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Simultaneous perchlorate and nitrate removal coupled with electricity generation in autotrophic denitrifying biocathode microbial fuel cell

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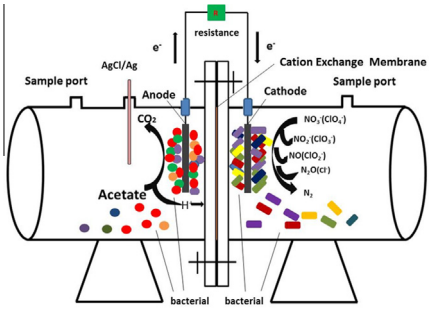
HIGHLIGHTS

- Perchlorate and nitrate was bioreduced on autotrophic denitrifying biocathode.
- Substrate bioreduction and electricity generation reached optimum at NO_3^-/ClO_4^- 1:1.
- High concentration perchlorate inhibited the biological activity of biocathode.
- *Thauera* related to β-*Proteobacteria* was the major genus on biocathodes.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, an autotrophic denitrifying biocathode was investigated to couple the reduction of nitrate or/and perchlorate with electricity generation. Results showed that when the current density in microbial fuel cell (MFC) with sole perchlorate and sole nitrate as the substrate stabilized at 3.00 and 1.52 mA/m^3 respectively, the perchlorate and nitrate removal efficiency achieved 53.14% and 87.05%. As influent molar ratio of NO_3^-/ClO_4^- was 1:1, the stable current density reached the a peak value (3.10 A/m^3) accompanied by the maximum integral mixed substrate removal (40.97% for ClO_4^- and 86.03% for NO_3^-). Open and close circuit experiments demonstrated that the nitrate and perchlorate removal should be ascribed to the bioreduction of autotrophic denitrifying biocathode. Cyclic voltammetry (CV) curves showed that all of biocathodes had strong electrochemical activity and there were few clear distinctions of redox potential between the biocathodes fed with different substrates. Results of 16S rRNA sequencing revealed a predominance of β-*Proteobacteria* in the autotrophic denitrifying biocathode, which is a well-known environmental nitrogen cycler.

1. Introduction

Perchlorate (ClO_4^-) is widely used in production of rocket propellants, highway safety flares, air bag inflators, fireworks and matches [1]. Because of its high solubility, low-reactivity and weak adsorption on minerals, the extensive presence of perchlorate has been detected in ground and surface water, as well as food, vitamins, and drinking water [2]. Perchlorate has been proven to have

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negative impacts on human health, particularly on the thyroid gland, so it is identified as an emerging environmental contaminant [3]. In many perchlorate-contaminated groundwater, nitrate (NO_3^-) is often found with a certain concentration. The nitrate in groundwater mainly comes from agricultural runoff and wastewater discharges [4]. Excessive nitrate in drinking water can cause several health problems such as liver damage and cancer. Meanwhile, nitrite, its immediate reduction product, has more toxicity [5]. Therefore, the maximum contaminant level (MCL) of perchlorate and nitrate in drinking water sources recommended by the US Environmental Protection Agency (EPA) is set as 15 μg/L and 45 mg/L, respectively [6]. Several methods have been explored to remove perchlorate and nitrate, including ion exchange, reverse osmosis, catalysis reduction and bioremediation [7–9]. But the presence of some disadvantages such as concentrated brine disposal, toxic byproducts production, slow process and high cost, limits their applications in practice [10].

Biological electrochemical system (BES), including microbial fuel cell (MFC) and microbial electrolysis cell (MEC), is a promising technology for wastewater treatment. BES provides the potential to directly generate electricity (MFC) or hydrogen (MEC) and simultaneously remove the organic and inorganic contaminants in wastewater with minimal energy requirements [11,12]. In BES, when some specific microorganisms attach to the cathode, the microbial cathode (biocathode) is formed. These microorganisms can accept electrons from a solid surface (cathode) and bioreduce the nitrate, nitrite or perchlorate through biocatalysis [13–15]. Compared with other technologies, biological reduction offers complete conversion of perchlorate and nitrate into Cl^- and N_2. Clauwaert et al. successfully realized the simultaneous carbon (acetate) and nitrogen (nitrate) removal in anode and cathode respectively regardless of the C/N ratios in the waste streams under anaerobic condition [14]. Phylogenetic microarray (PhyloChip) and fluorescence in situ hybridization (FISH) using 16S rRNA indicated that *Proteobacteria* and *Firmicutes* were dominant and active members of the cathodic denitrifying biofilms [14]. Perchlorate can be bioreducted to chloride by dissimilatory of perchlorate-reducing bacteria (PCRB). Many PCRB are facultative anaerobes and denitrifiers [1]. So some researchers developed the MFC with a denitrifying biocathode to reduce perchlorate [12,16]. Butler and co-workers performed a better perchlorate reduction in the MFC with denitrifying biocathode by increasing the perchlorate loading while decreasing nitrate loading, in which a maximum perchlorate removal of 24 mg/L·d and cathodic conversion efficiency of 84% was achieved [16]. They also found that the bacterial community of perchlorate reducing biocathode shared little overlap with a purely denitrifying biocathode community. It had been reported that the presence of nitrate inhibited perchlorate reduction, particularly, when the supply of electron donor was limited [17]. However, the inhibition of nitrate on perchlorate reduction was negligible if sufficient electron donor was available [18]. So it is essential to investigate the optimal molar concentration ratio of nitrate to perchlorate to achieve the better bioreduction performance on biocathodes.

