Prompt nitrogen removal by controlling the oxygen concentration in sediment microbial fuel cell systems: the electrons allocation and its microbial mechanism

Weiping Sima, Ruixiang Ma, Feixian Yin, Haodong Zou, Hong Li, Hainan Ai and Tao Ai

ABSTRACT

It has been proved that the nitrogen can be removed from the sediment in a sediment microbial fuel cell system (SMFCs), but the competition between nitrate and oxygen for electrons would be a key factor that would affect the removal efficiency, and its mechanism is not clear. Based on organic sediment fuel, an SMFC was constructed, and the influence of dissolved oxygen (DO) on nitrogen transformation and cathodic microbial communities was investigated. The results showed that the best total nitrogen removal efficiency of 60.55% was achieved at DO level of 3 mg/L. High DO concentration affected the removal efficiency through the electrons’ competition with nitrate, while low DO concentration suppressed the nitrification. Comamonas, Diaphorobacter and Brevundimonas were the three dominant genera responsible for denitrification at DO concentration of 3 mg/L in this study. The establishment of SMFCs for nitrogen removal by regulating DO level would offer a promising method for sediment treatment.

Key words | cathodic microbial communities, DO concentration, electrons allocation, nitrogen transformation

INTRODUCTION

The increased serious pollution of urban water by the emission of untreated industrial and domestic wastewater now is a widespread problem around the world. The destruction of natural water resources has not only reduced the stability of the ecological system (Waycott et al. 2009; Poncecampos et al. 2013) but also further increased adverse effects on human health (Viswanathan & Schirmer 2015). Sediment is recognized as a sink of major contaminants due to the accumulation of large amounts of inorganic and organic water pollutants, including the nutrient elements of N and P (Yan et al. 2017) and the complex refractory organic pollutants (Li et al. 2017a). What is worse, these pollutants in the sediment would release into the overlying water again so as to cause abrupt water pollution when the hydrodynamic and other physicochemical conditions are disturbed, though external pollution is cut off. Therefore, to eliminate water pollution fundamentally, sediment treatment must be carried out at the same time. Many traditional physicochemical methods, such as dredging (Song & Jiang 2011), ozonation (Zeng & Andrewwhong 2002) and electrochemical degradation (Li et al. 2017b), have been employed for sediment remediation. Nevertheless, the high cost, difficult operation and their possible secondary pollution to the environment limited their widespread application. Therefore, it is imperative to find a promising alternative method for sediment remediation in polluted rivers and lakes.

The sediment microbial fuel cell (SMFC) has recently attracted the attention of many researchers (Logan et al. 2006; Jiang et al. 2011) as a new renewable energy source during the pollutant removal (Clauwaert et al. 2007). Most SMFCs are open systems with anodes embedded in the anoxic sediment and cathodes suspended in the aerobic overlying water (Bond et al. 2002). In the anode side, those exoelectrogens attached on the anode oxidize organic matters in the sediment to generate electrons and protons, while in the cathode side, a terminal electron acceptor, such as nitrate, nitrite (Virdis et al. 2010), oxygen and heavy metal ions (Wu et al. 2017), receives the electrons
delivered from the anode by external circuit. A number of studies have demonstrated that the nitrogen was removed by the simultaneous nitrification and denitrification (SND) process occurring in the cathode chamber. In fact, nitrifiers and denitrifiers were simultaneously observed in a cathodic biofilm for the first time (Virdis et al. 2011), indicating that the SND process was the main mechanism for nitrogen removal. Furthermore, it was possible to accomplish nitrogen removal in the cathode chamber with pre-enrichment with nitrifying biofilm on the cathode (Yan et al. 2012). However, few studies have focused on the SND for nitrogen removal from the sediment in SMFCs.

