteria on electrode

The cathode and anode of the MFC were made of carbon felt (5.0 mm thickness) of 12.25 cm^2 (3.5 cm × 3.5 cm) surface area, respectively. Carbon felt and copper wire were soaked in H_2SO_4 and NaOH, respectively, and washed with ultrapure water before use. Copper wires were passed through carbon felts, and the anode and cathode carbon felts were soaked in solutions rich in electroactive microorganisms and denitrifying bacteria for about 3 d, respectively. The preparation steps of the anode electrode in experimental group could be referred in our previous reports [21,22]. Add an appropriate amount of electrically active microorganisms, an appropriate amount of water-based polyurethane, and a small amount of $FeCl_3$ solution to a beaker, and stir evenly. The carbon felt is then placed in a beaker and covered with an aqueous polyurethane solution. Potassium persulfate and N,N,N,N-4-methylethylenediamine solutions are then added to the beaker to allow polymerization to occur in the beaker, thereby immobilizing the bacteria on the electrodes. The preparation steps of the immobilized bacteria on the cathode are similar, except that the electroactive microorganisms are replaced by denitrifying bacteria. To ensure the same number of bacteria in the experimental group, the same amount of free bacteria was added into the control group chamber when MFC started.

2.2. Set up of MFC

The schematic diagram of the MFC in this study is shown in Fig. 1. The electrode chamber was composed of two plexiglass chambers separated by PEM (D117N), and the volume of the two electrode chambers was 125.0 mL (5.0 cm × 5.0 cm × 5.0 cm). The electrodes were placed in cathode and anode chamber, respectively, and the resistance (10.0 Ω) was connected. The distance between the cathode electrode and the PEM was approximately 3.5 cm. The bacteria immobilized on the electrode was labeled as experimental group (immobilizated electrode-MFC, IeMFC), and the free bacteria on electrode was labeled as control group (free bacteria-MFC, FbMFC). The concentration of NO_3^- was settled as 10.0 mg/L in the cathode chamber initially. A small