Recently, Xie et al. investigated the competitive microbial reduction of perchlorate and nitrate with a cathode directly serving as the electron donor [19]. However, an external potential (−0.50 V vs. SCE) was used in this study, where hydrogen may have been formed and utilized directly by conventional PCRB. In this research, a MFC with an autotrophic denitrifying biocathode was built to remove perchlorate and nitrate simultaneously. Meanwhile, the effect of NO_3^-/ClO_4^- on the reduction efficiency and electricity generation was investigated. Finally, the microbial community structure on biocathode was examined for insight into the key population involved in reduction processes.

2. Materials and methods

2.1. Experimental setup

The cylindroid MFC made of Perspex frames consisted of an anode chamber and a cathode chamber, both of which had a total volume of 230 mL (Fig. 1). The anode and cathode compartment were separated by a proton exchange membrane (PEM) (Hangzhou LeHou Environmental Protection Co., LTD, China). The width of PEM is 0.32 ± 0.02 mm, selective transmittance is not less than 96% and surface resistance is 4–5 Ω/cm^2. Two identical bare carbon felts (3.0 cm × 2.0 cm × 0.3 cm, Shanghai Lishuo composite material technology Co., Ltd) were packed into the MFC as the anode and cathode electrode and paralleled to the PEM respectively. Both electrodes were connected to an external resistance (R = 1000 Ω) with Ti wire (D = 0.6 mm) to form the circuit. Prior to use, the electrodes were washed by 1 M HCl and 1 M NaOH repeatedly to remove residual minerals and any biomass buildup, then thoroughly rinsed in deionized water and finally dried at 100 °C overnight. Each compartment reserved two sample ports (D = 6 mm) as inlet or outlet. The inlet sample port (on the top) connected a rubber hose with a syringe as sample injector, and the outlet sample port (on the side) bonded a rubber hose to the conical flask as collect bottle. To ensure strict anaerobic condition, each compartment was sealed with thick butyl rubber stoppers and aluminum crimp seal.

2.2. Inoculated sludge and synthetic wastewater

The activated sludge and excess sludge collected from a local municipal wastewater treatment plant in Changsha, China was mixed in 1:1 vol ratio as the inoculated sludge. Prior to inoculation, the mixed sludge was acclimated for almost 4 months under anaerobic autotrophic condition, with sulfide as electron donor and nitrate as electron acceptor.

In all experiments, both chambers of MFC were fed with synthetic wastewater containing 0.25 g K_2HPO_4, 2.70 g $NaHCO_3$, 0.005 g $Ca(OH)_2$ and 10 mL mineral solution (per liter in deionized water). The composition of mineral solution was according to Puig et al. [15]. The nitrate, perchlorate and sulfide were added into the cathode compartment in the form of potassium nitrate (KNO_3), potassium hyperchlorate ($KClO_4$) and sodium sulfide nonahydrate ($Na_2S{\cdot}9H_2O$), respectively. Their concentration would be changed according to the requirement of experiment conducted.

2.3. Operational procedure

The acclimated sludge were inoculated into cathode and anode chambers of three MFCs. The volatile suspended solids (VSS) and pH of inoculums were 86.50 g/L and 6.7 respectively. Then the synthetic wastewater containing sole perchlorate (ClO_4^-), sole nitrate (NO_3^-) and mixed substrates (ClO_4^-+NO_3^-) was added respectively into the cathode chambers. For all MFCs, the anode chambers were fed with the synthetic wastewater containing excessive amounts of sodium acetate and operated under the intermittent flow pattern. When the effluent concentration of cathode and voltage decreased and became stable, 90% of cathode and anode solutions were replaced. Simultaneously, the influent substrate concentration of cathode was elevated to the next level. During the experiment, the influent nitrate and perchlorate concentration were maintained 0.08–0.64 mmol/L and 0.033–1.20 mmol/L respectively. The influent NO_3^-/ClO_4^- changed from 9.6:1 to 1:3.7 according to the experimental requires. The operation temperature was controlled at 30 ± 1 °C in the incubator.