To our knowledge, dissolved oxygen (DO) was a critical factor in SND system as nitrification was inhibited by low DO concentration while denitrification might be enhanced (Virdis et al. 2011). However, other studies have shown that SND could also smoothly proceed even under aeration. Indeed, nitrate reduction in a bioelectroreactor occurred at DO concentration higher than 4 mg/L (Kuroda et al. 1999). Both nitrification and denitrification could be effectively coupled in an aerated cathode chamber (Virdis et al. 2010; Xie et al. 2011). In fact, some denitrifiers were observed under fully aerated conditions. More specifically, Pseudomonas stutzeri could synthesize nitrate reductase at DO concentration greater than 5 mg/L (Körner & Zumft 1989), and Pseudomonas aeruginosa could serve nitrate as the terminal electron acceptor at DO levels of 2.2 mg/L (Chen et al. 2006). However, the dependency of DO concentration on nitrogen removal and cathodic microbial community has not been clear so far. In addition, the residual DO in the overlying water might penetrate into the sediment. Moreover, it was demonstrated that ammonia oxidation and nitrate reduction could be accomplished on the anode that enriched Nitrosomonas, Comamonas and Paracoccus (Zhan et al. 2014). In short, the nitrogen could be removed both near to the anode side and in cathodic biofilm, but the contribution of each was not so explicit.

To date, most studies of SMFCs focused on wastewater treatment and few reports have investigated nitrogen removal in the sediment. From those researches mentioned above, it could be hypothesized that nitrogen in the sediment can be removed by using SMFCs technology. Therefore, in this study, a membrane-less SMFC was established, and the effects of DO concentration in the water column on nitrogen removal and cathodic microbial community were investigated. It would be of great theoretical significance for the future application of the SMFCs technology.

MATERIALS AND METHODS

Lake sediment and water sampling

The sediment and overlying water samples were collected from Xinhua Lake, a typical shallow eutrophic freshwater lake in Chongqing, China. Sediment samples were taken from approximately 5–10 cm below the sediment–water interface with a grab sampler (1 L). Then the collected sediment was pretreated to remove coarse debris and kept at 4 °C in a refrigerator before use as previously described (Zhang et al. 2011). Characteristics of the sediment sample were as follows: pH was 6.2 ± 0.1, total nitrogen (TN) 5.4 ± 0.03 mg/g, organic matter content 18.7 ± 0.1% (w/w), and moisture water content 45.59 ± 0.2% (w/w). The water sample was collected from a depth of 0–30 cm above the surface of sediment at the same time.

SMFCs configuration and operation

The schematic diagram of the SMFCs is shown in Figure 1. The single chamber SMFC reactor was made up of polymethyl methacrylate, with an inner diameter of 100 mm and height of 150 mm. Each reactor was equipped with a screw-sealed top cover with three oriﬁces, in which the rightmost was used to insert the DO probe, and the remaining two were used to connect a gas-guide tube and for sampling. The anode and cathode materials were both graphite felt (Φ 6 cm). Prior to use, the graphite felt was...
submerged overnight in 1 M HCl, washed with deionized water, then submerged overnight in 1 M NaOH, washed several times in deionized water, and finally burned and activated for 4 hours in a muffle furnace at 600 °C after stoving at 105 °C for 12 hours. The sediment was laid on the bottom of the reactor with an average thickness of 90 mm, in which the anode was embedded 30 mm above the bottom of the reactor. The cathode was hung about 120 mm above the anode after adding 60 mm overlying water in the SMFCs. The titanium wires were used to connect the circuit with a fixed external resistance of 500 Ω.

The anodic chamber was inoculated with the polluted lake sediment and water in the start-up phase to enrich electroactive biofilms in SMFCs. In the start-up phase, the real-time output voltage was monitored by the data acquisition card MCC (USB-1608G, American MCC) after the addition of 20 mL acetic acid (1 mol/L); when the voltage declined sharply another 20 mL acetic acid was added in the SMFCs again.

After successful start-up, the overlying water was replaced by the deionized water to better quantify the nitrogen removal in the system. To investigate the influence of DO concentration on nitrogen removal and power generation in the SMFCs, five initial DO levels of 0, 1, 3, 5 and 8 mg/L were used, which were named BR1, BR2, BR3, BR4 and BR5. The sediment, interstitial water and overlying water were sampled every 7 days for analysis. The sediment samples around the anode were taken out of the reactor from the top using a gravity core sampler. All experiments were conducted in duplicate at 25 °C, and each batch test was operated for 49 d.

