Impact of anodophilic biofilm bioelectroactivity on denitrification behavior of single-chamber air-cathode microbial fuel cell in steady state

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Abstract

Generally, high bioelectroactivity of anodophilic biofilm favors high power generation of microbial fuel cell (MFC), however, it is not clear whether it can promote denitrification of MFC synchronously. In this study, the impact of anodophilic biofilms bioelectroactivity on denitrification behavior of single-chamber air-cathode MFC (SAMFC) in steady state was studied for the first time. Anodophilic biofilms of various bioelectroactivity were acclimated at conditions of open circuit (OC), Rext of 1000Ω and 20Ω (denoted as SAMFC-OC, SAMFC-1000Ω and SAMFC-20Ω, respectively) and run for 100 days in the presence of nitrate. Electrochemical tests and microbial analysis results showed that the anode of the SAMFC-20Ω delivered higher oxidation and denitrification current response and had a higher abundance of electroactive bacteria, like Geobacter, Pseudomonas and Comamonas, which possessed bidirectional electron transfer function, demonstrating a higher bioelectroactivity of the anodophilic biofilm. Moreover, these electroactive bacteria favored the accumulation of denitrifers, like Thauera and Alicycliphilus, probably by consuming trace oxygen through catalyzing oxygen reduction. The SAMFC-20Ω not only delivered a 61.7% higher power than the SAMFC-1000Ω, but also achieved a stable and high denitrification rate constant (kDN) of 1.9, which was 50% and 40% higher than that of the SAMFC-OC and SAMFC-1000Ω, respectively. It could be concluded that the high bioelectroactivity of the anodophilic biofilms not only favored high power generation of the SAMFC, but also promote the growth of denitrifers at the anodes and strengthened denitrification. This study provided an effective method and important theoretical basis for enhancing power generation and denitrification performance of the SAMFC synchronously.

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Abstract

Generally, high bioelectroactivity of anodophilic biofilm favors high power generation of microbial fuel cell (MFC), however, it is not clear whether it can promote denitrification of MFC synchronously. In this study, the impact of anodophilic biofilms bioelectroactivity on denitrification behavior of single-chamber air-cathode MFC (SAMFC) in steady state was studied for the first time. Anodophilic biofilms of various bioelectroactivity were acclimated at conditions of open circuit (OC), R_{ext} of 1000Ω and 20Ω (denoted as SAMFC-OC, SAMFC-1000Ω and SAMFC-20Ω, respectively) and run for 100 days in the presence of nitrate. Electrochemical tests and microbial analysis results showed that the anode of the SAMFC-20Ω delivered higher oxidation and denitrification current response and had a higher abundance of electroactive bacteria, like *Geobacter* , *Pseudomonas* and *Comamonas,* which possessed bidirectional electron transfer function, demonstrating a higher bioelectroactivity of the anodophilic biofilm. Moreover, these electroactive bacteria favored the accumulation of denitrifers, like *Thauera* and *Alicycliphilus* , probably by consuming trace oxygen through catalyzing oxygen reduction. The SAMFC-20Ω not only delivered a 61.7% higher power than the SAMFC-1000Ω, but also achieved a stable and high denitrification rate constant *(* k_{DN}) of 1.9, which was 50% and 40% higher than that of the SAMFC-OC and SAMFC-1000Ω, respectively. It could be concluded that the high bioelectroactivity of the anodophilic biofilms not only favored high power generation of the SAMFC, but also promote the growth of denitrifers at the anodes and strengthened denitrification. This study provided an effective method and important theoretical basis for enhancing power generation and denitrification performance of the SAMFC synchronously.

Keywords: Denitrification, single-chamber air-cathode microbial fuel cell, bioelectroactivity, anodophilic biofilms, steady state