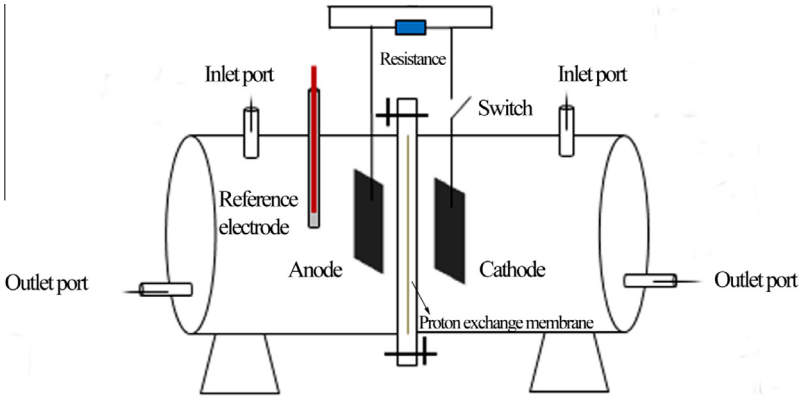

Fig. 1. Schematic of the double chambers autotrophic denitrifying biocathode MFC.

2.4. Analyses method

Cell potential (V) in the MFC circuit was measured and recorded every 1 min using a data acquisition system (Memograph M RSG40, Germany). Current (I) was determined according to Ohms law ($I = V/R$) [15], where R is a fixed external resistance (1000 Ω). Current density was calculated through dividing current by the net volume of biocathode compartment and theoretical current was determined by a previous described method [12]. Cyclic voltammetry (CV) curves were determined by the multichannel CHI1030 electrochemical workstation (Shanghai Chenhua instrument Co., LTD, China) with the 10 mV/s potential scan rate in the range of −1.8 V–1.8 V. The inoculated cathode, anode, and saturated calomel electrode (SCE) was as the working electrode, counter electrode, and reference electrode, respectively. The whole process was conducted at 25 °C and N_2 atmosphere.

Prior to chemical analysis, all samples were filtered using a syringe filter with 0.22 μm micropore filter membrane (LC + PVDF membrane, ANPEL Laboratory Technologies Inc., China). COD, NO_3^- and NO_2^- was determined following standard methods [20]. And perchlorate, chlorate, chlorite, chloride was analyzed by an ion chromatography (ICS-1000, Dionex, USA). The pH was monitored using a standard glass electrode and pH meter (pHS-3C model, Leici, China). All analyses were performed in triplicate to ensure reproducibility and the results were expressed as the mean value of these determinations. The surface morphology of initial electrode and biocathode were observed by scanning electron microscope (SEM) (LEO 1430 VP, U.S.A.).

2.5. Microbial community analysis

Microbial samples were obtained from the surface of cathode electrodes or the seed sludge. Total DNA was extracted from the microbial samples using the Powersoil DNA isolation kit (MoBio Laboratories, Inc., USA) following the manufacturer's instruction. The bacterial 16S rRNA genes were amplified by polymerase chain reaction (PCR) with universal primers: 8F (5′-AGAGTTT GATCCTGGCTCAG-3′) and 533R (5′-TTACCGCGGCTGCTGGCAC-3′) according to the previous research [21]. The PCR products were sequenced on the Roche GS FLX 454 pyrosequencing platform by Guhebio Bio-Pharm Technology Co., Ltd., Hangzhou, China. Finally, the sequences were trimmed using Mothur Software, qualified using the Silva alignment, clustered at 97% similarity with farthest algorithm to obtain the number of unique phylotypes and calculated rarefaction curves with the Mothur software to estimate the true total number of OTUs (Operational taxonomic unit) with infinite sampling.

3. Results and discussion

3.1. The reduction and electricity production of sole substrate system

In the sole perchlorate system, when the influent perchlorate concentration increased from 0.033 to 0.40 mmol/L in cathode compartment, the perchlorate removal efficiency maintained a higher level over 52.87% (Fig. 2a). The perchlorate removal at this stage was consistent with a previous study [12], which suggested that a community of perchlorate-reducing bacteria (PCRB) could be enriched from an autotrophic denitrifying biocathode community. However, with the perchlorate concentration exceeding 0.40 mmol/L, the COD removal efficiency had an obvious decline and only 10.93% perchlorate removal was achieved at influent perchlorate concentration 1.20 mmol/L, which may be ascribed to the lower electron demands of autotrophic denitrifying biocathode because its activity was inhibited by high concentration perchlorate (Table 1). It had been reported that once the substrate acetate in the anode chamber was depleted, the performance of perchlorate-reducing community could be limited [12]. Butler and co-workers also found that the perchlorate removal declined from 99% to 97% with the influent perchlorate concentration increasing from 10 to 20 mg/L [16]. Moreover, high concentration of perchlorate has adverse influence to the microorganism for its toxicity [18]. So it may conclude that the perchlorate reduction of autotrophic denitrifying biocathode was possibly inhibited by high concentration of perchlorate. Surprisingly, the current density had a closely relationship with the perchlorate removal amount as well as removal efficiency, the maximum and stable current density reached the peak 5.03 and 3.00 A/m^3simultaneously as influent perchlorate concentration was 0.40 mmol/L (Fig. 2b). However, pH value in cathode compartment was on the rise accompanied by the increasing influent perchlorate concentration (Table.1), which should be attributed to the consumption of protons during the reduction of perchlorate (Eq. (1)). The similar phenomenon also could be observed in the bioreduction of nitrate (Eq. (2)).