**Analysis and calculation methods**

DO, NH$_4^+$-N, NO$_2^-$-N, NO$_3^-$-N, TN and loss on ignition (LOI) were analyzed during the tests. The DO concentration was measured by a DO meter (HQ30d, Hach). The sediment was centrifuged at 6,000 r/min for 10 minutes to collect the interstitial water. The content of NH$_4^+$-N, NO$_2^-$-N and NO$_3^-$-N in the interstitial water and overlying water were measured according to the standard method (APHA 2012). Concentrations of NH$_4^+$-N, NO$_2^-$-N and NO$_3^-$-N in the sediment were determined by the corresponding standard method. The LOI of the sediment was determined by weighing the sample before and after combustion at 550 °C for 4 h (Song et al. 2010). The output voltage of the SMFCs was monitored by the MCC data acquisition card (USB-1608G, American MCC), with a record frequency of 0.01 Hz. The current was calculated according to Ohm’s law: $I = \frac{U}{R}$, where $U$ is voltage and $R$ is external resistance. Power (P) density was calculated according to $P = IU/A$, where $A$ is the area of anode.

**High-throughput microbial community analysis**

The microbial community on the cathodic biofilm was collected and measured by high-throughput pyrosequencing at the end of the experiment. Total DNA extraction and 16S rDNA analysis were done as described previously (Zhang et al. 2009). The polymerase chain reaction (PCR) products were subjected to electrophoresis using a 2.0% agarose gel after total DNA extraction. Based on the preliminary quantitative results of electrophoresis, the PCR products were detected and quantified again by the blue fluorescent quantitative system (QuantiFluor™ ST), and then the MiSeq sequencing was conducted by the Promega Company. Finally, the biodiversity was analyzed by the operational taxonomic units (OTUs) at a sequence identity level of 97% using the RDP classifier Bayes algorithm.

**RESULTS**

**Power generation at different DO level in the SMFCs**

The variation of output voltage and power density is shown in Figure 2, and the process of power generation could be divided into three phases: the early phase (0–7 d), steady phase (7–17 d) and attenuation phase (17–28 d). The similar trend was observed for output voltage and power density during the whole process. In the early phase, the output voltage and power density under different DO concentrations began to increase sharply after addition of substrate. Then in the steady phase, the maximum output voltage of the SMFCs under five cathodic DOs were 101.2 mV, 117.1 mV, 221.8 mV, 222.4 mV and 308.4 mV, respectively. The corresponding maximum power density under five cathodic DOs were 7.24 mW/m$^2$, 9.69 mW/m$^2$, 34.77 mW/m$^2$, 34.96 mW/m$^2$ and 67.21 mW/m$^2$, respectively. Both the maximum output voltage and maximum power density increased with the increase of cathodic DO level. Finally, in the attenuation phase, the output voltage and power density under each cathodic DO began to decline rapidly from the plateau to the minimum.

**LOI and TN removal in the SMFCs**

The LOI was measured to indicate the extent of organic pollution in the sediment. The removal efficiency of LOI in the
SMFCs during the 49 d batch test is shown in Figure 3(a). It is apparent that the LOI removal efficiency was higher than 10% in all the batch tests. The lowest and highest LOI removal efficiency of 12.3 ± 1.25% and 33.4 ± 2.46% was achieved at DO of 0 mg/L and 8 mg/L, respectively. The removal efficiency of LOI were 15.6 ± 1.68%, 18.9 ± 0.78% and 27.8 ± 1.65% at DO of 1 mg/L, 3 mg/L and 5 mg/L, respectively. As shown in Figures 2(a) and 3(a), the voltage produced by SMFCs and the LOI removal efficiency depended on the DO concentration applied, with the highest voltage and the highest LOI removal efficiency both obtained at the highest DO concentration of 8 mg/L.

