Response of antimicrobial nitrofurazone-degrading biocathode communities to different cathode potentials

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HIGHLIGHTS

- NFZ bioelectrodegradation degree was related to different cathode potentials.
- Different cathode potentials enriched specific biocathode communities respectively.
- Dominant genera possess nitroaromatics reduction and electron transfer functions.
- Higher potential modes enriched a Gram negative nitroaromatic reducer *Klebsiella*.
- Lower potential mode enriched a Gram positive nitroaromatic reducer *Enterococcus*.

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ABSTRACT

Bioelectrodegradation of various organic pollutants has been extensively studied. However, whether different cathode potentials could alter the antimicrobial-degrading biocathode community structure and composition remain poorly understood. Here, the microbial community structure and composition of the nitrofurans nitrofurazone (NFZ) degrading biocathode in response to different cathode potentials (-0.45 ± 0.01 , -0.65 ± 0.01 and -0.86 ± 0.05 V vs standard hydrogen electrode, with applied cell voltages of 0.2, 0.5 and 0.8 V, respectively) were investigated. The bioelectrodegradation efficiency and degree of NFZ were highly related to different cathode potentials. The 0.2 and 0.5 V performed biocathode communities were similar but significantly differed from those of the 0.8 V and open circuit biofilms. The bacteria possessing functions of nitroaromatics reduction and electrons transfer (e.g. *Klebsiella*, *Enterococcus*, *Citrobacter* and *Desulfovibrio*) were selectively enriched in different biocathode communities. This study offers new insights into the ecological response of antimicrobial-degrading biocathode communities to different cathode potentials.

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1. Introduction

Antimicrobial agents have led to serious pollution of various environmental settings globally, including surface water and groundwater, estuary sediment and soils, for excessively abused in humans, domestic animals, and aquaculture (Justino et al., 2016; Van Boeckel et al., 2015; Yun et al., 2017b; Zhang et al., 2015). Nitrofurazone (5-nitro-2-furaldehyde semicarbazone, NFZ) is a broad-spectrum antimicrobial agent that belongs to nitrofurans. Due to its potential carcinogenicity, genotoxicity and mutagenicity to human health, the European Union and U.S. Food and

Drug Administration have banned the utility of NFZ in food-producing animals. However, in some developing countries including China, it is still widely used in the livestock and fishery aquaculture owing to its low production cost, easy availability and high efficiency (Hong et al., 2015; Kwon, 2017; Vass et al., 2008). As a result, NFZ residues and its toxic biological metabolite semicarbazide are often detected in diverse aquatic species and products as well as aquaculture pond water and sediments (Vass et al., 2008; Yu et al., 2013). In order to eliminate the ecological impact of NFZ on water environment, the efficient degradation of NFZ and elimination of its antibacterial activity are very important during livestock wastewater treatment and aquaculture pond water remediation. Thus, NFZ was chosen as the model nitrofurans in this study.