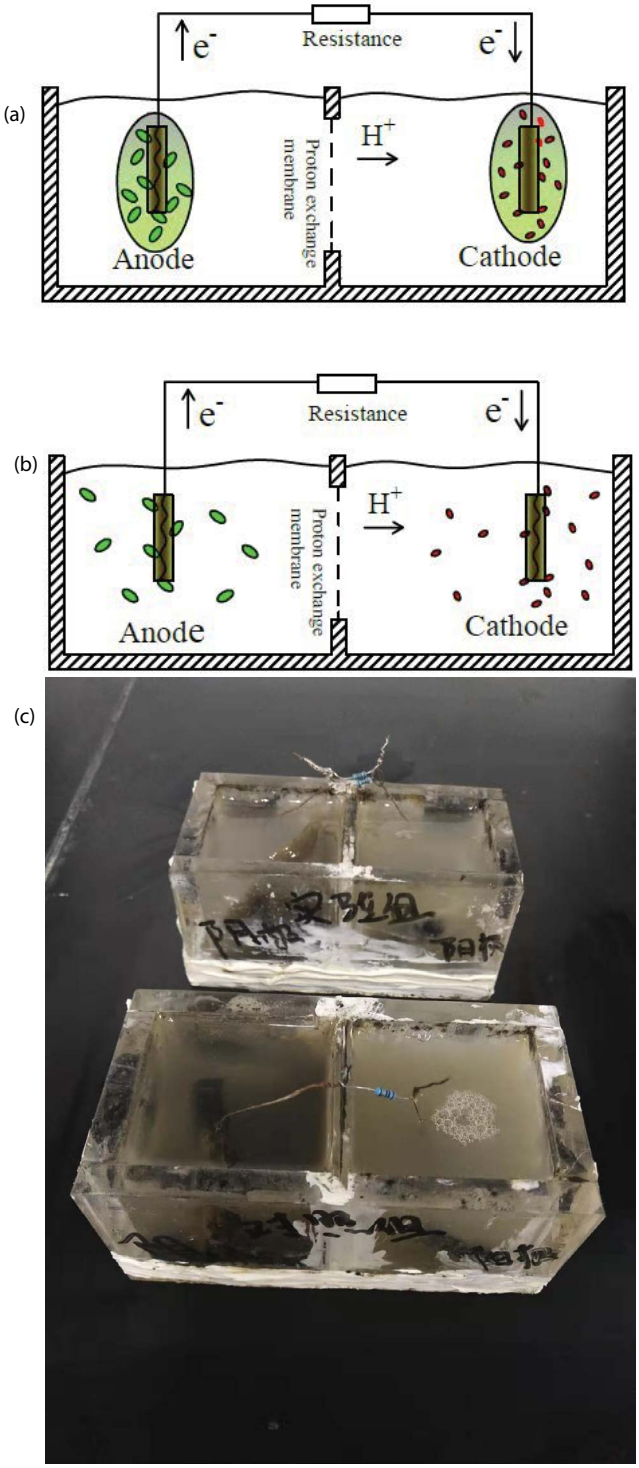

Fig. 1. Schematic diagram and real reactor image of microbial fuel cells (A) IeMFC, (B) FbMFC and (C) reactor image.

amount of glucose solution was added into the anode chamber to maintain the growth of bacteria. Water lost due to evaporation was routinely replenished with water for maintaining a constant water level [23], and all MFCs were operated under about 27.0°C throughout the experiments.

2.3. Test methods

Water samples were collected periodically for water quality measurements and analyzed within each experimental cycle. The concentrations of nitrate and COD in MFC were analyzed according to Standard Methods for the Examination of Water and Wastewater. Before analysis of the parameters in liquid, samples were membrane filtered (0.45 μm), and all tests were repeated at least twice. The voltage of MFC was measured by using a voltmeter (Victor VC890D, China).

2.4. High throughput sequencing analysis

High-throughput sequencing of 16S rRNA gene fragments was used to analyze the microbial communities in electrode. The initial electroactive microorganisms in anode was labeled S1 while the initial cathodic denitrifying bacteria was marked as S2. DNA sequencing was performed by Shanghai Shenggong Co., Ltd of China.

3. Results and discussion

3.1. Electricity generation in MFC

A voltmeter is used to regularly measure the voltage to characterize the power generation performance of the MFC and Fig. 2 shows the voltage of MFC in this study. The voltage of IeMFC increased slowly from 0.04 V in the experimental group, the maximum open circuit voltage was about 0.139 V with the maximum power density was 19,321.0 mW/m^3 (the total anode chamber volume of MFC). In the control group, the voltage of FbMFC increased slowly from 0.02 V, the maximum open circuit voltage was about 0.106 V, the maximum power density was 11,236.0 mW/m^3 (the total anode chamber volume of MFC). The voltage fluctuated greatly and the start-up time of FbMFC system was longer through the voltage changes. It can be seen from Fig. 2 that the voltage of experimental group was significantly higher than that in the control group, and the voltage was more stable.

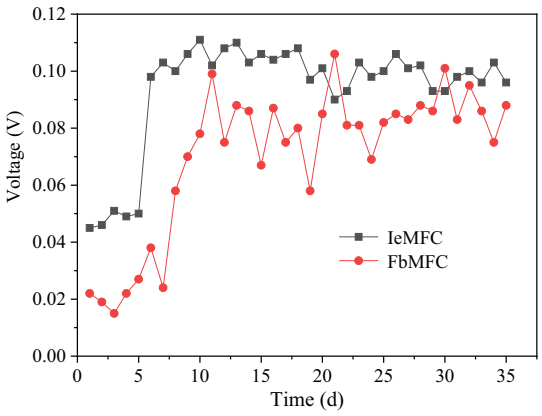

Fig. 2. Voltage of IeMFC and FbMFC.

In this study, before constructing MFC, the anode carbon felt of IeMFC was immersed in a suspension of electrically active microorganisms, electroactive microorganisms enriched on carbon felt and immobilized on the electrode, so the number of electroactive microorganisms on the electrode was large. The electrons produced by the microorganisms were not easy to diffuse freely in the anode chamber, and most electrons could be transported to the cathode. Therefore, the start-up time of IeMFC was short and the electricity production was higher and stabler. In the control group, although the anode carbon felt electrode was also immersed in the suspension of electroactive microorganisms, the microorganisms were not fixed on the electrode and could flow freely in the anode chamber, there were few electroactive microorganisms on the anode electrode of FbMFC. On the other hand, it takes some time for the electroactive microorganisms to accumulate to a higher density on the electrode, so the startup time of FbMFC was longer. At the same time, the electrons were scattered in the anode chamber, and the electrons may overflow and combine with oxygen in the air, resulting in the relative reduction of available electrons on the electrode and the low utilization rate of electrons, so the voltage of FbMFC was lower than that of the experimental group, here the electron utilization efficiency refers to the proportion of electrons transmitted to the MFC cathode through wires and used by the MFC cathode to all electrons generated in the anode chamber. The electroactive microorganisms immobilized on the electrode which improved the ability of bacteria to resist the impact of water fluctuation and ensured the stable electricity production performance.