The TN removal efficiency in the SMFCs is shown in Figure 3(b). The best TN removal efficiency of 60.55% was achieved at DO of 3 mg/L, while the lowest TN removal efficiency of 38.35% was obtained at the highest DO concentration. The TN removal efficiency was 42.11%, 55.22% and 56.00% at DO concentration of 0, 1 and
5 mg/L, respectively. With the increase of DO concentration, power generation and LOI removal efficiency increased, but it did not necessarily mean an increase in nitrogen removal. Indeed, the nitrogen removal efficiency in the first 7 days was both low, which might be related to the growth of biofilms.

**Nitrogen transformation in the SMFCs**

Figure 4(a)–4(e) and Figure S1 (Supplementary Material) show the nitrogen transformation in the SMFCs at different DO concentrations, and the initial content of different substrate in the sediment and interstitial water is shown in Table 1.

When DO was 0 mg/L, in the first 7 days, the NH$_4^+$-N content in the sediment, interstitial water and overlying water sharply increased to 180.61 mg, 45.26 mg and 79.41 mg, respectively. The NO$_3^-$-N content in the sediment declined from 61.97 mg to 54.87 mg, while it increased to 6.39 mg and 11.02 mg in the interstitial water and overlying water, respectively. These results demonstrated that the anoxic environment prompted the NH$_4^+$-N and NO$_3^-$-N to migrate from sediment to water. In the longer operation time, the
Figure 4 | The nitrogen transformation at DO of 0 mg/L (a), 1 mg/L (b), 3 mg/L (c), 5 mg/L (d), and 8 mg/L (e); left: NH$_4^+$-N; right: NO$_3^-$-N.
content of NO\textsubscript{3} -N and NO\textsubscript{2} -N in the sediment, interstitial water and overlying water declined all the time. This reduction might be due to the denitrification in the cathode.

When DO was 1 mg/L, in the first 7 days, the variation of nitrogen forms was similar to that of DO at 0 mg/L. In the longer operation time, in the sediment, the content of NH\textsubscript{4} -N declined from 176.37 mg to 106.31 mg, the content of NO\textsubscript{3} -N firstly increased from 22.52 mg to 29.89 mg and then declined to 18.57 mg. The content of NO\textsubscript{2} -N firstly increased from 0.08 mg to 0.86 mg and then declined to 0.54 mg. The decrease of NH\textsubscript{4} -N was a combined result of nitrification and diffusion to the overlying water.

When DO was elevated to 5 mg/L, in the first 7 days, the NH\textsubscript{4} -N content increased in the sediment, interstitial water and the overlying water, while the NO\textsubscript{3} -N content in the sediment declined from 61.97 mg to 36.36 mg. In the longer operation time, the NH\textsubscript{4} -N content was sharply decreased from 212.92 mg, 46.43 mg and 104.57 mg to 9.79 mg, 40.41 mg and 40.41 mg in the sediment, interstitial water and the overlying water, respectively. This result indicated that the nitrification process could proceed smoothly. Meanwhile, the decline of NO\textsubscript{3} -N content in the overlying water indicated that the denitrification was also strong under this DO level. The NO\textsubscript{2} -N content decreased with the decrease of NH\textsubscript{4} -N, indicating that SND in the cathodic side was the main pathway for nitrogen removal during this period.

When DO was 5 mg/L, in the first 7 days, the NH\textsubscript{4} -N and NO\textsubscript{3} -N content increased to 104.57 mg and 16.95 mg in the overlying water, respectively. In the longer operation time, the NH\textsubscript{4} -N content constantly decreased from 212.92 mg, 46.43 mg and 104.57 mg to 25.27 mg, 11.59 mg and 17.2 mg in the sediment, interstitial water and the overlying water, respectively. At the same time, the NO\textsubscript{3} -N content in the overlying water firstly increased from 16.95 mg to the maximum 57.76 mg and then gradually declined to 33.62 mg, which remained at high levels compared with low DO concentration. The accumulation of NO\textsubscript{3} -N in the overlying water at DO level of 5 mg/L indicated that denitrification was the rate-limiting step for nitrogen removal.

