Effects of temporary external voltage on the performance and community of microbial fuel cells
Jing Guo, Jianping Cheng, Jiaquan Wang, Zerui Zhang, Xiaoyun Xie and Pengpeng Chu

ABSTRACT
This study evaluated the effects of temporary external voltage on the performance of two-chambered microbial fuel cells (MFC) that use nitrate wastewater as a substrate. Results indicate that the external voltage affected the performance of the MFC during their operation, and this effect remained even after the voltage was removed. The degradation efficiency of the chemical oxygen demand increased in the MFC under external voltages of 0.5, 0.8, and 1.1 V, and the optimal applied voltage was 1.1 V. Compared with the control group without external voltages, the MFC under a voltage of 1.1 V achieved higher current densities and efficiency of nitrate removal during their operation. The MFC with an applied voltage of 1.1 V also achieved the highest maximum power density of 2,035.08 mW/m³. The applied voltages of 0.5 and 0.8 V exerted a positive effect on the performance of the MFC. High-throughput sequencing was used to explore the anode and cathode biofilms. Results showed that the influence was highly associated with microbial community in bio-anode. The predominant functional family changed from Methanotrichaceae during start-up to Flavobacteriaceae in a steady phase.

Key words | bioremediation, microbial fuel cells, nitrogen removal, temporary external voltage

HIGHLIGHTS
- A high voltage has a positive impact on microbial fuel cells (MFC) performance.
- MFC at a temporary external voltage of 1.1 V is the optimum for nitrate removal.
- External voltage affected the MFC performance and remained after the voltage removal.
- Voltage affects performance by controlling the enrichment of associated microorganisms in bio-anode.
- Nitrogen removal efficiency in cathode was influenced by electron transferring in the anode.

INTRODUCTION
Microbial fuel cells (MFC) are devices that use bacteria as catalysts to oxidize organic and inorganic matter and generate current (Davis & Yarbrough 1962; Berk & Canfield 1964; Rao et al. 1976). Electrons and protons produced by bacteria from substrates are transferred to the anode and flow to the cathode linked by a conductive material containing a resistor or operated under a certain load condition (Logan 2006). These simultaneous reactions consequently generate electrical power (Li et al. 2009). Numerous factors influence the electricity generation capacity and efficiency of removing pollutants in MFC, including proton transport via the membrane, electron acceptors, and electrode surface properties (Cheng & Logan 2006; Oh & Logan 2006; Kyu et al. 2008; Zhang et al. 2008).

doi: 10.2166/wst.2020.251
Nitrate pollution in groundwater has been a serious concern in most parts of the world because of the discharge of domestic and industrial wastewater and increased usage of nitrogenous fertilizers (Park et al. 2005). Excessive nitrate can harm humans and animals and be reverted to nitrite with increased toxicity by microorganisms in the human body; thus, protecting ecosystems by reducing nitrogen is essential (Gabriel et al. 2009). Nitrate and nitrite, which can be degraded and removed by electrochemical reaction and autotrophic denitrification, can be used as electron acceptors of the cathode (Zhang & Angelidaki 2012). Several methods are employed to improve the efficiency of bacterial denitrification for nitrate removal, including the addition of nutrient elements in the anode and electron donor adjustment (Oh et al. 2009). Moreover, proper electrical stimulation can promote microbial metabolism, leading to enhanced biochemical performance (Thrash & Coates 2008).

Stimulation by electrolysis-driven bioremediation has been reported in several studies (LeDuc & Terry 2005; Busalmen et al. 2008; Owsianiak et al. 2009; Wang et al. 2009). The application of an external voltage affects the performance of MFC presumably with the microorganism (Wang et al. 2009). A permanent electric field enhances the electricity generating capacity of MFC. A permanent positive potential of 0.5 V promotes the denitrification rate and induces high electrochemical activity in single-chamber microbial electrolysis cells (Zhu et al. 2016). However, the sustained effects of removing the electric field and a change in bacteria need to be elucidated. Studies have been conducted on the roles of a sustained external electric field in MFC; however, the effect on MFC of electrolysis-driven removal remains complex and needs further demonstration.