INTRODUCTION

As one of newly developed microbial electrochemical technologies, microbial fuel cell (MFC) attracts special attentions due to its double functions of waste removal and electrical energy recovery from wastewater. MFC utilizes the electrode respiration of exoelectrogens (also referred to as electroactive microorganisms and anode respiring bacteria) (Borole et al., 2011; Logan, 2009; Lovley, 2012) to convert the pollutants from water to available electrical energy or resources (Falk Harnisch & Schroder, 2010; Rabaey & Verstraete, 2005). Based on the electrode respiration of the exoelectrogens, multiple pollutants in the wastewater can be synergistically removed. As one of important pollutants in wastewater that usually cause serious water eutrophication, nitrate can serve as electron acceptor and is (bio)electrochemical reduced at the cathode of MFC (Falk Harnisch & Schroder, 2010; Li, Yu, & He, 2014), this provide a new idea for nitrate removal. The simultaneous organic oxidation at bioanode and bioelectrochemical denitrification (BEDN) at biocathode was firstly performed in a dual-chamber MFC (Clauwaert et al., 2007), in which autotrophic denitrifers at cathode conducted BEDN by uptaking electrons from the organic oxidation of exoelectrogens at the anode. However, the autotrophic denitrifers usually reproduced slowly and thus led to a poor denitrification performance (H. Huang et al., 2019; Patureau, Zumstein, Delgenes, & Moletta, 2000). Recently, it had been found that the exoelectrogens enriched at the anode of MFC could not only conduct organics oxidation, but also be able to catalyze cathodic oxygen reduction (Ka Yu Cheng, Ho, & Cord-Ruwisch, 2010) and bioelectrochemical denitrification (BEDN) (K. Y. Cheng, Ginige, & Kaksonen, 2012). It meant that the BEDN could also be carried out at the anode of MFC. It had been reported that exoelectrogens can transfer electrons directly to denitrifers by direct attachment or through electrode (Liang et al., 2019). The heterotrophic environment of bioanode could facilitate the reproduction of the exoelectrogens thus would substantially increase the denitrification performance of MFC. Anodic BEDN was firstly conducted in dual-chamber MFC (Zhang et al., 2013), however, the use of ion-exchange membrane decreased the performance of MFCs on nitrate removal. Moreover, the high operating cost sourced from the use of ion-exchange membrane also limits the application of dual-chamber MFC in practical wastewater treatment(Haobin Huang et al., 2018). In comparison, single-chambered air-cathode (SAMFC) would be more favorable for practical wastewater treatment due to the merits of membrane-less configuration, low internal resistance, low cost. In SAMFC, the BEDN mainly occurs at bioanode (Sukkasem, Xu, Park, Boonsawang, & Liu, 2008), might be due to the presence of dissolved oxygen which restrict the enrichment of denitrifers on the air-cathode.

In recent years, many investigations on BEDN of the SAMFC have been advanced in recent years. (H. Huang et al., 2019; Haobin Huang et al., 2018; Sukkasem et al., 2008). In one study, Liu et al compared the denitrification performance under electricity generation (EG) (with loads of 1000 and 270 Ω) and open circuit (OC) conditions and found that the denitrification activity of the SAMFC under current generation conditions was slightly lower than that under OC state. They thought that the possible reason was attributed to the electron competition between the electrode and nitrate under the EG condition (Sukkasem et al., 2008). However, it should be noted that this study was based on transient state, in which the operation of SAMFC under specific condition was in a relatively short time, e.g. several hours, and difficult to reflect the real denitrification performance of SAMFC. In another study, Huang et al also compared the denitrification performance of SAMFC under the EG (with load of 1000 Ω) and OC conditions in steady state and achieved the opposite result that the denitrification performance under the EG was higher than that under OC state (H. Huang et al., 2019). Generally, MFC with lower external resistance favored the acclimation of anodophilic biofilms with higher bioelectroactivity, thus favors higher power generation, however, it is not clear whether it can promote denitrification of MFC synchronously? How does the bioelectroactivity of anodophilic biofilms affect the denitrification behavior of the SAMFC in steady state?

In this study, we studied the impact of anodophilic biofilms bioelectroactivity on the denitrification behavior of the SAMFC in steady state for the first time, trying to achieve high power generation and denitrification performance of SAMFC synchronously. Anodophilic biofilms of the SAMFC with various bioelectroactivity was acclimated at conditions of open circuit, R_{ext} of 1000Ω and 20Ω (denoted as SAMFC-OC, SAMFC-1000Ω and SAMFC-20Ω, respectively) and run for 100 days in the presence of nitrate. The anode potentials, voltage of the SAMFCs were monitored in the whole operation process, then the bioelectroactivity of the anodophilic biofilms, microbial community and denitrification performance of the SAMFCs were analyzed and compared.

2. MATERIALS AND METHODS

2.1. SAMFC construction and microorganism acclimation

Cube-shaped plexiglass with a volume of 84 mL was used as the setup of SAMFCs. The anodes were graphite brushes with 2 cm in diameter and 2.5 cm in length. The air-cathodes were fabricated by rolling method as previously described (Liu et al., 2014) using activated carbon as oxygen reduction reaction catalyst. Nine SAMFCs were built and divided into three groups with three in each group. The three groups of SAMFCs run at conditions of open-circuit (OC), 1000Ω load and 20Ω load (denoted as SAMFC-OC, SAMFC-1000Ω and SAMFC-20Ω), respectively. These SAMFCs were inoculated from SAMFC that inoculated with domestic wastewater from treatment plant (Qingshan, Nanchang) and run for more than one year. The SAMFCs were firstly operated by feeding 50 mM phosphate buffer solution (PBS) containing 1.64 $g \cdot L^{-1}$ sodium acetate (equal to 1280 $mg \cdot L^{-1}$ COD) for 30 days to acclimate exoelectrogens onto the anodes, then run by feeding the 50 mM PBS containing 1.64 $g \cdot L^{-1}$ and extra 1.21 $g \cdot L^{-1}$ sodium acetate (200 $mg \cdot L^{-1} NO_3^-$-N) to acclimate denitrifers. The detailed acclimation and characterization process of the SAMFCs is shown in Figure S1.