At the highest DO of 8 mg/L, high accumulative content of NO\textsubscript{3} -N but with little residue of NH\textsubscript{4} -N was found in the reactor, indicating that nitrification was promoted but denitrification was inhibited under aerobic environment.

### Microbial community analysis on cathodic biofilm

The cathodic biofilm was observed in BR1, BR2, BR3 and BR4 experiments (Figure S2, Supplementary Material), and no biofilm was observed when DO was 8 mg/L due to continuous aeration. The biofilms were taken out and analyzed by high-throughput pyrosequencing after the experiment. The Venn diagram that described the relationships between the four samples is shown in Figure 5(a). The number of OTUs in all the samples fluctuated between 190 and 521. All the four samples shared 128 OTUs, accounting for 24.6%–67.4% of the OTUs in each sample, indicating that the compositions of microbial communities in the biofilm were highly similar under different DO concentrations.

It was found that Proteobacteria, Bacteroidetes and Firmicutes were the three dominant phyla in all the samples (Figure 5(b), with relative abundance >0.1%). Moreover, a high similar microbial community composition was observed at low DO of 0 mg/L and 1 mg/L, with Bacteroidetes (45.9% and 46.7%), Proteobacteria (28.6% and 35.5%) and Firmicutes (14.2% and 13.6%) found in both biofilms. At DO of 3 mg/L, the relative abundance of Proteobacteria (79.1%) and Bacteroidetes (19.1%) was very high but there was a low abundance (1.4%) of Firmicutes. At DO of 5 mg/L, Bacteroidetes, Proteobacteria and Firmicutes were the three dominant phyla with a relative abundance of 52.1%, 29.7% and 16.6%, respectively. It was reported that Proteobacteria is a typical nitrifying phylum that contains a large number of nitrobacteria (Sun et al. 2017).

Those functional microorganisms related to nitrogen removal were further investigated at the genus level (Figure 5(c)). Brevundimonas was the most abundant bacteria group at DO of 5 mg/L. Srinandan et al. (2011) reported that Brevundimonas was able to reduce nitrate to nitrite. Comamonas, a denitrifying bacteria (Xing et al. 2010), was detected with 2.9% abundance at DO of 3 mg/L. The genus Azospirillum, with the ability to reduce nitrate into nitrogen gas under anaerobic or anoxic conditions (Gao et al. 2017), was much higher in the reactor with DO of 0 mg/L. Diaphorobacter was identified as a denitrifying bacteria that had the ability to accumulate nitrite (Chakravarthy et al. 2011), and had a relative abundance of 14.4% and 1.4% at DO of 3 mg/L and 1 mg/L, respectively.

### Table 1 | The initial content of different substrate in the sediment and interstitial water

<table>
<thead>
<tr>
<th>Index</th>
<th>LOI (%)</th>
<th>TN (mg)</th>
<th>NH\textsubscript{4} -N (mg)</th>
<th>NO\textsubscript{2} -N (mg)</th>
<th>NO\textsubscript{3} -N (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interstitial water</td>
<td>/</td>
<td>14.16</td>
<td>5.59</td>
<td>0.89</td>
<td>0.02</td>
</tr>
<tr>
<td>Sediment</td>
<td>6.23</td>
<td>864.43</td>
<td>95.84</td>
<td>61.97</td>
<td>0.13</td>
</tr>
</tbody>
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Figure 5: (a) Venn diagram based on the OTUs of biofilms at different DO concentrations (a), the relative abundance of bacterial communities at the phylum level (b) and the genus level (c).
The bacteria belonging to *Flavobacteria* sp. and *Rhizobium* sp. were also observed in the biofilm, which were isolated from a denitrifying reactor and a bio-cathode MFC, respectively (Sun et al. 2011). *Flavobacterium* was more abundant in the biofilm at DO of 1 mg/L, and was responsible for both the degradation of organic matters and NH4⁺-N removal (Liu et al. 2017). The fermentative bacteria of *Macellibacteroides* was the dominant genus in the anaerobic system (Jabari et al. 2012). Those typical nitrifying bacteria, such as *Nitrosomonas* and *Nitrosospira*, had low abundance in this study.