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Bioelectrochemical systems (BES), employing electrochemically active microorganisms to catalyze oxidative or reductive reactions in the anode or cathode respectively, have attracted a considerable amount of attentions in recent decades (Pandey et al., 2016; Pant et al., 2010; Wang et al., 2015; Yun et al., 2017a), especially its merits on the bioelectrodegradation of various organic pollutants including nitro-, azo-, halo-aromatics and antimicrobial agents based on the microbial electrode-respiration process (Cui et al., 2014, 2016; Feng et al., 2015; Gao et al., 2016; Jiang et al., 2016a; Li et al., 2016; Liang et al., 2013; Sun et al., 2013; Wang et al., 2011, 2016; Zhang et al., 2017). Generally, operational factors could impact the catalytic efficiency and functioning stability of abiotic cathode/biocathode reactors (e.g. temperature, pH, buffer solution type and concentration, targeted pollutants concentration and analogue, co-substrate addition, and electrode potential) (Jiang et al., 2016a,b; Kong et al., 2014, 2015b; Liang et al., 2014, 2016; Shen et al., 2013; Yun et al., 2016, 2017a). Our previous studies have indicated that nitrofurans derivatives NFZ and furazolidone could be efficiently reduced by the abiotic cathode under different cathode potentials (−0.15 to −1.25 V vs standard hydrogen electrode, SHE). Moreover, different cathode potentials significantly affected the NFZ and furazolidone degradation efficiency and the elimination of degradation products (Kong et al., 2015a,b). However, whether the acclimated biocathode communities could enhance NFZ degradation has not yet been reported. Our recent studies have shown that the polarity inversion (decrease electrode potential from −0.2 to −0.45 or −0.40 V) could significantly alter the bioanode community structure and composition (Yun et al., 2016, 2017a). Interestingly, a previous study has shown that different anode potentials (from −0.25 to 0.81 V vs SHE) did not affect bioanode community structure and composition (Zhu et al., 2014). However, whether the lowered cathode potentials (below −0.45 V vs SHE) could alter the functional biocathode community structure and composition and whether different cathode potentials could selectively enrich distinct electroactive nitroaromatic reducers remain poorly understood.

This study investigated the effect of different cathode potentials on the bioelectrodegradation efficiency and degree of NFZ in a dual-chamber BES with biocathode. The NFZ bioelectrodegradation characterization, products formation dynamics, and biocathode communities diversity, structure and composition were studied. The objectives of this study were to (I) determine the impact of different cathode potentials on the bioelectrodegradation of NFZ, formation and further elimination of corresponding degradation products and (II) elucidate the ecological response of the NFZ-degrading biocathode communities to different cathode potentials. Our results indicated that the bioelectrodegradation efficiency and degree of NFZ were highly related to different cathode potentials, and lower and higher cathode potentials enriched different electroactive nitroaromatic reducers respectively.

2. Materials and methods

2.1. BES reactor setup

The BES reactors were constructed by assembling two equal-sized Lexan plates ($4 \times 4 \times 3 \text{ cm}^3$) with a cylindrical cavity (3 cm in diameter and 4 cm in length) and two equal-sized Lexan plates ($4 \times 4 \times 1 \text{ cm}^3$) as outside baffles and separated by a cation exchange membrane (Ultrex CMI-7000, Membranes International, Ringwood, NJ, U.S.) as described elsewhere (Kong et al., 2015a). The internal volume of each chamber was 28 mL. Graphite fiber brush (2.5 cm in diameter and 2.5 cm in length, TOHO TENAX Co., Ltd., Japan) was used as both anode and cathode. A 10 Ω resistor with the external power (0.5 V) in series was employed for the

connection. A saturated calomel reference electrode (SCE, 0.247 V vs SHE, model-217, Shanghai Precise. Sci. Instrument Co., Ltd., China) was inserted into the cathode chamber. All the potential data showed herein were against SHE. The open circuit control was also carried out in the same reactor by directly inoculating NFZ-reducing consortium under non-electrochemical anaerobic condition (the external power source and the resistor were disconnected).

2.2. Microbial inoculum and BES tests

Three biocathodes were parallel established with applied cell voltage of 0.5 V and glucose (additional carbon and electron sources) supply. In order to determine the effect of different cathode potentials on the bioelectrodegradation efficiency and degree of NFZ, two of the three established biocathodes were switched to supply the additional voltages of 0.2 and 0.8 V. While one biocathode still maintained 0.5 V supply. An open circuit and a 0.8 V-supplied abiotic cathode BES reactors both worked as controls to assess the contribution of pure microbial or electrochemical degradation of NFZ respectively. These reactors were operated for 6 fed-batch cycles (48 h for each cycle). Then the three biocathode BESs were run without the additional electron donor glucose (with 10 mM NaHCO_3 instead) for another 3 cycles. For the glucose-fed biocathode mode, NFZ (50 mg/L), glucose (600 mg/L) and cathode were the cathodic electron acceptor, intracellular and extracellular electron donors for cathodophilic microbes, respectively. For the NaHCO_3 -fed biocathode mode, inorganic NaHCO_3 and electrode served as carbon source and electron donor for cathodophilic microbes, respectively. All tests were carried out at room temperature ($23 \pm 3^\circ \text{C}$).