All the SAMFCs were operated in fed-batch mode at a constant temperature of 35±1°C. During the acclimation process, the anode potentials and cell voltages were automatically recorded every 5 min using a data acquisition system (digital multimeter, Keithley 2700, USA) connected to a personal computer. Medium was refreshed when the anode potential rose to over 0 V. All the electrode potentials were measured versus the Ag/AgCl reference electrode (+0.198 V versus SHE, saturated KCl). The concentrations of NO_3^--N in the SAMFCs were measured at the 20th (t_{20}), 40th (t_{40}), 60th(t_{60}), 80th (t_{80}), and 100th (t_{100}) day, respectively. The microbial electrocatalytic activity and community analyses of the microorganisms at the anodes were conducted at the initial (t_0), 20th (t_{20}) and 100th (t_{100}) day, respectively. Microbial community analysis can damage the anode biofilm, therefore one of the three SAMFCs in each group was used for NO_3^--N concentration, CV characterization and final microbial community analysis, while the other two SAMFCs were only used for microbial community analysis.

2.2 Measurements and calculations

The concentrations of NO_3^--N in the SAMFCs were measured regularly with the time interval of 20 days. For example, the first and second denitrification tests were performed at t_{20} and t_{40} respectively. During the whole period for denitrifers biofilm acclimation, the concentrations of sodium nitrate keep at 1.21 g L^{-1}, which is corresponding to the NO_3^--N concentration of 200 mg L^{-1}. In order to obtain optimal C/N ratio, three different concentrations of sodium acetate of 0.82 g L^{-1} (640 mg L^{-1} COD), 1.64 g L^{-1} (1280 mg L^{-1} COD) and 2.05 g L^{-1} (1600 mg L^{-1} COD) were used to control the C/N weight ratio of 1.2, 2.4 and 3, respectively. The denitrification tests under different C/N ratios were performed at t_{20}. Three different SSAMFCs were sampled at 1h, 2h, 3h, 4h and 5h and immediately filtered through disposable Millipore filter with the pore size of 0.22μm for the analyses of nitrate, nitrite at the same condition simultaneously. NO_3^--N concentration measurements were conducted by applying a spectrophotometer (SpectroDirect, Lovibond, Gremany) using standard methods.

Denitrification rate (r_{DN}) was fitting by pseudo-first order reaction with respect to concentration and calculated as follows according to Jia et al(Jiang et al., 2018),Ln $\left(\frac{C_t}{C_0}\right) = -\mathrm{Kt}$, where C_0 is the initial concentration of nitrogen in the form of nitrate (NO_3^--N) (mg/L), t is the time, C_t is the NO_3^--N econcentration(mg/L) at t h. K is the observed reaction rate constant.

2.3 Bioelectroactivity analysis anodophilic biofilms

The bioelectroactivity of the anodophilic biofilms in the SAMFCs at the period of initial (t_0), 20^{th} day (t_{20}) and 100^{th} day (t_{100}) was analyzed by cyclic voltammetry (CV) using potentiostatically-controlled three-electrode system, in which the anode served as the working electrode, the air-cathode served as the counter electrode and an Ag/AgCl electrode served as the reference electrode. The CV measurements were controlled by a potentiostat (CHI1040B, Shanghai Chenhua Instrument Co., Ltd, China) and carried out in the potential range from +0.4 V to -0.5V at scanning rate of 1 mV s^{-1}. All the SAMFCs were set at open-circuit condition for 1h before CV implementation.

2.4 Microbial community analysis

Biofilm samples were also collected from the anodes of SAMFCs at the stages of t_0, t_{20} and t_{100} by using a 10-mL syringe with a 0.9×150 mm stainless steel needle for microbial community analysis. High-throughput sequencing analysis based on all bacterial 16S rRNA was performed to know the microbial communities present in the biofilms as described in ref. (Chen et al., 2019) , detailed procedure see supplemental information.

3. RESULTS AND DISCUSSION

3.1 Power generation and bioanode potential variation of the SAMFCS

Figure 1A show that the SAMFC-20Ω, SAMFC-1000Ω and SAMFC-OC generate a voltage of 0.093, 0.525 and 0.698V during nearly one month operation process. The SAMFC-20Ω generated a steady power of 0.432 mW, which is 61.7% higher than the SAMFC-1000Ω with 0.267 mW (Figure 1B). Moreover, the SAMFC acclimated under load of 20Ω could generated a maximum power density of 976.6 mW m^{-2} without power overshoot, while that acclimated under load of 1000Ω delivered a power of only 637 mW m^{-2} with an obvious power overshoot (Figure 1C).