**DISCUSSION**

**DO played a key role in electron distribution between oxygen and nitrate**

The DO concentration played a key role in nitrogen removal and final nitrogen forms in the SMFCs. Theoretically, DO has two different pathways to influence the nitrogen transformation: one affects the nitrification process by suppressing the activity of ammonia oxidizing bacteria and nitrite oxidizing bacteria (NOB), and the other one inhibits the denitrification process by competing for the electrons with nitrate and nitrite.

The high DO concentration was more advantageous for power output as the electrons were derived from the LOI degradation (Figure 2(a)). However, excessive DO concentration in the water column would exert a negative effect on nitrogen removal (Figure 3(b)). When DO was 5 mg/L, the denitrification process in the cathodic biofilm was blocked as verified by the accumulation of NO3⁻-N and NO2⁻-N in the water column (Figure 4(d)). This could be because high DO was sufficient for nitrifiers, but the residual molecular oxygen would still prevent NO2⁻-N or NO3⁻-N being used as electron acceptors. The content of NO3⁻-N in the longer operation time was relative stable (Figure 4(d)) might also be caused by the concentration gradient gradually decreasing between the sediment and overlying water. Moreover, the accumulation of NO3⁻-N led to the decrease of pH so as to produce more protons, which would further prompt the reduction of oxygen. The accumulation of NO2⁻-N might be due to the toxicity of free nitrous acid to NOB (Aee et al. 2018), for which the NO2⁻-N was more competitive for protons than oxygen. However, the TN removal efficiency of 38.35% was still achieved at DO of 5 mg/L. Thus nitrogen may be removed by the following two ways: (I) nitrogen was removed by the aerobic denitrifying bacteria in the cathodic biofilm. *Pseudomonas* spp. could reduce NO3⁻-N to N2 under aerobic conditions (Su et al. 2001); (II) a proportion of TN was most likely removed by the denitrifiers in the sediment.

At DO level of 0 mg/L and 1 mg/L, the competition between nitrifiers and oxygen reducing bacteria for the limited oxygen would be more intensive, which would then exert a negative influence on nitrification. As shown in Figure 4(a) and 4(b), the accumulation of NH4⁺-N content in the water column indicated the suppression of nitrification. In the longer operation time, the continuous decrease of NH4⁺-N content might be attributed to the microbial assimilation in the cathode biofilm.

The possible nitrate removal proportions in the biofilms and in the sediment under different DO concentrations is shown in Figure 6. The nitrogen was mainly removed by the aerobic denitrifying bacteria in the sediment at DO of 8 mg/L. However, when DO concentration was 5 mg/L, the aerobic denitrifying bacteria in the cathodic biofilm and the denitrifying bacteria in the sediment were both responsible for nitrogen removal. Moreover, the autotrophic biocathodic denitrification also might be responsible for the nitrate reduction as a high output voltage was obtained at DO of 5 mg/L (Cecconet al. 2019). It is quite interesting to look into why there is an optimal DO concentration for nitrogen removal. The best performance of nitrogen removal was obtained at DO of 5 mg/L, which might be because the DO can not only meet the oxygen required for nitrification but also make appropriate electron allocations between nitrate and oxygen. When DO was 0 mg/L, nitrogen may
be removed by the following two ways: (I) most nitrogen was removed by anaerobic ammonia oxidizing bacteria or the short-cut nitrification and denitrification, where *Brevundimonas* was able to reduce nitrate to nitrite at low DO level (Ding *et al.* 2018); (II) only a small part of TN removal was assimilated by microorganism’s growth and reproduction in the cathodic biofilm (Kang *et al.* 2018).