3.2. NO_3^-–N removal in MFC

To investigate the denitrification performance of MFC, the nitrate concentration variation in cathode chamber was analyzed. Nitrate removal efficiency refers to the proportion/ratio of nitrate concentration in water to be converted and degraded. In this study, the initial nitrate concentration in the cathode chamber was set at 10.0 mg/L. With the system running, the nitrate concentration was measured every 8 h. As shown in Fig. 3, when the nitrate concentration in the influent was 10.0 mg/L, the effluent of IeMFC was 4.92 mg/L while in FbMFC was 6.05 mg/L after 8 h. The nitrate was reduced to 0 after less than 24 h, and the nitrate removal efficiency was 100% in IeMFC. In the control group, the nitrate concentration was 1.69 mg/L after 24 h with the nitrate removal efficiency was 83.1%, and the nitrate concentration was still 0.73 mg/L after 32 h. The removal rate of nitrate in the experimental group was significantly faster than that in the control group.

According to Fig. 3, the experimental group can completely remove nitrate after 24 h, so the HRT is set to 24 h to study the removal performance of nitrate during long-term operation. To verify the experimental effect, repeated experiments were carried out, and the typical cycle was selected to calculate the nitrate removal rate. It can be seen from Fig. 4 that after the MFC run stably, IeMFC could completely remove nitrate after 24 h, while the removal efficiency

of FbMFC for nitrate was only 85%, and the removal efficiency of IeMFC was higher than that of FbMFC.

To investigate the flexibility of the immobilized bacterial electrode, multiple anode and cathode with immobilized bacteria were put into anode chamber and cathode chamber in a new MFC, respectively, and the resistance and wire were connected to build a new multi-electrode immobilized bacteria MFC (Fig. 5). It was found that the new MFC could start up and operation in a short time, generated a voltage immediately, and the nitrate could be removed gradually. The NO_3^- concentration in the cathode chamber decreased from 20.0 to 3.7 mg/L within 6 h, and less than 8 h to remove all 20.0 mg/L nitrate. The number of electrodes affects the performance of MFC [24], and the flexible use of multiple electrodes in this study improved the denitrification performance in MFC. The bacteria immobilized on the electrode in this study could increase the flexibility of the electrode, realize a flexible use of MFC electrode. It is conducive to shorten the start-up time of MFC and could ensure nitrate removal efficiency, which has great advantages in the wastewater treatment industry.

3.3. Analysis of microbial community on electrode

3.3.1. Analysis of anode bacteria

Electroactive microorganisms can carry out extracellular electron transfer, which converts chemical energy stored in organic matter into electrical energy. The results of high-throughput sequencing showed (Fig. 6a) that the anode bacteria inoculated in MFC were mainly *Shewanella* (59.11%) and *Stenotrophomonas* (38.57%) of *Proteobacteria* (99.33%) phylum. After 30 d operation, the main bacteria phylum in the anode of IeMFC were *Proteobacteria* (95.96%), *Bacteroidetes* (2.15%), *Firmicutes* (1.62%); while in the anode of FbMFC were *Proteobacteria* (96.52%), *Firmicutes* (2.77%). Most of the bacteria phylum in the two groups of anodes belong to *Proteobacteria* phylum, and a few belong to *Firmicutes* phylum in this study. *Proteobacteria* and *Firmicutes* both use carbohydrates as carbon sources for metabolism and produce electrons. The electrons generated in the body of *Firmicutes* need to pass through the cell wall to transfer so the electrons generation ability of this kind of bacteria was relatively weak, and *Proteobacteria* was a common electricity producing bacteria phylum.