The potentials of the SAMFC bioanodes during the whole acclimation process shown in Figure 2 display that, after running for about one week, the potentials of the SAMFC anodes change periodically with periodical refresh of the medium containing acetate. Once the medium is refreshed, the anode potential drops rapidly and then level off at a negative value due to the bioelectrochemical oxidation of acetate. Figure 2C displays that the anodes of the SAMFC-20Ω, SAMFC-1000 and SAMFC-OC could achieve a negative potential of -100, -450 and -480 mV, respectively. When the acetate is depleted, the anode potential increases and approaches the cathode potential (about 0 V) gradually. Figure 2B, 2D and 2E show that the SAMFCs fed with media containing both acetate and nitrate substrate display similar trend of anode potential variation to that fed with media containing only acetate substrate. However, a conspicuous potential platform appears in the curves of anodes fed with medium containing both acetate and nitrate (Figure 2D and 2E). Once the medium is refreshed, the anode potential of the SAMFC-20Ω, SAMFC-1000Ω and SAMFC-OC firstly drops from about 0V to negative and level off at -0.09, -0.369 and -0.386 V, respectively, for about 3.6 h.

The evident platform of the potential curve might be a consequence of concurrent acetate oxidation and nitrate reduction at the anodes of the SAMFCs, which result in a mixed anode potential. Figure 2B, 2D and 2E reveal that the potential platform periodically exists in every cycle during denitrifers acclimatization. Subsequently, the anode potentials of the SAMFC-20Ω, SAMFC-1000Ω and SAMFC-OC drop again and level off at -0.11, -0.45 and -0.5V, respectively (Figure 2D), which are basically equal to that of the SAMFCs fed with media containing only acetate. This potential decline is believed to be caused by the depletion of nitrate. The nitrate concentration in a complete cycle at the 40$^{\text{th}}$ day is measured and shown in Figure 1G, it confirms that the nitrate is depleted in about 3 to 4 h in all the SAMFCs, which is well matched with the duration of the anode potential platform (Figure 2F). Thereby, the potential decline could be a signal of nitrate depletion and would be used for monitoring the nitrate concentration in wastewater. Finally, the anode potentials of SAMFC-20Ω increases gradually to positive potential values (Figure 2D and 2E) due to the depletion of acetate, which is similar to that of acclimation of exoelectrogens by feeding medium containing only acetate (Figure 2C).

3.2 Bioelectroactivity of anodophilic biofilms

After acclimation of the SAMFCs in medium containing acetate for 30 days, the bioelectroactivity of the biofilms enriched at the bioanodes are measured by CVs and the results are shown in Figure 3. Figure 3A1 shows that all the CVs of anodophilic biofilms acclimated in the presence of acetate (turnover condition) present the typical positive sigmoidal shape (Liang et al., 2019; Pous et al., 2016), indicating that exoelectrogens are established successfully in all the SAMFCs. Obvious differences are observed from the CVs of different SAMFC bioanodes. The CVs of the SAMFC-20Ω bioanodes display a high bioelectrocatalytic oxidation current of 7.8 mA, which is much higher than that of the SAMFC-1000Ω (5.5 mA) and SAMFC-OC (0.3 mA), respectively. These results indicate that the biofilms enrich at the bioanodes of the SAMFC-20Ω possesses higher bioelectrocatalytic activity than that of the SAMFC-1000Ω and SAMFC-OC. It has been reported that the bioelectrocatalytic activity of exoelectrogens at the anode is highly associated with the anode potential and current density (Aelterman, Freguia, Keller, Verstraete, & Rabaey, 2008; Zhao & Chen, 2018). Higher bioelectrocatalytic activity could be obtained at comparatively more positive anode potential and higher current density (Aelterman et al., 2008; Hong, Call, Werner, & Logan, 2011; Jiang et al., 2018), because electron transfer from bacteria to anode is accelerated substantially when SAMFC operated under lower external resistances (Zhu, Tokash, Hong, & Logan, 2013). In this study, the SAMFC-20Ω could deliver comparatively more positive anode potential and higher current density than the SAMFC-1000Ω and SAMFC-OC, thereby a much higher anodic electrocatalytic activity of exoelectrogen biofilms are enriched the bioanodes. Furthermore, the high current density in the SAMFC-20Ω enable anode-respiring bacteria to outcompete aerobic heterotrophs for substrate to release much more electrons (Ren, Zhang, He, & Logan, 2014), thus SAMFC operated under 20Ω is an efficient means to enrich exoelectrogens. The CVs in Figure 3A2 show that the exoelectrogens enriched in medium containing only acetate substrate could also catalyze BEDN, which is in accordance to that of previous reports (K. Y. Cheng et al., 2012) .