Concretely, anodic and cathodic biofilms were enriched by inoculating municipal sludge from a wastewater treatment plant (Harbin, China) and acclimated NFZ-reducing consortium, respectively as described elsewhere (Liang et al., 2013; Wang et al., 2011). Cathode biofilms were established by inoculating NFZ-reducing consortium three times into the BES reactor. For the NFZ-reducing consortium enrichment, wastewater and sludge samples (about 5 mL) from a wastewater treatment plant (Shijiazhuang, China) were mixed with 70 mL anaerobic medium, which was prepared by boiling and purged with N_2 gas (99.9% purity) for 15 min. The medium composition was described previously but amended with NFZ (50 mg/L) and glucose (600 mg/L) (Wang et al., 2011). The mixed culture was incubated in a rotary shaker at 160 rpm and 25°C for 4 d, and then was transferred to a fresh medium (70 mL) with 10% dilution for another 4 d. This procedure was repeated six times. Finally, the NFZ acclimated consortium is capable of completely reducing NFZ to the antibacterial inactive products within 72 h.

2.3. Chemicals and analytical methods

NFZ (98% purity) was purchased from Aladdin (Shanghai, China). HPLC grade methanol was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade.

All samples taken from the cathode chamber within 72 h were filtered through 0.22 μm filters (Shanghai XingYa Purification Material Co., China). The concentrations of NFZ and its three main products, [(5-hydroxyamino-2-furyl)-methyl]-hydrazinecarboxamide (AMH), [(5-amino-2-furyl)-methylene]-hydrazinecarboxamide (AMN) and N-N fraction product (5-nitro-2-furyl) methanamine (NFF) were measured using a reverse-phase HPLC system with a UV detector (model e2695, Waters Co., Milford, MA, USA) (Kong et al., 2015a). The NFZ reduction products were identified by a HPLC-QTOFMS as described recently (Kong et al., 2015a).

The standard samples for NFZ degradation products NFF, AMN, and AMH are not available commercially. Therefore, we used the HPLC peak area to describe the concentration or mass change of these products during NFZ degradation. The rate constant of NFZ reduction (k_{NFZ}) was calculated by fitting the data to apparent first-order reaction models: $C = C_0 e^{-kt}$ and $C = C_0 (1 - e^{-kt})$, where C represents the NFZ (mg/L) concentration at time t (h) and C_0 is the initial NFZ concentration (50 mg/L). The electric current (I) was calculated by the Ohm's law. NFZ reduction efficiency ($E_{\text{r-NFZ}}$, %) was evaluated based on the concentration difference between influent and effluent. Differences between treatments were statistically analyzed by the two-tailed unpaired t -test.

2.4. Electrochemical and morphological analysis

To characterize the bioelectrochemical activity of biocathode communities under different cathode potentials, CV analysis was performed using an electrochemical workstation (model-660D, CH Instruments Inc., Austin, TX, U.S.) as described previously (Liang et al., 2013). The electrochemical impedance spectroscopy (EIS) test was carried out over a frequency range of 0.01–10⁵ Hz and amplitude of 5 mV (model-660D, CH Instruments Inc., Austin, TX, U.S.) for the analysis of the polarization internal resistance of biocathode communities under different cathode potentials. In order to exclude the additional effect of glucose on the biocathodic metabolism, in the CV and EIS experiments, inorganic NaHCO₃ was selected as carbon source and electrode as electron donor. For the uniformity and stability of the experimental conditions, the second cycle of the scanned cyclic voltammograms were shown. The concentration of NFZ and NaHCO₃ were 50 mg/L and 10 mM, respectively in the CV and EIS analysis at 25 °C. The cathodic biofilms were observed by scanning electron microscopy (SEM) (HELIOS Nanolab 600i, Hillsboro, OR, USA) as described elsewhere (Wang et al., 2012).