In this study, temporary external voltages ranging from 0.5 V to 1.1 V were imposed on MFC inoculated with mixed cultures on the bio-anode and biocathode in the start-up phase, which use nitrate as an electron acceptor. The effect of different initial voltage on MFC was investigated in the present research. The performance of the proposed MFC system with different start-up external voltage was evaluated, including chemical oxygen demand (COD) removal efficiency, nitrogen removal efficiency, and current generation. This study aimed to assess whether temporary external voltages continuously influence the performance of MFC, identify the reasons underlying this effect, and determine the optimal cell voltage. Moreover, the microbial communities enriched during treatment were characterized via sequencing of the 16S rRNA gene to explore the dynamic changes in the microflora.

**EXPERIMENTAL**

**MFC setup and operation**

Four double-chamber MFC reactors were used in this study. The anodic and cathodic compartments had a working volume of 400 mL each. The two compartments were separated via a cation exchange membrane (Nafion 117, Dupont, USA). Each chamber used a self-made carbon brush (with an effective volume of 80 mL) as the electrodes, which were soaked overnight in deionized water to remove organic contamination on the surface of the carbon brushes. The MFC circuits were connected via an external copper circuit under 1,000 Ω load. The experiment was performed by applying the three voltages (0.5 V (MFC0.5), 0.8 V (MFC0.8), or 1.1 V (MFC1.1)) in duplicate, including using a DC-regulated power supply (APS3003S-3D, Gratten Technology, China). A parallel identical control MFC (MFCcontrol) was constructed in the experiment, which was operated under similar conditions but without an external voltage (Friman et al. 2012). The external voltage was applied to MFC in the start-up period for 28 days until the voltage output period stabilized. After the start-up phase, the supply powers were removed and the devices were steadily operated for three months before the study, which was the stabilizing phase. An external resistor of 1,000 Ω was set for the start-up and stabilizing phase until a constant maximum voltage was reached, and the effective biofilms had been fully enriched on the electrode surface. After stabilizing, the MFC were operated under open circuit until a consistently stable maximum voltage was obtained, which was one cycle of operation, and repeated for at least three cycles. The power density curve and polarization curves for the MFC were tested by varying the external resistance from 5 Ω to 9999 Ω. Current production was monitored using a PC-connected data logger (USB-4716, Yanhua Corp., China), and data were recorded every 2 min (Figure 1). All tests were run at 25 ± 0.5 °C in a controlled biochemical incubator.

The mixed solution of anolyte or catholyte and inoculated sludge in a volume ratio of 4:1 was used as a solvent. The anode and cathode were inoculated with raw sludge from an aeration tank of the Wangxiaoayong Sewage Treatment Plant in Hefei City, China. The anode growth media (500 mg/L COD, pH 7.4) contained 0.641 g/L of acetate, phosphate buffer solution (PBS, including 2.883 g/L KH₂PO₄, 6.571 g/L K₂HPO₄, 0.13 g/L KCl, and 0.31 g/L NH₄Cl; pH 7.4), a mineral solution, and a vitamin solution.
The mineral solution included the following constituents (in grams per liter of deionized water): N(CH₂COOH)₃, 1.5; CaCl₂·2H₂O, 0.1; CuSO₄·5H₂O, 0.01; FeSO₄·7H₂O, 0.1; H₂BO₃, 0.01; MgSO₄·H₂O, 0.5; Na₂MoO₄, 0.025; NiCl₂·6H₂O, 0.024; Na₂WO₄·2H₂O, 0.025; ZnCl₂, 0.13; AlK(SO₄)₂·12H₂O, 0.01; CoCl₂·6H₂O, 0.1; and NaCl, 1.

The vitamin solution contained the following constituents (in milligrams per liter of deionized water): vitamin B₅, 5; vitamin H, 2; vitamin B₆, 10; niacin, 5; folic acid, 2; riboflavin, 5; vitamin B₁₂, 0.1; vitamin B₁, 5; p-aminobenzoic acid, 5; lipoic acid, 5. The cathode chamber was fed with 0.722 g/L of KNO₃ solution at pH 7.4, 12.5 mL/L of the vitamin solution, and 5 mL/L of the mineral solution. Also, 1 g/L of NaHCO₃ was added to catholyte to provide a carbon source for cathode microorganisms.