When the nitrate is supplied to the SAMFCs accompanying with the acetate, denitrifers are enriched at the bioanodes after acclimating for a period time. The CVs of the anodophilic biofilms at the 20$^{\text{th}}$ day are performed in the PBS medium containing only nitrate and shown in Figure 3B2. They all display a negative sigmoidal shape which is extremely accordance with that in ref. (Pous et al., 2016) , proving that anodophilic biofilms could successfully catalyze nitrate reduction (K. Y. Cheng et al., 2012). Notably, the CVs of the SAMFC-20Ω anode display a more negative bioelectrocatalytic reduction current response of-1.15 mA, which is four and eight times as that of the SAMFC-1000Ω (-0.28 mA) and SAMFC-OC (-0.13 mA) bioanodes, respectively, representing a higher BEDN performance. The CVs of the anodophilic biofilms at the 20$^{\text{th}}$ day shown in Figure 2B1 display typical positive sigmoidal shape but with increased bioelectrocatalytic oxidation peak current of 11.5, 6.6 and 1.3 mA for the SAMFC-20Ω, SAMFC-1000Ω and SAMFC-OC, respectively, indicating that the bioelectrocatalytic activity of exoelectrogens at the anodes is enhanced even in the presence of denitrifers, indicating the good coexistence of exoelectrogens and denitrifers. Moreover, a higher negative denitrification current is observed for all the SAMFCs at the 100$^{\text{th}}$ day (Figure 3C2), demonstrating an increase of BEDN performance with increase of acclimation time. In addition, the enrichment of denitrifers at the anode result in a distinctive "duck head" shape (Figure 3B1 and 3C1) in the CVs of the SAMFC-20Ω anodes measured in the presence of acetate. The CVs measured under non-turnover conditions (absence of substrate) can further characterize the electrochemical features and extracellular electron transfer (EET) modes of the biofilm. The non-turnover CVs of the anodophilic biofilm acclimated by feeding media containing acetate shown in Figure 3A3 present two major clear redox systems with the formal potential ranged from -0.260 to -0.350 V, which is reported to be related to membrane bound c-type cytochromes and also highly consistent with the CV characteristic that described in the literature(Fricke, Harnisch, & Schroder, 2008; F. Harnisch & Freguia, 2012). The two major redox systems at the formal potentials of -0.256 V and -0.360 V in SAMFC-20Ω are basically equal to that of -0.260 V and -0.350 V in SAMFC-1000Ω as well as -0.263 V and -0.347 V in SAMFC-OC. The similar two major clear redox systems with the formal potential ranged from 0.235 to 0.370 V can also be observed in the non-turnover CVs of anodophilic biofilm acclimated by feeding media containing both acetate and nitrate (Figure 3B3 and 3C3), hinting that the bioelectrochemical features and EET modes of the exoelectrogen biofilms is preserved even in the presence of denitrifers.

3.3 Denitrification performance of SAMFCs

The denitrification performance of the SAMFC-20Ω, SAMFC-1000 and SAMFC-20Ω at different stages is investigated. The C/N ratio of the feeding media is firstly optimized by feeding PBS containing 1.64 g L^{-1} acetate but different nitrate concentration. Figure S2 shows that when the C/N ratio is over 2.4, the SAMFCs achieved the optimum denitrification performance, which was basically in accordance well with the value of 2.2 reported in literature (Dhamole, Nair, D'Souza, Pandit, & Lele, 2015). The denitrification performance tests in following are based on the media with a C/N ratio of 2.4. Figure 4 shows that all the SAMFCs display comparatively poor denitrification performance at initial stage. But a gradual enhancement of denitrification performance was observed along with the rise of acclimation time. For example, In SAMFC-20Ω, the initial nitrate concentration of 200 mg $L^{-1}NO_3^-$-N drops to 127, 110, 95, 66 and 56 mg L^{-1} at the 20th, 40th, 60th, 80thand 100th day, respectively, in one hour (Figure 4A1, B1, C1, D1 and E1). The decrease of NO_3^--N concentration from 200 mg L^{-1} to lower than 30 mg L^{-1} takes about 4h at the 20th, 40th day, but 3h at the 80th and 100th day. The similar NO_3^--N concentration variation trend could also be observed in SAMFC-1000Ω and SAMFC-OC. This gradual enhancement of denitrification rate with the rise of acclimation time in all the SAMFCs is probably ascribed to the gradual maturation of denitrifying biofilms at the anodes which could find the proof from microbial community analysis results in Figure S4.