2.5. 16S rRNA gene sequencing and data analysis

A detailed description of DNA extraction, DNA purity and quantity determination, PCR amplification, 16S rRNA gene Illumina MiSeq sequencing and data analysis was provided in our previous study (Cheng et al., 2015; Liang et al., 2016). The raw data from 16S rRNA gene sequencing was analyzed according to a recent study (Qu et al., 2015). Microbial diversity indices including Chao1 value, richness (identified OTU numbers in each sample), Shannon-Weaver index (H), Simpson reciprocal ($1/D$) and Pielou evenness were calculated as described recently (Liang et al., 2016; Schloss et al., 2009). For indices, higher number represents higher diversity. Hierarchical clustering analysis was performed using CLUSTER v3.0 and visualized by TREEVIEW.

3. Results and discussion

3.1. NFZ-reducing consortium capability

After the 6th transfer, the NFZ-reducing capability of the acclimated consortium was determined. CV analysis preliminarily indicated that almost no reductive current was recorded for the enrichment within 72 h, which was quite close to the cyclic voltammogram of PBS control. In contrast, NFZ containing medium (50 mg/L) showed three obvious reduction peaks those were consistent with our previous study (Kong et al., 2015a). CV result indirectly showed the added NFZ could be efficiently reduced by the acclimated NFZ-reducing consortium (Fig. S1A). Further HPLC analysis demonstrated the acclimated consortium is able to reduce NFZ (50 mg/L) to three main products of NFZ reduction (Fig. S1B). Inter-

estingly, these three main products (retention time at 3.2 min, 3.5 min and 3.6 min respectively) were the same as the NFZ reduction products from the pure electrochemical process. They were preliminarily identified as NFF, AMN and AMH (Kong et al., 2015a). These results suggest that microbial nitroreductase and N-N bond reductase catalyze the reduction of NFZ.

3.2. Bioelectrodegradation of NFZ under different operational conditions

After inoculating the NFZ-reducing consortium for three times, cathodic biofilms were established after 196 h. During the biofilm establishment process, the cathode potential kept between −0.75 and −0.80 V with the applied cell voltage of 0.5 V and currents of the three reactors obviously increased over the first cycle. After 3 cycles, the current increased from approximately 0.2 to 0.3 mA, suggesting that the electroactive NFZ-degrading cathodic biofilm was preliminarily established (Fig. 1a). The current and cathode potential of the established biocathodes changed obviously after applying different cell voltages (0.2, 0.5 and 0.8 V). As a result, the 0.2, 0.5 and 0.8 V groups achieved the mean cathode potential of -0.45 ± 0.01 , -0.65 ± 0.01 and -0.86 ± 0.05 V vs SHE, respectively. The 0.8 V-supplied biocathode showed the highest current among the three biocathode groups, indicating that NFZ could be more efficiently reduced under a lower cathode potential (Fig. 1b). With NaHCO₃ and cathode as carbon source and electron donor respectively, the variation trends of cathode potential and current were similar to those with glucose as carbon source and additional electron donor. The higher applied voltage resulted in lower cathode potential and higher corresponding reaction current (Fig. 1c). This indicated that the use of NaHCO₃, an inorganic carbon source instead of glucose, did not change the reactor performance significantly.