**Cathodic microbial community structure changed by DO microenvironment**

Low nitrogen removal efficiency without biofilm at DO of 8 mg/L indicated that the biological process was important for nitrogen removal. As shown in Figure 5(b), *Bacteroidetes, Proteobacteria* and *Firmicutes* were the three most dominant phyla in all the biofilms, suggesting that these bacteria played a dominant role in nitrogen removal (Kondaveeti *et al.* 2014). The DO concentration caused a diverse community that integrated members of different functional groups involved in nitrogen removal (Figure 5(c)). When DO was 0 mg/L, more anaerobic nitrogen-fixing bacteria of *Azospirillum* and the denitrifiers of *Macellibacteroides* were observed. Moreover, the genus of *Diaphorobacter*, a typical denitrifying bacteria, was more abundant at DO level of 3 mg/L (41.3%) than that of 0 mg/L (5.0%).

As mentioned above, DO was a critical factor in the SND system. However, the aerobic nitrifying bacteria and anaerobic denitrifying bacteria co-existed in the cathodic biofilm even at high DO level in this study. Therefore, it can be assumed that a DO gradient was formed along the biofilm, as nitrifiers and denitrifiers would position themselves according to the suitable DO concentration. At DO level of 0 mg/L and 1 mg/L, the nitrifiers were inhibited as the limited DO was almost depleted by the heterotrophic bacteria and cathodic oxygen reduction, whereas at higher DO of 5 mg/L, the DO was too much for nitrifying bacteria and oxygen reduction, thus resulting in the residual DO reaching the deeper layer of the biofilm, and thus compressing the space of the anaerobic microenvironment and reducing denitrifying bacteria. At DO level of 3 mg/L, the special structure of biofilm provided a suitable inner space for denitrifiers, although the activities of denitrifiers could be inhibited by high DO concentration. In fact, a macroscopic anaerobic environment was not so strictly needed any more, because the facultative anaerobic microorganisms could reduce nitrate even under air condition with headspace containing 21% oxygen (Jiang *et al.* 2018). In addition, *Nitrosomonas* could complete the conversion of ammonium to dinitrogen with hydroxylamine as the main substrate under anoxic conditions (Vilajeliupons *et al.* 2017). These results supported a scenario in which nitrifiers would preferentially be located on the outer layers of the biofilm, while denitrifiers would be positioned instead on the portions closer to the electrode.

Other factors, such as the biofilm thickness, biofilm structure, electric stimulation and nutrients gradient, could also exert influence on the bacteria stratification of different types of respiration (Uemoto & Saiki 2000). It is apparent that a thicker biofilm would block DO, organic carbon, and other substances from being transferred into the inner part of the biofilm. The biofilms with diameters of less than 100 μm would be fully penetrated by oxygen (Meyer *et al.* 2005). Stimulation from the electric field would directly or indirectly change bacteria community (Mena *et al.* 2016). Moreover, the ammonium fluxes diffusing from the sediment to overlying water might increase with the increase of output voltage (Kim *et al.* 2008), thus changing the microbial structure in the biofilm. Further studies have yet to determine how the DO concentration would influence the biofilm thickness and biofilm structure for nitrogen removal.

**Environmental application of SMFCs for sediment treatment**

Regulating electron distribution allocation between nitrate and oxygen and developing more efficient and robust biofilms by controlling the oxygen supply would further promote nitrogen removal. The results obtained in this study are of great practical significance for us to restore those polluted aquatic environments. For those heavily polluted aquatic environments with seasonal or perennial DO level in natural systems, it is necessarily to control the DO concentration at a suitable level to achieve the best performance of pollutant removal, whereas for those slightly polluted aquatic environments, combining with other ecological repairing techniques, such as phytoremediation and constructed wetlands, would be more reasonable.

**CONCLUSIONS**

Different DO concentrations exerted significant influence on nitrogen removal and cathodic microbial communities in the SMFCs. Nitrogen was effectively removed at the optimal DO of 3 mg/L as oxygen played an appropriate role in electron distribution among nitrate, nitrite and oxygen. The distinct communities between the biofilms further explained the effects of different DO concentrations on microbial diversity and pollutant removal. Those dominant functional
microorganisms related to nitrogen removal varied greatly at different DO levels due to the oxygen gradient forming along the biofilm. Regulating the DO level would be of great theoretical significance for the future application of the SMFCs to pollutant removal.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this paper is available online at https://dx.doi.org/10.2166/wst.2020.222.

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