$$ClO_4^- + 8e^- + 8H^+ \rightarrow Cl^- + 4H_2O \quad (1)$$

$$NO_3^- + 5e^- + 6H^+ \rightarrow 1/2N_2 + 3H_2O \quad (2)$$

In the sole nitrate system, the concentration of nitrate (0.08–0.64 mmol/L) was gradually elevated in cathode chamber. As shown in Fig. 2c, the nitrate removal efficiency was above 87.05% when the influent nitrate was less than 0.32 mmol/L, but the nitrate removal efficiency showed a declining trend with the nitrate concentration exceeding 0.32 mmol/L. Due to the removal amount improved continually, the current density went up along

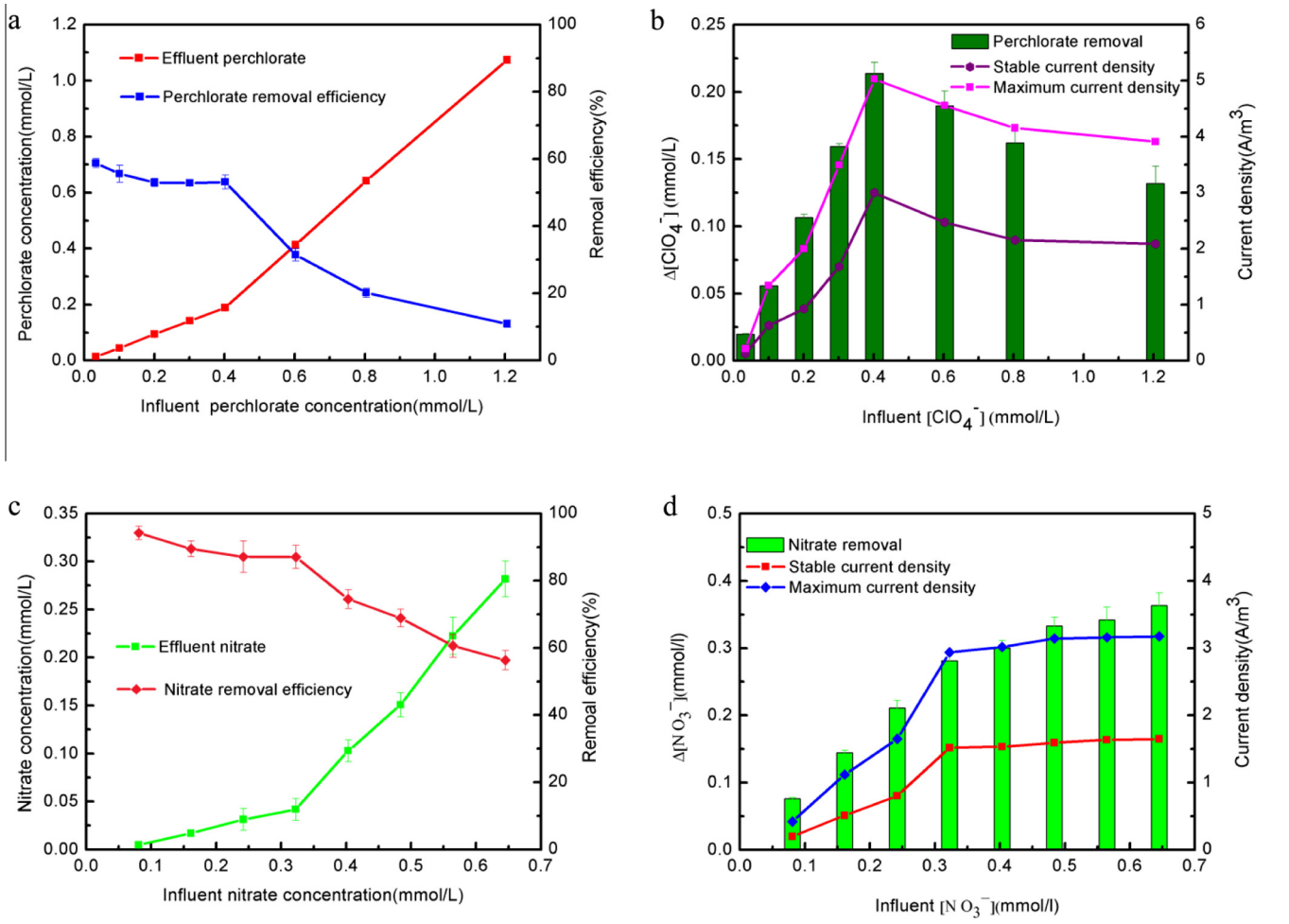

Fig. 2. Perchlorate (a) and nitrate (c) removal, and their relationship with current density in the autotrophic denitrifying biocathode MFC fed with sole perchlorate (b) and nitrate (d).