When the applied cell voltage was 0.2 V, the reduction rate constant k and reduction efficiency at 1 h (E_{r1h}) of NFZ were $0.677 \pm 0.069 \text{ h}^{-1}$ and $42.25 \pm 1.35\%$ with glucose addition. When the applied cell voltage was increased to 0.8 V, the cathode potential was approximately −0.9 V, and the k and E_{r1h} values increased to $1.202 \pm 0.124 \text{ h}^{-1}$ and $70.60 \pm 4.21\%$, respectively. However, the k and E_{r1h} values of the abiotic cathode control (0.8 V supply) were $0.825 \pm 0.024 \text{ h}^{-1}$ and $53.82 \pm 1.80\%$, respectively, and the corresponding values were $0.457 \pm 0.032 \text{ h}^{-1}$ and $16.19 \pm 2.23\%$ (Table 1) for the open circuit control. Therefore, compared to the open circuit and abiotic cathode controls, the 0.8 V-supplied biocathode can significantly improve the reduction rate and efficiency of NFZ ($P \leq 0.03$).

In order to exclude the additional effect of glucose on the biocathodic metabolism, the inorganic NaHCO₃ worked as carbon source and electrode as electron donor were investigated. Similarly, with the increase of the applied voltage, k and E_{r1h} values accordingly increased. The NFZ degradation curve of the open circuit control did not accord with the apparent first-order kinetic equation, indicating that NFZ was difficult to be biodegraded without additional electron donor (e.g. glucose or electrode) (Fig. 2b). When the applied cell voltage was 0.2 V, the k and E_{r1h} values were $0.597 \pm 0.084 \text{ h}^{-1}$ and $46.62 \pm 4.95\%$, respectively. When the applied cell voltage was 0.8 V, the k and E_{r1h} values increased to $1.102 \pm 0.045 \text{ h}^{-1}$ and $65.27 \pm 0.13\%$, respectively. The k and E_{r1h} values of the open circuit control were $0.456 \pm 0.032 \text{ h}^{-1}$ and $7.33 \pm 5.43\%$, respectively (Table 1). The k and E_{r1h} values of the three biocathode modes (0.2, 0.5 and 0.8 V) were not significantly differed between the glucose and NaHCO₃ performed groups ($P > 0.05$), in spite that NaHCO₃ performed biocathodes had relatively lower k and E_{r1h} values. Importantly, compared to the abiotic cathode, the NaHCO₃-fed biocathode still showed significant biocatalytic capacity ($P < 0.01$).

Fig. 1. Variation trends of cathode potential and current in the stage of biocathode start up (a) and bioelectrodegradation of NFZ under different applied cell voltages with glucose (b) and NaHCO_3 (c) as carbon sources.

Table 1

The E_{r1h} and k values of NFZ reduction under different conditions.

	Applied voltage/V	Carbon source	$E_{r1h}/\%$	$k \text{ (h}^{-1}\text{)}$	r^2
Biocathode	0.2	Glucose	42.25 ± 1.35	0.677 ± 0.069	0.9906 ± 0.0066
	0.2	NaHCO_3	46.62 ± 4.95	0.597 ± 0.084	0.9948 ± 0.0025
	0.5	glucose	56.69 ± 2.38	0.913 ± 0.049	0.9930 ± 0.0013
	0.5	NaHCO_3	53.44 ± 1.39	0.804 ± 0.055	0.9995 ± 0.0004
	0.8	Glucose	70.60 ± 4.21	1.202 ± 0.124	0.9990 ± 0.0008
	0.8	NaHCO_3	65.27 ± 0.14	1.102 ± 0.045	0.9997 ± 0.0002
Abiotic cathode	0.8	–	53.82 ± 1.80	0.825 ± 0.024	0.9968 ± 0.0012
Open circuit	–	Glucose	16.19 ± 2.23	0.456 ± 0.032	0.9648 ± 0.0019

3.3. Formation of NFZ reduction products under different operational conditions

The degradation characterization and pathway of NFZ with the abiotic cathode under different cathode potentials had reported in our previous study. Though the degradation rate, efficiency and degree of NFZ were obviously affected by different cathode potentials, three mainly identified degradation products including AMH, AMN and NFF were consistent (Kong et al., 2015a). In this study, the three products of both the biocathode and open circuit biofilms were the same as previously identified based on the HPLC peak sequence and retention time.