As shown in Figure 4A2, the SAMFC-20Ω possess a lowk_{DN} of 1.07, which is lower than that of the SAMFC-1000Ω (of 1.26) at the 20th day. However, after acclimation for another 20 days, the k_{DN} of the SAMFC-20Ω increases greatly and is up to 1.57, which surpass that of the SAMFC-1000 of about 1.28 at the 40th day (Figure 4B2). Moreover, Figure 4B2, C2, D2 and E2 reveal that the denitrification rate of the SAMFC-20Ω outperforms that of the SAMFC-1000Ω and SAMFC-OC from the 40th day and achieves a stable denitrification rate with a high k_{DN} of 1.9 after acclimation for 80 days, which is much higher than that of SAMFC-1000Ω and SAMFC-OC with similar k_{DN} of 1.3. The slightly lowerk_{DN} in SAMFC-20Ω at the 20thday compared with that in SAMFC-1000Ω is probably due to the competition of electrons between the denitrification of the denitrifers in the anode and oxygen reduction (ORR) in the air-cathode. In the primary stage of operation, the electrons released by the exoelectrogens at the bioanodes are tend to be accepted by the air-cathode for ORR in the SAMFC-20Ω due to the higher electrode potential than that in the SAMFC-1000Ω, thus shows slightly lower denitrification rate. The SAMFC-20Ω facilitates the accumulation of exoelectrogens which usually are capable of bidirectional electron transfer including release electrons by oxidation of acetate, and accept electrons by reduction of oxygen and denitrification. Therefore, the exoelectrogens can enhance the denitrification performance of SAMFC by (a) direct bioelectrochemical denitrification and (b) promoting the accumulation of denitrifers through catalyzing oxygen reduction to remove the trace oxygen diffused from air-cathode.

3.4 Microbial community analysis

The sum of the relative abundance of the denitrifers at the anodes of SAMFCs is summarized in table S3 and displayed in Figure 5. A great increase on the total abundance of denitrifers can be observed during the 100 days acclimation. The total relative abundance of denitrifers increases from 41.6±6.9%, 42.4±9.2% and 32.6±9.4% for the SAMFC-20Ω, SAMFC-1000Ω and SAMFC-OC at the 20^{th} day, to 65.5±9.8%, 48.3±10.1% and 48.9±8.2% for the SAMFC-20Ω, SAMFC-1000Ω and SAMFC-OC at the 100^{th} day, respectively. In fact, exoelectrogens with the feature of bidirectional electron transfer such as *Geobacter* , *Pseudomonas* and *Comamonas* are the contributor for BEDN, they can not only release electrons by the organic oxidation, but also enable denitrification by accepting electrons from electrode (Gregory, Bond, & Lovley, 2004; Yu, Wu, Cao, Gao, & Yan, 2015). It has to be noted that *Geobacter* in the anodes of SAMFC-20Ω and SAMFC-1000Ω with relative abundance of 4.2% and 2.1% at the initial stage survive in the denitrification process and remains 2.5% and 0.6% at the 100^{th} day, respectively.*Pseudomonas* in the anode of SAMFC-20Ω and SAMFC-1000Ω with the relative abundance of 2.2% and 1.5% at the initial stage is preserved during the 100 days acclimation, and became 2.3% and 1.3% at the 100th day, respectively. A high relative abundance of 10% *Pseudomonas*is enriched in the SAMFC-OC at the initial stage, but could not be detected at the 100^{th} day. The successful inheritance of *Geobacter* and *Pseudomonas* from anterior biofilm indicated that exoelectrogens could coexist well with denitrifers during denitrification process despite a tiny drop of the relative abundance was observed. In addition, *Comamonas* that has already existed in acetate-acclimated biofilm is typical exoelectrogens equipped with the ability of denitrification (H. Huang et al., 2019; Liang et al., 2019). The *Comamonas* at the initial stage has a low relative abundance of 1~2% in all SAMFCs, its proportion in the SAMFC-20Ω rises remarkably to 14.0% after acclimation for 100 days in the presence of nitrate, but there is no obvious change in the SAMFC-1000Ω and SAMFC-OC. Thus, the notable enrichment of *Comamonas* in SAMFC-20Ω might highly contribute to the consumption of nitrate. Most critically, the sum of relative abundance for *Geobacter* ,*Pseudomonas* and *Comamonas* at the 100th day is 18.9% in SAMFC-20Ω, which is three times higher than that in SAMFC-1000Ω (5.7%) and nearly five times higher than that in SAMFC-OC (3.8%). The high relative abundance of exoelectrogens in the anodophilic biofilms is considered to be the key factor to endow the SAMFC-20Ω with excellent denitrification performance. As shown in Figure S4, in one aspect the exoelectrogens can directly catalyze the bioelectrochemical denitrification, in another aspect the exoelectrogens are also able to catalyze oxygen reduction reaction, thus they could consume the trace oxygen diffused from the air-cathode and thus promote the propagation of denitrifers, e.g. *Alicycliphilu* s. That is why a high relative abundance of *Alicycliphilu* s (18.2±1.8 %) could be observed in the SAMFC-20Ω at the 100^{th} day (as shown in Figure 4), which is much higher than that in the SAMFC-1000 (9.8±1.9%) and SAMFC-OC (5.5±2.1%).