Measurement and analysis

Current density (J) was calculated as $J = U/(R \times V)$, where $U$ is the cell voltage (mV), $R$ is the external resistance ($\Omega$), and $V$ is the effective volume of the cathode chamber ($m^3$). Power density (P) was determined as $P = U^2/(R \times V)$. Polarization and power density curves were used to evaluate the power performance of MFC by varying the external resistance over a range of 5–9999 Ω using a resistor box when stable maximum voltages were produced in a steady and repeatable voltage cycle. A pH meter (8692, AZ Instrument Co. Ltd, Taiwan, China) was used to measure changes in anolyte and catholyte pH. NO₃⁻, NO₂⁻, NH₄⁺, and COD concentrations were measured based on standard methods. Total nitrogen (TN) was measured using a multi N/C3100 analyzer (Analytik Jena, Germany).

Amplification of 16S rRNA genes, sequencing, and sequence analyses

A piece of the electrode was cut off with a sterile razor after MFC performance tests and chemical analysis, and the genomic DNA was extracted using the Qubit 3.0 DNA for Soil kit (Omega Bio-tek, GA, USA) following the instructions provided by the manufacturer. The same extraction kit was used to extract DNA from the aeration tank of the sewage treatment plant that was used as a microbial inoculum. The V₃–V₄ hypervariable regions of the 16S rRNA gene were polymerase chain reaction (PCR)-amplified and sequenced with a MiSeq Illumina sequencer (Illumina, Inc., San Diego, CA, USA) using a 250 bp × 2 paired-end protocol (Muyzer et al. 1993). Multiplexed libraries were prepared using 341F and 805R primers modified by adding external barcodes to allow parallel processing of multiple samples. PCR was performed in 2 × 15 μL reactions with Taq Master Mix and 10 μM of each primer. Amplification was conducted under the following conditions: 95 °C for 5 min, 5 cycles with 94 °C for 20 s, 55 °C for 20 s, 72 °C for 30 s, and a final elongation step of 72 °C for 5 min. The amplicons were then purified using the Wizard SV Gel and PCR Clean-up System (Promega Corporation, Madison, WI, USA) under the instructions provided by the manufacturer and then quantified using Qubit (Life Technologies, Carlsbad, CA, USA). All DNA samples were tested for amplification inhibition by sample dilution. Reads from sequencing were demultiplexed based on the internal barcodes. Forward and reverse reads were merged with perfect overlapping and quality filtered with default parameters. Singleton sequences (sequencing appearing only once in the entire data set) were removed both from the whole dataset and from each sample dataset. Operational taxonomic units (OTU) were defined in the entire data set, clustering the sequences at 97% sequence identity and defining a representative sequence for each cluster (Daghio et al. 2019). A subset of 10,000 random sequences was chosen from each sample and the abundance of each OTU was estimated by mapping the sequences of each sample against the representative sequence of each OTU at 97% sequence identity. The sequences representative of each OTU were classified at different taxonomic ranks using the Ribosomal Data Project classifier (≥80% confidence) (Wang et al. 2007). To compare the microbial diversity between the samples, we performed weighted and unweighted analyses.
with Unifrac and calculated clustering using the unweighted pair group method with arithmetic mean.

The method in which the Chao calculator returns the Chao1 richness estimate for an OTU definition is commonly used in ecology to estimate the total number of species (Chao 1989). The Shannon calculator returns the Shannon diversity index for an OTU definition with a higher value, indicating higher diversity (Schall et al. 2018).

**RESULTS AND DISCUSSION**

**Electrical performance and nitrate degradation**

**Electrochemical analysis of MFC**

The electricity generation capacity of the MFC was influenced by the external voltage. Figure 2 presents the output voltage of the MFC and the polarization and power density curves when system performance was maintained at the optimal stage. The voltage changes considerably throughout the entire cycle, and the trend can be divided into three phases: ascending, stationary, and declining phases (Min et al. 2005; Kaewkannetra et al. 2011; Piepenbrock et al. 2014).