The effect of different applied cell voltages on the formation of NFZ degradation products by the glucose-fed biocathodes was shown in Fig. 2. When NFF was accumulated to the maximum, it was further reduced to (5-amino-2-furan) methylamine (Fig. 2c). Importantly, the formation of NFF strongly suggests that the reduction of N–N and C–N bonds in NFZ could avoid the production of the high toxic biological metabolite of NFZ, semicarbazide (Kwon, 2017). Instead of semicarbazide, the organic nitrogen source urea could be generated during the NFZ degradation process, which may be used for the cathodophilic microbes. Moreover, the open-loop reactions happened to produce straight chain hydrocarbons (5-hydroxycadaverine and 5-amino-pentanamide) at lower cathode potentials (Kong et al., 2015a), which may be acted as the potential organic carbon source for the cathodophilic microbes. In all the performed modes, AMH showed a tendency to increase first and then decrease (Fig. 2gh). Since it is a transition state of nitro to amine group of NFZ reduction, this intermediate can be rapidly reduced to the amine product, AMN. Apart from the 0.2 V supply condition, AMN showed the tendency to increase first and then decrease, which was further reduced and even open-loop happened (Fig. 2e). Additionally, the higher applied voltage resulted in the faster product generation rate or the subsequent degradation rate. When the applied voltage was 0.8 V, the three products almost disappeared within 48 h, indicating that the intermediate product was faster degraded by ring opening at a lower cathode potential. Importantly, the three products in the abiotic

cathode BES were still markedly accumulated compared to the 0.8 V-supply biocathode within 48 h ($P < 0.01$). The NFF and AMN products increased gradually and accumulated in the open circuit biofilm, strongly indicating that the pure biodegradation process was difficult to destroy the heterocycle product to generate straight chain hydrocarbons (Fig. 2ce).

The effect of different applied voltages on the formation of NFZ degradation products by the NaHCO_3 -fed biocathodes was also shown in Fig. 2. When 0.2 V voltage was provided, the NFF and AMN products were obviously accumulated within 72 h (Fig. 2df), indicating that they were difficult to be further degraded under this condition. When applied with an increased voltage, the overall trends of the three products degradation dynamics were consistent with the glucose-fed biocathodes. Compared with the abiotic cathode, the biocathode still had a faster rate of product formation and further degradation at 0.8 V. This indicated that the cathodophilic microbes had a relatively higher catalytic capacity even with NaHCO_3 as carbon source and electrode as electron donor. The three products of the open circuit experiment were slowly accumulated. Combined with the glucose-fed open circuit experiment, we concluded that NFZ was difficult to be reduced because NaHCO_3 only worked as carbon source but not electron donor (Wang et al., 2011). In addition, microbes could not get electrons from electrode with the circuit disconnected, and thus could not complete the normal microbial respiration. Collectively, the transformation of NFZ to the three main products maintained in the NaHCO_3 -fed biocathode although NFZ reduction rate and efficiency were relatively decreased compared to those of the glucose-fed biocathode. The degradation degree of the NFZ degradation products depended on the cathode potential but the composition of the NFZ degradation products were consistent at different operational conditions.

3.4. CV, EIS, and SEM characterization

The CV results showed that the biofilms had a similar feature and the onset potential was approximately -0.35 V at 0.5 and 0.8 V applied voltages. The peak potential of NFZ reduction was approximately -0.45 , -0.60 and -0.75 V for 0.2, 0.5 and 0.8 V

Fig. 2. Bioelectrodegradation of NFZ and formation dynamics of products (NFF, AMN and AMH) with glucose (aceg) and NaHCO_3 (bdhf) as carbon sources under different operational modes.