4. CONCLUSION

The impact of anodophilic biofilms bioelectroactivity on denitrification behavior of single-chamber air-cathode microbial fuel cell (SAMFC) in steady state was studied. Results showed that the denitrification rates of the SAMFCs increased with time and achieved a stable level after running for about 80 days. The SAMFC-20Ω showed lower denitrification rate than the SAMFC-1000Ω at the 20^{th} day, but surpassed it after running for over 40 days. The SAMFC-20Ω not only generated 61.7% higher power than the SAMFC-20Ω, but also achieved a 50% and 40% higher k_{DN} than the SAMFC-1000Ω and SAMFC-OC, respectively. The enhanced power generation and denitrification performance was attributed to the high bioelectroactivity of the anodophilic biofilms, which not only promote current generation, but also improved BEDN facilitated the enrichment of other denitrifers. This study provides an important theoretical basis for enhancing power generation and denitrification performance of SAMFC synchronously, would promote its practical application for wastewater treatment.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

REFERENCE

Aelterman, P., Freguia, S., Keller, J., Verstraete, W., & Rabaey, K. (2008). The anode potential regulates bacterial activity in microbial fuel cells. *Appl Microbiol Biotechnol, 78* (3), 409-418. doi:10.1007/s00253-007-1327-8

Borole, A. P., Reguera, G., Ringeisen, B., Wang, Z.-W., Feng, Y., & Kim, B. H. (2011). Electroactive biofilms: Current status and future research needs. *Energy & Environmental Science, 4* (12), 4813-4834. doi:10.1039/c1ee02511b

Chen, S., Brown, R. K., Patil, S. A., Huber, K. J., Overmann, J., & Schröder, U. (2019). Aerobic microbial electrochemical technology based on the coexistence and interactions of aerobes and exoelectrogens for synergistic pollutant removal from wastewater. *Environmental Science: Water Research & Technology, 5* (1), 60-69. doi:10.1039/c8ew00530c

Cheng, K. Y., Ginige, M. P., & Kaksonen, A. H. (2012). Ano-cathodophilic biofilm catalyzes both anodic carbon oxidation and cathodic denitrification. *Environ Sci Technol, 46* (18), 10372-10378. doi:10.1021/es3025066

Cheng, K. Y., Ho, G., & Cord-Ruwisch, R. (2010). Anodophilic Biofilm Catalyzes Cathodic Oxygen Reduction. *Environmental Science & Technology, 44* (1), 518-525. doi:10.1021/es9023833

Clauwaert, P., Rabaey, K., Aelterman, P., De Schamphelaire, L., Pham, T. H., Boeckx, P., . . . Verstraete, W. (2007). Biological Denitrification in Microbial Fuel Cells. *Environmental Science & Technology, 41* (9), 3354-3360. doi:10.1021/es062580r

Dhamole, P. B., Nair, R. R., D'Souza, S. F., Pandit, A. B., & Lele, S. S. (2015). Denitrification of high strength nitrate waste from a nuclear industry using acclimatized biomass in a pilot scale reactor. *Appl Biochem Biotechnol, 175* (2), 748-756. doi:10.1007/s12010-014-1317-0

Fricke, K., Harnisch, F., & Schroder, U. (2008). On the use of cyclic voltammetry for the study of anodic electron transfer in microbial fuel cells. *Energy & Environmental Science, 1* (1), 144-147.

Gregory, K. B., Bond, D. R., & Lovley, D. R. (2004). Graphite electrodes as electron donors for anaerobic respiration. *Environ Microbiol, 6* (6), 596-604. doi:10.1111/j.1462-2920.2004.00593.x

Harnisch, F., & Freguia, S. (2012). A basic tutorial on cyclic voltammetry for the investigation of electroactive microbial biofilms.*Chem Asian J, 7* (3), 466-475. doi:10.1002/asia.201100740

Harnisch, F., & Schroder, U. (2010). From MFC to MXC: chemical and biological cathodes and their potential for microbial bioelectrochemical systems. *Chemical Society Reviews, 39* (11), 4433-4448.