As shown in Fig. 6b, the main genera and relative abundance in the anode of IeMFC at the genus level after 30 d operation were *Shewanella* (10.75%), *Stenotrophomonas*

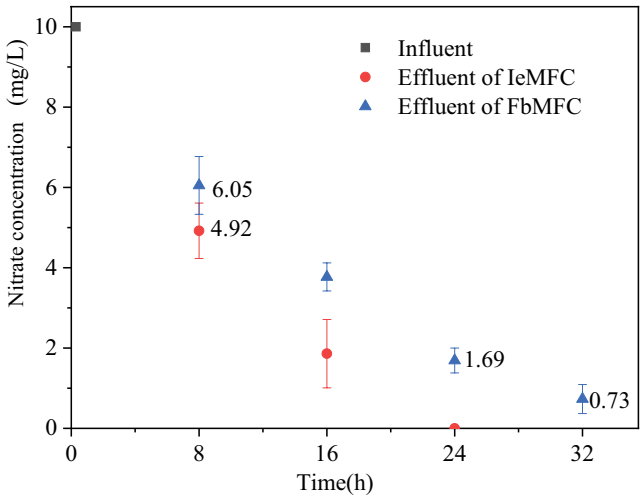

Fig. 3. Denitrification performance of IeMFC and FbMFC in a typical cycle.

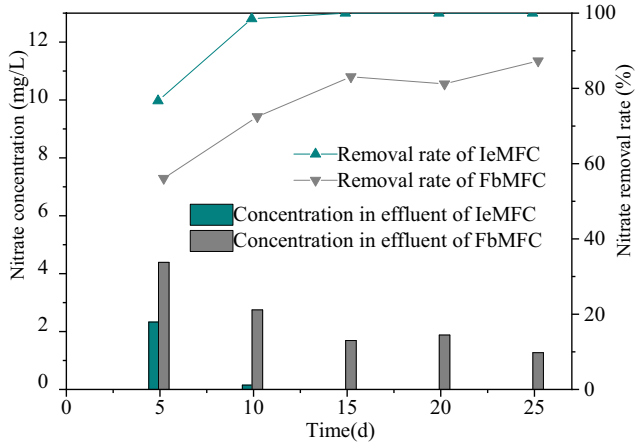

Fig. 4. Denitrification performance of IeMFC and FbMFC in long-term operation process.

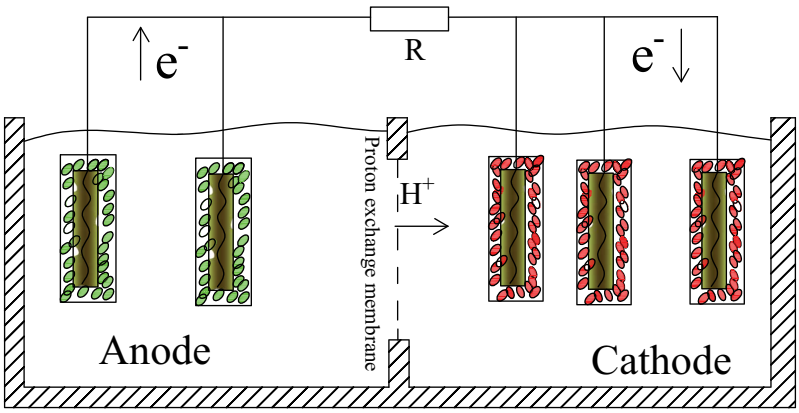

Fig. 5. Schematic diagram of multi-electrode immobilized bacteria microbial fuel cells.