The voltages increased (Figure 2(a)) at the ascending phase rapidly. The voltage output of the MFCcontrol reached a maximum of 443 mV after 7.8 h of operation and then decreased rapidly. The maximum output voltages under external voltages of 0.5, 0.8, and 1.1 V were 400, 428, and 440 mV, respectively, which were lower than those of MFCcontrol. However, the output of MFCcontrol declined significantly, whereas those of the others were relatively stable. This deviation is attributed to the variation of microorganisms on both diversity and growth stimulated by the external voltages (Lian et al. 2016). Denitrification might have exhibited different behaviors, resulting in differences in voltage (Zhang et al. 2014). Both possibilities were worth investigating.

Polarization and power density curves during power generation cycles were obtained to study electron discharges in various initial external voltage microenvironments. After a short ascent stage, the power density rose to the peak (Figure 2(c)). The maximum power density increased with an increase in external voltage. The MFC exposed to a voltage of 1.1 V exhibited the highest maximum power density of 2,035.08 mW m$^{-3}$ after the operation. The polarization curves (Figure 2(b)) were fitted well by a linear phase, exhibiting a linear relationship, with its slope representing the internal resistance. The internal resistance was
estimated to be 153.8 Ω, 271.0 Ω, 175.4 Ω, and 91 Ω with an increase in the power supply. The resistance of MFC_{0.1,1} was lower than MFC_{control}. On the other hand, the resistance under 0.5 V and 0.8 V was higher than that of control.

At a higher temporary electric field, the voltage could effectively reduce the internal resistance of the MFC, thereby improving the maximum power density. When the external voltage was lower than a certain value (such as 0.8 V), the internal resistance of MFC was greater than that of the control group which does not have an external short-term electric field. Furthermore, the maximum power density was slightly lower than MFC_{control}. Applying a voltage of 1.1 V visibly improved the electrochemical performance of the MFC. This observation is consistent with those reported previously (Finkelstein et al. 2006). The application of suitable voltage facilitates the migration of a large number of protons and electrons via a proton exchange membrane and an external circuit, respectively (Wang et al. 2016).

**Effect of applied voltage on nitrate degradation**

Tests were conducted to determine the effect of an applied voltage on the microbial transformation of nitrate. The anode COD removal efficiency and the average conversion rate of compounds containing nitrogen in the cathode during the MFC start-up are shown in Table 1. The consumption of nitrogen yielded minimal changes in the quantity of NO₂⁻ concentration (data not shown). The denitrifying bacteria used NO₃⁻ as the electron acceptor to induce denitrification to form NO₂⁻. NO₂⁻ is a primary intermediate product of denitrification, and its existence indicated the denitrification process in these reactors. Moreover, NH₄⁺-N and NO₂⁻-N generated nitrogen by anaerobic ammonium oxidation (anammox) reaction, and the nitrogen was discharged into the air. Simultaneous denitrification and anammox led to a low NO₂⁻-N concentration.

The use of carbon sources in the MFC operated under an external voltage increased relative to that in the control MFC (i.e. operated without an external voltage). The results showed that the trend in COD removal improved substantially when an external voltage was added and continued to rise with an increase in applied voltage (Wang et al. 2009; Daghio et al. 2016). Under external voltages of 0.5, 0.8, and 1.1 V, the COD removal efficiency reached 64.83%, 72.64%, and 78.09%, respectively, whereas in MFC_{control}, the removal efficiency was limited to 60.27%. Based on the study, the applied voltage increases the COD degradation efficiency. This finding correlates with the report by Zhu et al. (2016). It might also be attributable to the increased external voltage, which could increase the activity of anaerobic microorganisms producing electricity, and promote anaerobic microorganisms to use the substrate (Duteanu et al. 2010). The degradation rate of NO₂⁻-N and the conversion rate of NH₄⁺-N positively correlated with power supply. The results were inconsistent with the finding that suitable electrical stimulation can promote microbial metabolism, leading to enhanced biochemical performance (Thrash & Coates 2008) which, after adding electrodes, led to the utilization of more organics for denitrification. There is no obvious promotion of the degradation effect on MFC_{control}. The reason might be that during start-up, denitrifying microorganisms that were not fully domesticated exhibited different behaviors, which is worthy of further study.