Hong, Y., Call, D. F., Werner, C. M., & Logan, B. E. (2011). Adaptation to high current using low external resistances eliminates power overshoot in microbial fuel cells. *Biosens Bioelectron, 28* (1), 71-76. doi:10.1016/j.bios.2011.06.045

Huang, H., Cheng, S., Li, F., Mao, Z., Lin, Z., & Cen, K. (2019). Enhancement of the denitrification activity by exoelectrogens in single-chamber air cathode microbial fuel cells. *Chemosphere, 225* , 548-556. doi:10.1016/j.chemosphere.2019.03.052

Huang, H., Cheng, S., Yang, J., Li, C., Sun, Y., & Cen, K. (2018). Effect of nitrate on electricity generation in single-chamber air cathode microbial fuel cells. *Chemical Engineering Journal, 337* , 661-670. doi:10.1016/j.cej.2017.12.150

Jiang, X., Ying, D., Ye, D., Zhang, R., Guo, Q., Wang, Y., & Jia, J. (2018). Electrochemical study of enhanced nitrate removal in wastewater treatment using biofilm electrode. *Bioresour Technol, 252* , 134-142. doi:10.1016/j.biortech.2017.12.078

Li, W.-W., Yu, H.-Q., & He, Z. (2014). Towards sustainable wastewater treatment by using microbial fuel cells-centered technologies. *Energy & Environmental Science, 7* (3), 911-924.

Liang, D., He, W., Li, C., Yu, Y., Zhang, Z., Ren, N., & Feng, Y. (2019). Bidirectional electron transfer biofilm assisted complete bioelectrochemical denitrification process. *Chemical Engineering Journal, 375* , 121960. doi:10.1016/j.cej.2019.121960

Liu, Q., Chen, S., Zhou, Y., Zheng, S., Hou, H., & Zhao, F. (2014). Phosphorus-doped carbon derived from cellulose phosphate as efficient catalyst for air-cathode in microbial fuel cells. *Journal of Power Sources, 261* , 245-248. doi:10.1016/j.jpowsour.2014.03.060

Logan, B. E. (2009). Exoelectrogenic bacteria that power microbial fuel cells. *Nature Reviews Microbiology, 7* (5), 375-381. doi:Doi 10.1038/Nrmicro2113

Lovley, D. R. (2012). Electromicrobiology. *Annual Review of Microbiology, 66* (1), null. doi:doi:10.1146/annurev-micro-092611-150104

Patureau, D., Zumstein, E., Delgenes, J. P., & Moletta, R. (2000). Aerobic Denitrifiers Isolated from Diverse Natural and Managed Ecosystems. *Microb Ecol, 39* (2), 145-152.

Pous, N., Carmona-Martinez, A. A., Vilajeliu-Pons, A., Fiset, E., Baneras, L., Trably, E., . . . Puig, S. (2016). Bidirectional microbial electron transfer: Switching an acetate oxidizing biofilm to nitrate reducing conditions. *Biosens Bioelectron, 75* , 352-358. doi:10.1016/j.bios.2015.08.035

Rabaey, K., & Verstraete, W. (2005). Microbial fuel cells: novel biotechnology for energy generation. *TRENDS in Biotechnology, 23*(6), 291-298.

Ren, L., Zhang, X., He, W., & Logan, B. (2014). High Current Densities Enable Exoelectrogens to Outcompete Aerobic Heterotrophs for Substrate. *Biotechnology and Bioengineering, 111* . doi:10.1002/bit.25290

Sukkasem, C., Xu, S., Park, S., Boonsawang, P., & Liu, H. (2008). Effect of nitrate on the performance of single chamber air cathode microbial fuel cells. *Water Res, 42* (19), 4743-4750. doi:10.1016/j.watres.2008.08.029

Yu, Y., Wu, Y., Cao, B., Gao, Y.-G., & Yan, X. (2015). Adjustable bidirectional extracellular electron transfer between Comamonas testosteroni biofilms and electrode via distinct electron mediators. *Electrochemistry Communications, 59* , 43-47. doi:10.1016/j.elecom.2015.07.007

Zhang, J., Zheng, P., Zhang, M., Chen, H., Chen, T., Xie, Z., . . . Abbas, G. (2013). Kinetics of substrate degradation and electricity generation in anodic denitrification microbial fuel cell (AD-MFC). *Bioresour Technol, 149* , 44-50. doi:10.1016/j.biortech.2013.09.043

Zhao, W., & Chen, S. (2018). Critical parameters selection in polarization behavior analysis of microbial fuel cells. *Bioresource Technology Reports, 3* , 185-190. doi:10.1016/j.biteb.2018.07.010

Zhu, X., Tokash, J. C., Hong, Y., & Logan, B. E. (2013). Controlling the occurrence of power overshoot by adapting microbial fuel cells to high anode potentials. *Bioelectrochemistry, 90* , 30-35. doi:10.1016/j.bioelechem.2012.10.004

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