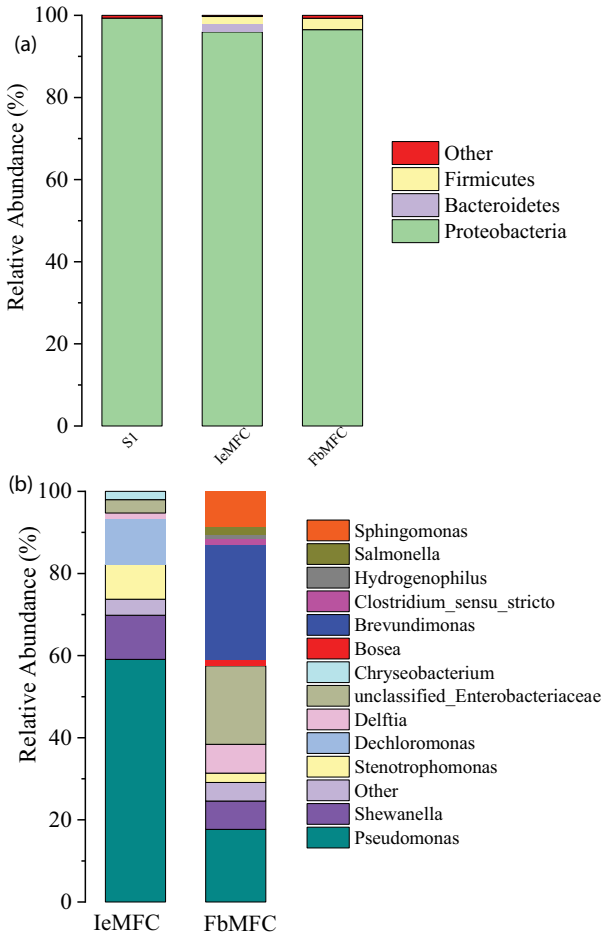

Fig. 6. High throughput sequencing results at the (a) phylum level and (b) genus level of anode.

(8.51%), *Pseudomonas* (59.08%), *Dechloromonas* (11.17%), *Chryseobacterium* (2.02%), *Delftia* (1.3%), while in the anode of FbMFC that were *Shewanella* (6.91%), *Stenotrophomonas* (2.26%), *Pseudomonas* (17.68%), *Bosea* (1.68%), *Brevundimonas* (27.94%), *Clostridium_sensu_stricto* (1.46%), *Delftia* (7.03%), *Hydrogenophilus* (1.07%), *Salmonella* (1.83%), *Sphingomonas* (8.49%). The types and relative abundance of microorganisms on the anodes of the two MFCs have changed compared with the initial values, and the changes in the bacterial structure in the control group are more obvious than those in the experimental group. After 30 d operation, the relative abundance of *Shewanella* in the experimental group changed from 59.11% to 10.75%, while in the control group it was only 6.91%. *Schwarzenella* [25] was a facultative anaerobic bacterium belonging to *γ-Proteobacteria* phylum. It was gram-negative and can use a variety of chemical substances including iron as substrates to produce electrons [26], and it was a kind of electricity producing bacteria that widely exists in water and sediment and was often used for inoculating in MFC. *Stenotrophomonas* bacteria could still be detected in the two groups of MFC after 30 d of operation, but their relative abundance in the two MFC was quite different. The relative abundance of *Pseudomonas* in IeMFC was 59.08% while in FbMFC was only 17.68%, indicating that *Pseudomonas* has become the dominant bacteria in the anode of IeMFC. The bacteria on the anode were immobilized to maintain the relative stability and ensure the stable electricity production performance of MFC.

In this study, bacteria inoculated in MFC were still relatively abundant in the experimental group after 30 d, which indicated that bacteria immobilization had a certain role in maintaining the stability of bacteria, making the dominant bacteria not easy to lose and solving the problem of low and unstable biomass on electrode, improving the ability of MFC anode to resist water fluctuations and ensuring the stable electricity production performance of MFC.

3.3.2. Analysis of cathode bacteria

The performance of denitrifying bacteria on cathode affects the nitrogen removal efficiency of MFC, and the stability of denitrifying bacteria was of great significance to maintain the long-term stable nitrogen removal. High-throughput sequencing showed that the bacteria inoculated on cathode initial were mainly *Pseudomonas* (68.39%), *Brevundimonas* (6.97%), *Rhizobium* (9.04%) and *Trichococcus* (2.15%) at the phylum level. The main phylum in the cathode of IeMFC were *Proteobacteria* (96.66%) and *Bacteroidetes* (2.25%) after 30 d operation (Fig. 7a), and the relative abundance of the *Proteobacteria* had little change compared with initially. *Proteobacteria* (86.04%) and *Bacteroidetes* (12.93%) were the main phylum in the cathode of FbMFC, the species and relative abundance at phylum level changed significantly during MFC operation process in the control group.