The continuous influences of temporary external voltage on MFC performance is researched in this paper. The concentration variations in NO₂⁻-N, NO₃⁻-N, and NH₄⁺-N in the solution over time in a typical cycle were measured. A relatively reduced NO₂⁻-N concentration was detected. Figure 3 shows the conversion results for NO₃⁻-N and NH₄⁺-N as well as the TN average removal rate (the conversion of nitrite was too low and thus was not listed). As shown in the figure, the applied voltage significantly influences the ability of removing nitrate compared with that of MFC_{control}. The rate of nitrate removal was proportional to the applied voltage. The removal rate of contaminants increased when the voltage ranged from 0 V to 1.1 V. Ammonium and TN removal rate also exhibited inconsistent trends with that of nitrate. Under high applied voltage conditions, NO₃⁻-N production and TN removal efficiency were higher, which demonstrated that electrode denitrification by anaerobic denitrification bacteria occurred in the cathode of the MFC.

The average removal rate of TN did not increase gradually with the increase of external voltage. The average removal rates in MFC_{0.5} (0.408 mg/(L/h)) and MFC_{0.8} (0.562 mg/(L/h)) were lower than the control group.

### Table 1 | Measurement of the anode COD removal efficiency and average conversion rate of compounds containing nitrogen

<table>
<thead>
<tr>
<th>Reactor type</th>
<th>COD removal</th>
<th>NO₂⁻-N average conversion rate</th>
<th>NH₄⁺-N average conversion rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>efficiency (%)</td>
<td>mg/(L/h)</td>
<td>mg/(L/h)</td>
</tr>
<tr>
<td>MFC_{control}</td>
<td>60.27</td>
<td>0.78</td>
<td>0.25</td>
</tr>
<tr>
<td>MFC_{0.5}</td>
<td>64.83</td>
<td>0.31</td>
<td>0.15</td>
</tr>
<tr>
<td>MFC_{0.8}</td>
<td>72.64</td>
<td>0.39</td>
<td>0.24</td>
</tr>
<tr>
<td>MFC_{1.1}</td>
<td>78.09</td>
<td>1.01</td>
<td>0.32</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>7.95</td>
<td>0.33</td>
<td>0.07</td>
</tr>
</tbody>
</table>
(0.606 mg/(L/h)). Under 1.1 V, the TN average removal rate of the cathode chamber reached 0.847 mg/(L/h). Comparing the power generation performance of each MFC, the result shown that the removal of TN by MFC mainly depends on the electron acceptor in the cathode chamber accepting electrons.

NH4\(^+\)-N concentration decreased sharply at first, and subsequently increased over time before finally decreasing. The phenomena can be explained that: (1) simultaneous nitrification and denitrification existed in the cathode chamber, and in the initial operation, the accumulated NH4\(^+\)-N in the cathode reacted with oxygen; (2) as the reaction progressed, the nitrification rate decreased and NO3\(^-\)-N reduction reaction produced hydroxylamine, which can be rapidly converted to NH4\(^+\)-N under alkaline conditions. The reduction could be attributed to the suitable temperature in the cathode (25 ± 0.5 °C), which was beneficial for the growth of normal ammonia-oxidizing bacteria.

The effect of external voltage on the performance of MFC is continuous. The various effect of bio-electrochemical and contaminant removal under different start-up external voltage suggested that external voltage influenced bacteria directly rather than nitrate migration. To further elaborate on the aforementioned experimental results, we analyzed the composition and structural changes in the micro-environment in the MFC.

Characterization of microbial communities

To investigate the structure of the microbial community, high-throughput sequencing was conducted. The bacterial DNA was extracted from the electrode surface and microbial inoculum. Microbial communities were characterized by high-throughput sequencing of the 16S rRNA gene.