Table 1
Characteristics of anolyte and catholyte with different substrates.

Sole perchlorate			Lagging time (h)	Sole nitrate			Mixed nitrate and perchlorate		
Influent ClO_4^- (mmol/L)	COD removal (%)	pH		Influent NO_3^- (mmol/L)	COD removal (%)	pH	Influent NO_3^-/ClO_4	COD removal (%)	pH
0.033	74.23	7.8	44	0.08	82.56	7.7	9.6:1	90.89	7.7
0.10	72.65	8.1	46	0.16	82.12	7.9	3.2:1	88.24	7.8
0.20	75.11	8.2	56	0.24	80.89	8.3	1.6:1	89.59	8.0
0.30	74.78	8.3	62	0.32	79.21	8.4	1:1	87.37	8.2
0.40	75.45	8.3	62	0.40	79.67	8.5	1:1.2	84.17	8.3
0.60	74.78	8.4	70	0.48	79.13	8.6	1:1.9	83.56	8.5
0.80	69.98	8.5	72	0.56	78.02	8.7	1:2.5	78.23	8.7
1.20	66.58	8.6	72	0.64	76.79	8.9	1:3.7	71.13	8.8

with the increasing nitrate concentration, it different from sole perchlorate system (Fig. 2a). When nitrate was 0.65 mmol/L, the peak values of the maximum and stable current density were 3.17 and 1.64 A/m^3, respectively (Fig. 2d). As shown in Table 1, the COD removal in anode compartment maintained around 80%, suggesting that a good carbon removal performance of this system. Furthermore, compared with the sole perchlorate system, the trend of COD removal efficiency was much smoother and the average stable time was much longer in sole nitrate system, it may implicit that the nitrate as the substrate was more beneficial to the stability of autotrophic denitrifying biocathode (Table S1). Zhang and co-workers also used the duration time to maintain stable voltage to compare the stability of different cathodes [22].

3.2. The reduction and electricity production of mixed substrates system

In the mixed system, both nitrate with a constant concentration of 0.32 mmol/L and perchlorate with a varying concentration from 0.033 to 1.20 mmol/L were introduced to the cathode compartment as the mixed substrates, in which the influent molar ratio of nitrate and perchlorate (NO_3^-/ClO_4^-) changed from 9.6:1 to 1:3.7. The perchlorate removal in this system was not obviously improved with the influent perchlorate increasing from 0.033 to 0.07 mmol/L (accordingly NO_3^-/ClO_4^- declining from 9.6:1 to 4.8:1), meanwhile the denitrification rate maintained higher level (over 85%), suggesting that the coexistence of low concentration

perchlorate did not influence the nitrate reduction of autotrophic denitrifying bacteria. When NO_3^-/ClO_4^- gradually decreased from 4.8:1 to 1:1, a progressive increase in perchlorate removal (40.97%) was observed and the performance of denitrification (86.03%) still was better (Fig. 3a). The improved perchlorate reduction should be ascribed to the enrichment of PCRB and adaption of autotrophic denitrifying bacteria to the higher concentration perchlorate [12,23]. Some researchers also reported that nitrate can be used as a primary electron acceptor supporting the growth of per(chlorate) reducing bacteria [23]. With the NO_3^-/ClO_4^- further declining to 1:3.7, the denitrification rate was only 42.36% at NO_3^-/ClO_4^- ratio of 1:3.7, indicating that the nitrate removal was obviously inhibited by the high concentration of perchlorate. It's somewhat different from the point that nitrate reduction outcompeted perchlorate reduction for electrons from the electron donor such as electrode or acetate [19]. Meanwhile, the perchlorate removal in this system also fell to the minimum value (3.94%). Besides, the reduction rate of nitrate and perchlorate in mixed system with NO_3^-/ClO_4^- ratio of 1:1 were very close to that in sole system, although the effluent concentrations were not same (Fig. S1a). In briefly, the aforementioned results demonstrated that perchlorate and nitrate competed for the electrons from biocathode and their weakest competitiveness occurred at 1:1 (NO_3^-/ClO_4^-). From the Table 1 and Table S1, it could find that the COD removal efficiency and flow rate of CO_2 emission decreased during the latter phase, suggesting that the weaker perchlorate and nitrate removal maybe led by the limited production of electron in this system [12]. From the perspective of electricity production, it was found that stable current reached the maximum (3.10 A/m^3) at 1:1, but the maximum current reached the peak (6.17 A/m^3) at 1:1.2 (Fig. 3b). Cai and co-workers observed the stable voltage was related to the efficiency of substrate removal and stable voltage was more representative than maximum voltage in the MFCs [24]. And the anodic potential reached the lowest point (−916.56 mv) at 1:1, suggesting that a negative poised anodic potential enhanced the performance of microbial fuel cell and lower negative anodic potential had higher voltage [25]. At the same time, the maximum power density (2.19 W/m^3) was achieved as the NO_3^-/ClO_4^- was 1:1, which also demonstrated the above view (Table 2). Furthermore, the total substrate removal gradually increased as the NO_3^-/ClO_4^- was greater than 1:1.2, the nitrate removal amount did not change significantly from 9.6:1 to 1:1. However, the actual current in the mixed system was greater than the theoretical current in sole system with same nitrate or perchlorate concentration (Table 2), demonstrating that perchlorate reduction on biocathode played a major role in the variation of stable current density within this range. When the molar ratio varied from 1:1 to 1:3.7, the stable current density was on the decline, and drop rate became quicker, especially from 1:1.2 to 1:3.7, this result was similar with the removal situation