The main genus and relative abundance on cathodes of IeMFC after 30 d were *Pseudomonas* (50.51%), *Brevundimonas* (9.83%), *Rhizobium* (11.24%), *Marinobacter* (13.61%). While in FbMFC were *Pseudomonas* (25.99%), *Brevundimonas* (19.96%), *Rhizobium* (14.42%), *Enterobacter* (16.61%). It can be seen from Fig. 7b that the species and relative abundance of MFC on cathode have changed during operation process. The changes in FbMFC were more obvious than that in IeMFC, the bacterial in cathode of IeMFC was more stable. The relative abundance of *Pseudomonas* which was the main denitrifying bacteria in experimental group changed from 68.39% to 50.51% after 30 d, while that in control group was only 25.99% after 30 d, which indicated that the immobilization of bacteria on electrode was conducive to maintaining the stability of functional bacteria. High throughput sequencing analysis showed that bacteria immobilization could effectively maintain the stability of functional bacteria on MFC electrode and improve the nitrogen removal efficiency of MFC. In this study, bacteria were immobilized on the electrode, and electron utilization rate in anode was improved, and the nitrogen removal performance was improved in the cathode. Bacteria immobilization in MFC could realize electrode flexible using, that is, the anode of immobilized electroactive microorganisms was put into the anode chamber, and the cathode was put into the cathode chamber that contained nitrate. After connecting the resistance and wire, the nitrate removal could be realized shortly in MFC, which shortens the start-up time of MFC reactor, ensures the power generation performance and nitrate removal efficiency. The novel MFC with immobilized bacteria will effectively promote the application of MFC in the field of wastewater treatment.

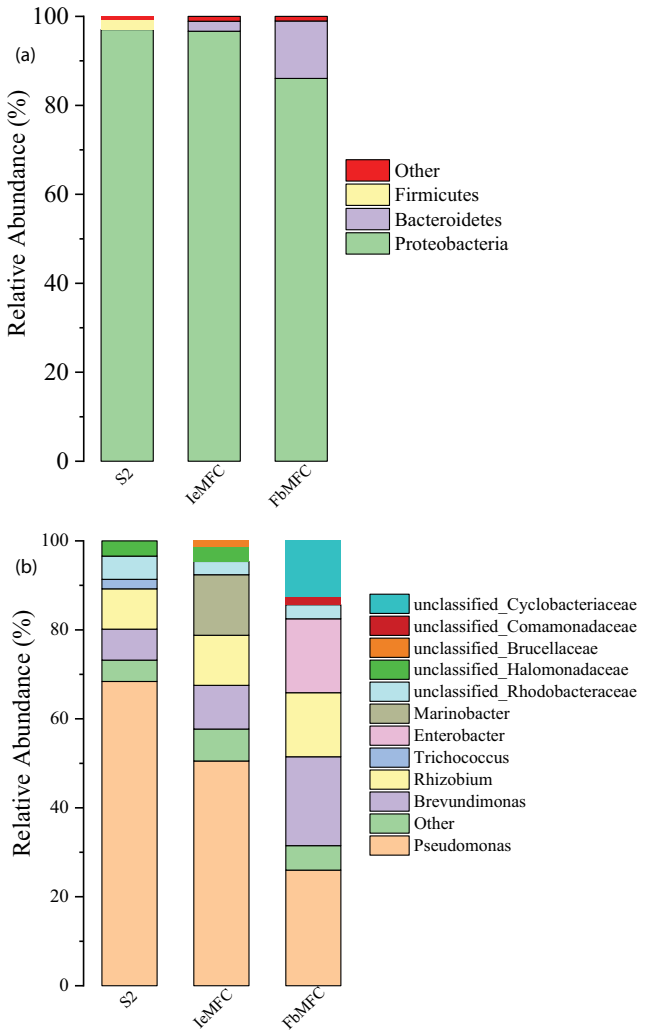

Fig. 7. High throughput sequencing results at the (a) phylum level and (b) genus level of cathode.

4. Conclusions

In this paper, a new type of MFC with bacteria immobilized on the electrode was prepared, and it was found that the anode immobilized electroactive microorganisms was beneficial to improve the electron utilization efficiency, and the denitrification performance of the immobilized denitrifying bacteria on the cathode was improved at the same time. High-throughput sequencing analysis indicated that bacterial immobilization on the electrodes was beneficial to maintain the stability of the community structure of functional bacteria in MFC. The novel MFC with immobilized bacterial in this study is a highly potential novel setup for efficient nitrate removal and will effectively promote the application of MFC in wastewater treatment.

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Declaration of competing interest

There are no conflicts of interest to declare.

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