Microbial community evolution

To understand the mechanism of nitrogen conversion and removal in the MFC, the abundance and diversity of anode-attached and cathode-attached microbial communities during start-up and stable operation are shown in Table 2. The Shannon index could be used to estimate the abundance and uniformity of microbial structure, and the Chao1 index evaluates the richness of the microbial communities. The indexes of the stable production operation were lower than that of the starting period. During the start-up, microorganisms were not fully domesticated. With the running time increased, microorganisms that were not adapted to the environment were eliminated due to competition with dominant bacteria. In the steady operation, as the external voltage increased, the Shannon index for the cathode and anode were 5.36, 5.66, 5.54, 5.46, and 4.92, 4.11, 5.39, 5.07, respectively. This was inconsistent with the electrical performance of each MFC. There was no positive correlation between the diversity and performance, and the results are consistent with Kiely et al. (2010).

Bray tree plots were used to reflect the similarity and diversity among samples (Figure 4). Genetic similarities were calculated using a simple matching coefficient, and the phenogram was constructed using the unweighted pair group method with arithmetic mean (UPGMA) method (Drummond & Rodrigo 2000). The length of the branches
represents the distance between the samples, with similar branch lengths indicating close distance. Cluster analysis revealed that the temporary applied voltage gave a more obvious effect in the microbial structure of anode. Furthermore, the effect remained after removal.

The composition of communities in different phases at the phylum level were shown in Figure 5. As might be expected, Proteobacteria was the predominant phylum, which agrees with previous studies (Li et al. 2016). Bacteroidetes, Chloroflexi, Firmicutes, Planctomycetes, and Actinobacteria also dominated in the cathode reactors. Previous studies have demonstrated that a large number of heterotrophic nitrifying bacteria and denitrifying bacteria belong to Proteobacteria, Firmicutes, and Chloroflexi and play an important role in the nitrogen cycle of the water environment (Wrighton et al. 2010). Euryarchaeota was significantly more abundant on the anodes when the voltages were applied but decreased from 0.5 V to 1.1 V, which was consistent with the report by Wang et al. (2016). Enrichment of Euryarchaeota was lower in the samples collected during steady operation (both from the anode and the cathode) and was negligible on the cathodic surface and in the microbial inoculum. The results indicated that the application of voltage accelerates the accumulation of Euryarchaeota, and the impact on accumulation will be immediate. The abundance of Planctomycetes was inhibited by the applied voltage relative to that of MFCcontrol. After the external voltage was removed, the concentration was significantly increased in the cathode chamber (from 11.66% on the cathode of 1.1 V to 5.29% on the cathode of 0 V).

The microbial community compositions at the family level are illustrated in Figure 6. This analysis showed that the microbial community compositions were distinct between samples. At the family level, Prolixibacteraceae was identified as dominant in the raw sludge (18.1%). However, microbial community shifts occurred after different treatment and cultivation. The three dominant families in the anode during the starting period are listed, including Methanotrichaceae, Anearcholineaceae, and Prolixibacteraceae. The dominant families changed to Flavobacteriaceae and Xanthomonadaceae after the temporary removal of applied voltage. There was no obvious difference in the microbial community composition of the cathode chamber. The microorganisms linked to the nitrogen cycle were enriched during the treatment in which Anaerolineaceae, Rhodocyclusaceae, Planctomycetaceae, and Flavobacteriaceae were detected and could degrade nitrogen compounds in the steady phase.

In the anode chamber, there was a significant difference in the abundance of predominant functional groups between

| Table 2 | Comparison of diversity parameters and species abundance between anode-attached and cathode-attached microbial communities