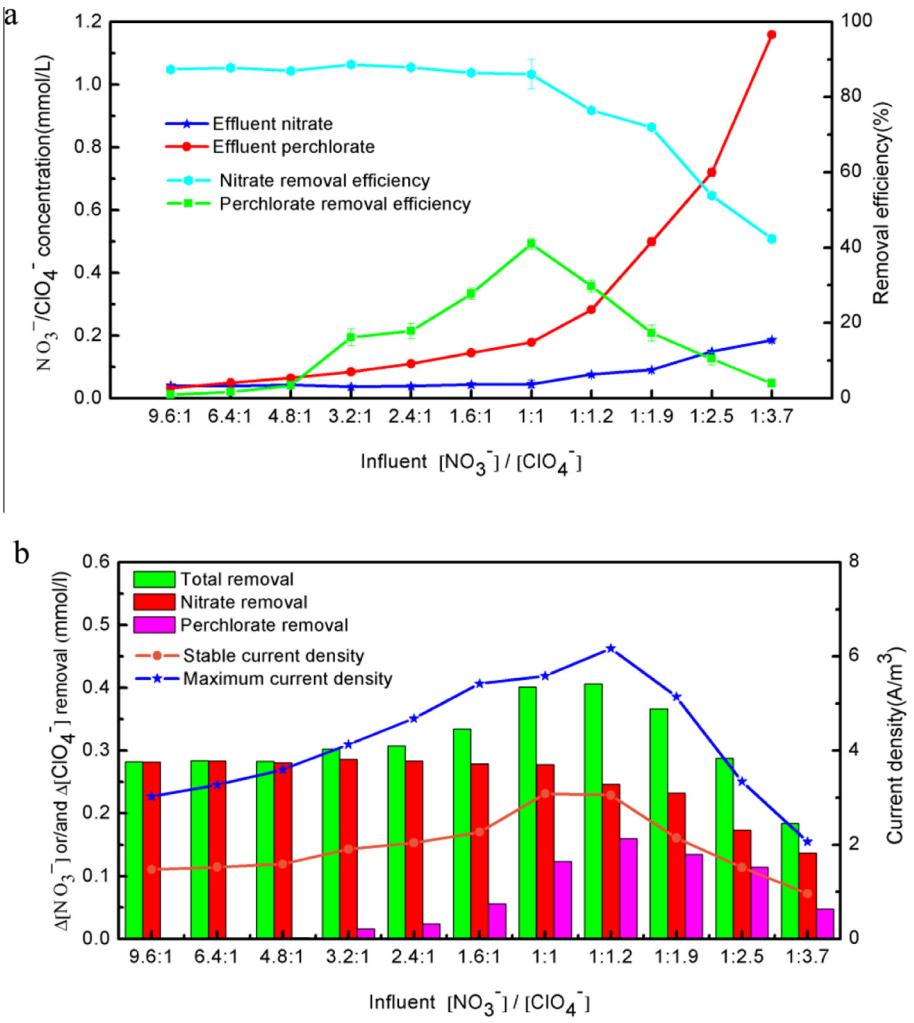

Fig. 3. Simultaneous perchlorate and nitrate removal under different influent $[NO_3^-]/[ClO_4^-]$ (a) and effect of influent $[NO_3^-]/[ClO_4^-]$ on the current density and substrate removal (b) in the autotrophic denitrifying biocathode MFC.

Table 2
The performance of power density, actual and theoretical current in MFCs fed with different substrates.