supply modes, respectively. The 0.2 V supply group also had a similar onset potential of -0.35 V, while it showed obviously higher peak current than the 0.5 and 0.8 V supply groups (Fig. 3a). This suggests that the biocathode with 0.2 V supply enriched more cathophilic microbe, which likely resulted in the decreased over-potentials. Although the microbial catalytic abilities at 0.5 and 0.8 V were weaker than that at 0.2 V, both of them were larger than that of the abiotic cathode. Additionally, the EIS results showed that the internal resistance of the cathodes at three applied cell voltages (0.2, 0.5 and 0.8 V) was 5.02, 21.94 and 23.15 Ω , respectively, which were significantly lower than that of the abiotic cathode (38.29 Ω) (Fig. 3b). The CV and EIS results together strongly indicate that cathophilic microbes enhanced the biocathodic electron transfer for the catalysis of NFZ. SEM results showed that the biofilms at open circuit, 0.2 and 0.5 V modes relatively enriched more cathophilic microbes than that

at 0.8 V mode, suggesting that the lower cathode potential and strong electrical stimulation could affect the formation of thick cathodic biofilm (Fig. S2).

3.5. Overall microbial phylogenetic diversity

Based on the Illumina high-throughput sequencing results, the number of 16S rRNA gene sequences for different applied voltages (0.2, 0.5 and 0.8 V) and open circuit samples were 105624, 56387, 110194 and 142992, respectively (Table 2). Sufficient sequences strongly indicated the reliability of sequencing results. The Chao1 values from different cathodic biofilms were relatively higher than that of the open circuit biofilm. Other biodiversity indexes, such as H' , $1/D'$ and richness (OTU numbers after resample homogenization) also indicated the open circuit biofilm had the lowest phylogenetic diversity (Table 2). This may be related to the fact that the source of

Fig. 3. Cyclic voltammograms (a) and nyquist plots (b) of three biocathodes and an abiotic cathode for NFZ reduction.

the electron donor in the open circuit biofilm was glucose, while electrode could be worked as the additional electron donor for cathophilic bacteria in biocathode communities.

3.6. Shifts in microbial community structure and composition

Microbial community structures are similar for the three cathodic biofilms but are different from the open circuit biofilm based on the hierarchical clustering analysis of classified OTUs from each sample (Fig. 4a). Concretely, microbial community structures are highly similar for the 0.2 and 0.5 V applied cathodic biofilms. A total of 19 phyla were classified and most of sequences belonged to 5 phyla, including *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Syner-*

gistes and *Actinobacteria* (Fig. 4b). The biofilms from 0.2 and 0.5 V supplied modes and open circuit control obviously enriched *Proteobacteria*, accounting for 84.81, 75.55 and 89.94%, respectively. Interestingly, the lower cathode potential mode (0.8 V supply group) obviously enriched *Firmicutes* (accounting for 60.07%), which was 4.07, 11.38 and 12.09 times higher than those of the 0.5 V group, 0.2 V group and open circuit control, respectively. A total of 45 classes were classified and most of sequences belonged to 9 classes (Fig. 4c). The biofilms from 0.2 and 0.5 V applied modes and open circuit control obviously enriched *Gammaproteobacteria*, accounting for 81.09, 71.52 and 88.23%, respectively. The 0.8 V mode only had 11.85% of *Gammaproteobacteria*, while *Bacilli* and *Actinobacteria* dominated by 56.41 and 21.26%, respectively. These clear differences strongly indicated that different cathode potentials shaped different bacteria communities. The lower cathode potential (0.8 V voltage supply) obviously led to a clear distinction compared to the 0.2 and 0.5 V performed biocathode communities.