<table>
<thead>
<tr>
<th>Index</th>
<th>Starting period</th>
<th>Steady operation</th>
<th>Anode</th>
<th>Cathode</th>
<th>Anode</th>
<th>Cathode</th>
<th>Anode</th>
<th>Cathode</th>
<th>Anode</th>
<th>Cathode</th>
<th>Anode</th>
<th>Cathode</th>
<th>Anode</th>
<th>Cathode</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw sludge</td>
<td>0 V</td>
<td>0.5 V</td>
<td>0.8 V</td>
<td>1.1 V</td>
<td>0 V</td>
<td>0.5 V</td>
<td>0.8 V</td>
<td>1.1 V</td>
<td>0 V</td>
<td>0.5 V</td>
<td>0.8 V</td>
<td>1.1 V</td>
<td>0 V</td>
</tr>
<tr>
<td>Shannon</td>
<td>6.05</td>
<td>4.14</td>
<td>6.17</td>
<td>5.72</td>
<td>5.46</td>
<td>6.05</td>
<td>4.14</td>
<td>6.17</td>
<td>5.72</td>
<td>5.46</td>
<td>6.05</td>
<td>4.14</td>
<td>6.17</td>
<td>5.72</td>
</tr>
<tr>
<td>Chao1</td>
<td>4,000</td>
<td>4,633</td>
<td>4,293</td>
<td>2,509</td>
<td>2,732</td>
<td>4,000</td>
<td>4,633</td>
<td>4,293</td>
<td>2,509</td>
<td>2,732</td>
<td>4,000</td>
<td>4,633</td>
<td>4,293</td>
<td>2,509</td>
</tr>
</tbody>
</table>
Figure 4 | Bray tree plots analysis by UPGMA. (a) Comparison of starting period and (b) comparison of steady phase. MFC1 – microbial characters of the anode; MFC2 – microbial characters of cathode.

Figure 5 | Composition of the bacterial community at the phylum level. (a) Composition of the bacterial community in the anode and (b) composition of the bacterial community in the cathode. MFC1 – MFC in starting period; MFC2 – MFC in steady phase.

Figure 6 | Taxonomic composition at the family level of microbial communities enriched during treatment. Families with a relative abundance of 3% (or higher) in at least one sample are reported. (a) Composition of the bacterial community in the anode and (b) composition of the bacterial community in the cathode. MFC1 – MFC in starting period; MFC2 – MFC in steady phase.
the starting period and the steady phase (Table 3). *Methanotrichaceae* within the archaeal phylum *Euryarchaeota*, which can compete with electrogenic bacteria for substrates and decreasing electron transfer to the cathode, also inhibits the denitrification reaction. When the 1.1 V voltage was applied, a large number of additional electrons were charged into the cathode to promote the degradation of nitrate. After the voltage application, the dominant changed to *Flavobacteria*, which can form a symbiotic and mutually beneficial system of the electrogenesis flora (Arora 2012) to induce more electrons to be extracted. The electro-chemistry performance of MFC during stable operation was directly proportional to the abundance of *Flavobacteria*, suggesting that the anode limits power production. The functional genus for denitrifying was dominant in the cathode reactor. The richness of the genus was higher during the steady phase. *Pirellula* is a typical ammonia-oxidizing bacteria, which can utilize NO$_3^-$ denitrification product NO$_2^-$ to oxidize NH$_4^+$ and generate N$_2$ (Astrid et al. 1996) under hypoxic environment; *Thermogutta* can use NO$_3^-$ or NO$_2^-$ as electron acceptors. NH$_4^+$ is the only product of nitrate reduction (Slobodkina et al. 2015); *Chryseobacterium* and *Ignavibacterium* are the novel nitrate-reducing (Kim et al. 2009) and nitrite-reducing (Liu et al. 2012) bacteria, respectively. The temporary external voltage had no distinct impact on dominant bacteria in the cathode chamber compared with the control group.

**CONCLUSIONS**

The application of an external voltage affected the performance and nitrate removal of MFC during their operation, and the effect remained even after the voltage was removed. Compared with the control, the MFC under a positive voltage application of 1.1 V performed better. Appropriate electrical stimulation during start-up could improve the efficiency of nitrate removal and the electrochemical performance of the MFC. However, the application of smaller voltages – that is, 0.5 and 0.8 V – caused a deterioration in performance during MFC operation. The temporarily applied voltage affected the performance of the MFC by influencing the enrichment of the associated microorganisms in bio-anode. The predominant functional family changed from *Methanotrichaceae* during start-up to *Flavobacteria* in a steady phase. Nitrogen removal efficiency in the cathode was influenced by electron transferring in the anode.
ACKNOWLEDGEMENTS

This research work was supported by the Natural Science Foundation of China (41572246), Natural Science Foundation of Anhui Province (1808085MD102).

REFERENCES

Effects of temporary external voltage on microbial fuel cells


First received 6 February 2020; accepted in revised form 11 May 2020. Available online 26 May 2020.