$[ClO_4^-]$ (mmol/L)	Sole perchlorate			$[NO_3^-]$ (mmol/L)	Sole nitrate			$[NO_3^-]/[ClO_4^-]$	Mixed nitrate and perchlorate		
	Power density (W/m^3)	Actual current (A)	Theoretical current (A)		Power density (W/m^3)	Actual current (A)	Theoretical current (A)		Power density (W/m^3)	Actual current (A)	Theoretical current (A)
0.033	0.01	0.03	0.06	0.08	0.01	0.04	0.10	9.6:1	0.50	0.34	0.43
0.10	0.09	0.14	0.22	0.16	0.06	0.12	0.19	3.2:1	0.84	0.35	0.45
0.20	0.20	0.21	0.29	0.24	0.15	0.18	0.28	1.6:1	1.18	0.37	0.57
0.32	0.65	0.39	0.51	0.32	0.46	0.33	0.36	1:1	2.19	0.44	0.77
0.40	2.07	0.69	0.91	0.40	0.47	0.33	0.50	1:1.2	2.14	0.47	0.81
0.60	1.41	0.57	0.79	0.48	0.58	0.37	0.67	1:1.9	1.06	0.52	0.88
0.80	1.07	0.49	0.73	0.56	0.64	0.38	0.98	1:2.5	0.53	0.71	0.93
1.20	1.00	0.48	1.02	0.64	0.66	0.39	1.18	1:3.7	0.21	0.70	1.09

(Fig. 3b). Moreover, as seen from Fig. S1b, the value of stable current density in mixed system with NO_3^-/ClO_4^- ratio of 1:1.9 were less than that in mixed system with NO_3^-/ClO_4^- ratio of 1:1. Previous study had also reported that nitrate reduction was the main control factor for the current production in mixed system and the denitrification was inhibited by high concentration of perchlorate, which also was confirmed by previous studies [12,16]. Above all, it further suggested that high concentration perchlorate inhibited the biological activity of biocathode as in the sole perchlorate system, which possibly led the total substrate efficiency go down quickly, further reduced the electrons utilization.

3.3. The reduction and electricity production of abiotic cathode MFC

Three series of MFCs without the inoculation of acclimated sludge were operated in open and close circuit respectively to confirm the adsorption and electrochemical reduction of substrate on electrode. Each MFC was fed with sole nitrate (0.32 mmol/L), sole perchlorate (0.40 mmol/L), and mixed substrate (0.32 mmol/L nitrate and 0.32 mmol/L perchlorate) in cathode compartment, respectively. Compared with the 87.05% of nitrate removal and 52.87% of perchlorate removal on biocathode, nitrate removal in open and close model was 3.45 and 7.21% as well as 3.01 and 7.81% for perchlorate. Similar results were obtained for the mixed substrate. Meanwhile, the stable current density in close circuit was not greater than 139.98 mA/m^3, which was rather low compared with the MFC with biocathode. From Table S1, it was obviously found that the autotrophic denitrifying biocathodes could maintain longer stable time (67 h–106 h) than abiotic cathode (<2 h) regardless of any biocathode, as a previous study concluded [22]. All of above demonstrated that nitrate or perchlorate removal was mainly through the bioreduction of autotrophic denitrifying biocathode, and the adsorption (open model) and electrochemical reduction (close model) on electrode were negligible. Zhang and his co-workers found that the sulfide removal in MFC was the synthesized results of multiple effects, in which the contribution of volatilization, adsorption, electrochemical and biological oxidations for sulfide removal accounted for 11.1, 14.6, 24.1 and 50.2%, respectively [26].

3.4. The electrochemical analysis of biocathode

The SEM pictures (Fig. S2) of initial cathode and denitrifying biocathode revealed that the cathode had a significant change in the aspect of surface morphology. Much more rod shaped bacteria grew as clusters on the biocathode after acclimation.

As a kind of electrochemical method, CV was performed to examine the catalytic behavior of the biocathode. As shown in Fig. 4, no distinct redox peak was observed from the fresh electrode without inoculation. On the contrary, all of biocathodes fed with sole nitrate, sole perchlorate or their mixture exhibited a much higher catalytic activity than the abiotic cathode. Surprisingly, three type of biocathodes performed an identical redox potential and three approximate redox peaks. An anodic peak at −0.16 V and a cathodic peak at −0.42 V were clearly visible in the CV curve. It indicated that the biocathode attached with autotrophic bacteria may have a great electrochemical activity, and the transfer mechanism of electron in three types of biocathode was similar.

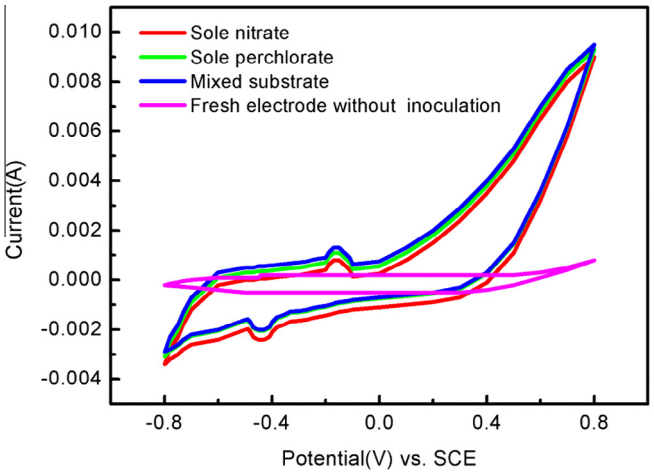

Fig. 4. Cyclic voltammetry curves for bicathodes and raw electrode.