3.7. Potential function of dominant genera

A total of 184 genera were classified among the four biofilm samples, of which 12 genera with relative abundance >1% were shown in Fig. 5. *Klebsiella* was dominant in the 0.2 V (accounting for 62.54%) and 0.5 V (accounting for 56.96%) applied groups, while it was obviously decreased to 6.36% in the 0.8 V supply group. Previous studies have demonstrated that *Klebsiella* has nitroaromatics-reducing ability and electrochemical activity (Roldan et al., 2008; Xia et al., 2010). The 0.8 V supply group and open circuit control mainly enriched *Enterococcus* (accounting for 56.34%) and *Pseudomonas* (accounting for 81.82%), respectively. Previous studies have found that *Enterococcus* is often enriched in the nitroaromatic nitrobenzene-reducing biocathode communities, and the relative abundance can reach 38.4% (Liang et al., 2014) or 74.7% (Wang et al., 2011). This was consistent with the fact that *Enterococcus* and *Pseudomonas* species own nitroaromatics-reducing ability (Rafi et al., 2003; Roldan et al., 2008). These results indicated that the microbial community structure with applied electrical stimulation was obviously different from that of the open circuit biofilm. Moreover, higher potential modes enriched a Gram-negative nitroaromatic reducer *Klebsiella*, and lower potential mode enriched a Gram-positive nitroaromatic reducer *Enterococcus*. Additionally, *Citrobacter* had relative abundances of 12.16% and 9.40% at 0.2 V and 0.5 V supply groups, respectively, while its relative abundances for the 0.8 V supply and open circuit modes were 0.61% and 5.80%, respectively. Previous studies have proved that *Citrobacter* is an electroactive nitroaromatic reducer (Liang et al., 2017; Xu and Liu, 2011). A recent study has found that *Citrobacter* was obviously enriched by 29.24% in a glucose-fed azo dye-reducing biocathode community (Sun et al., 2016). Relatively low abundances of *Desulfovibrio* (from 1.63 to 2.04% in the electrode biofilms), *Salmonella* (from 0.14 to 1.17% in the electrode biofilms) and *Clostridium* (4.35% in the open circuit biofilm) were also detected, which have been reported to carry out nitroaromatics reduction by nitroreductase (Roldan et al., 2008; Spain, 1995). Collectively, cathode potential regulation strategy stimulated the microbial electrode respiration activity, and enriched different electroactive nitroaromatic reducers (e.g. *Klebsiella*, *Enterococcus*, *Citrobacter* and *Desulfovibrio*).

Table 2
Microbial diversity indices of biocathode communities under different operational conditions.

	No. of sequences	Chao1	Richness	H	1/D	Pielou evenness
0.2 V supply	105624	378.38	304	1.98	2.92	0.35
0.5 V supply	56387	342.86	270	2.11	3.41	0.38
0.8 V supply	110194	446.13	359	1.90	3.07	0.32
Open circuit	142992	313.97	222	1.32	1.96	0.24

Fig. 4. Hierarchical clustering analysis of identified OTUs from cathodic biofilms under different operational modes. The OTU numbers were logarithmically transformed for the hierarchical cluster analysis. Brighter red coloring indicates higher OTU abundances as shown in the colorbar (a). Taxonomic classification of 16S rRNA gene sequences from microbial communities of the 0.2, 0.5, 0.8 V supply and open circuit modes at phylum (b) and class (c) levels. Relative abundance of each taxon was defined as the percentage of the same taxon to the corresponding total sequences for each sample. Some classes were <1% abundances and unclassified were summarized as others. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 5. The dominant genera of biocathode communities under different operational modes.

respectively. These dominant functional genera likely played critical roles in enhancing the NFZ reduction and electrons transfer.

4. Conclusions

Ecological response of NFZ-degrading biocathode communities to different cathode potentials (0.2, 0.5 and 0.8 V voltage groups) was investigated. Different cathode potentials obviously affected

the bioelectrodegradation efficiency and degree of NFZ and shaped different biocathode communities. The 0.2 and 0.5 V performed biocathode communities were similar (both enriched a Gram-negative nitroaromatic reducer *Klebsiella* >55%) but significantly differed from the 0.8 V supply (enriched a Gram-positive nitroaromatic reducer *Enterococcus* by 56%) and open circuit modes (enriched a Gram-negative nitroaromatic reducer *Pseudomonas* by 82%). The dominant functional genera likely played critical roles in enhancing the NFZ catalysis and electrons transfer.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biortech.2017.06.056>.

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