3.5. Microbial community analysis of biocathode

In this study, microbial communities in initial seed sludge (S1), the biocathode fed with sole nitrate (S2), sole perchlorate (S3) and their mixture (S4) were investigated using16S rRNA amplicon sequencing (Fig. 5). The numbers of OTUs and the richness as well as diversity of four samples were compared (Table.3). We observed that Shannon index and Simpson index decreased after acclimation, which illustrated that community diversity has shrunk due to the special bioreduction conditions. Fig. 6 illustrated ten phyla with highest relative abundance in each sample. Obviously, *Proteobacteria* and *Chloroflexi* were dominant in S1. *Proteobacteria* were still the major phyla in three types of biocathode, whose proportions achieved 55.22, 49.96 and 51.65% in S2, S3 and S4, respectively. However, *Chloroflexi* became slightly after acclimation, as well as *Acidobacteria*. Meanwhile, *Thermi*, as a new phyla occurred in biocathodes. Kondaveeti et al. reported that *Thermi* species were known as nitrate reducing microorganisms and prevalent in the bioelectrochemical denitrification system [27]. Among *Proteobacteria* phylum, β-*Proteobacteria* and α-*Proteobacteria* were identified as the most significant classes in biocathodes, accounting to 67.19 and 24.29% of all the Proteobacteria sequences in S2, 53.86 and

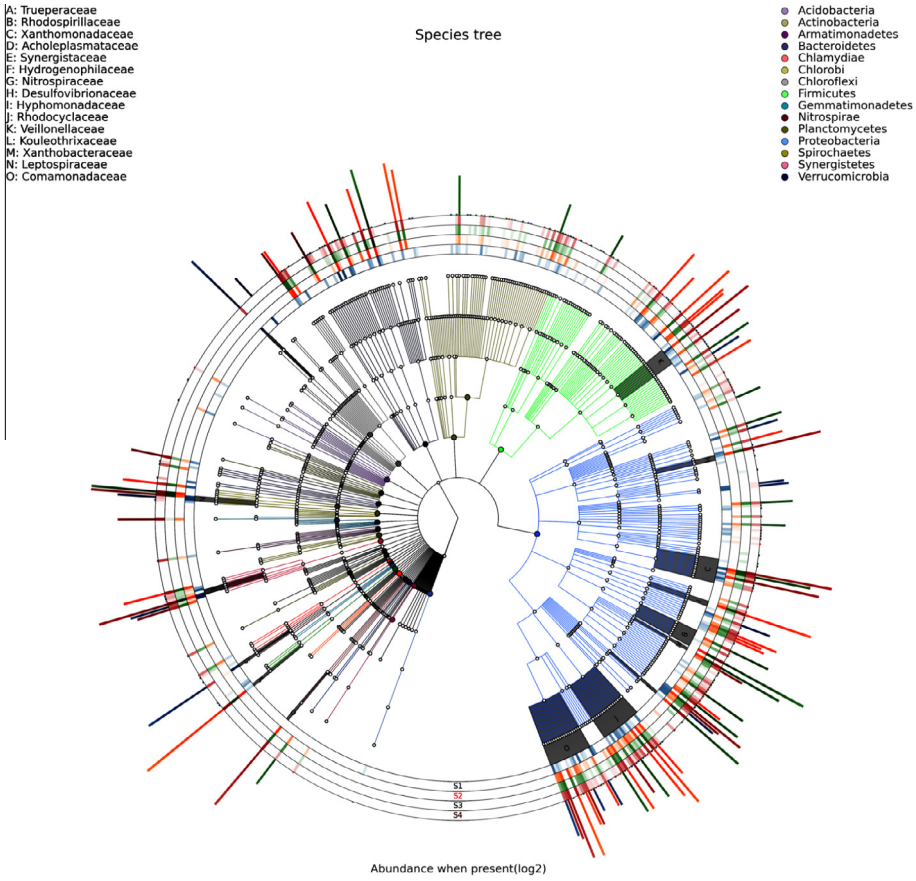

Fig. 5. The phylogenetic tree of relative abundance in four test samples.

Table 3
OTU number, richness and diversity indices of bacterial community in four samples.

Sample	OTUS Number	Coverage (%)	Chao	Ace	Shannon	Simpson
S1	3594	0.97	4326.14	4710.06	8.93	0.99
S2	6167	0.97	7109.49	7864.27	7.97	0.97
S3	5336	0.97	6818.53	7479.44	8.15	0.98
S4	5684	0.96	7934.85	8832.34	8.26	0.98

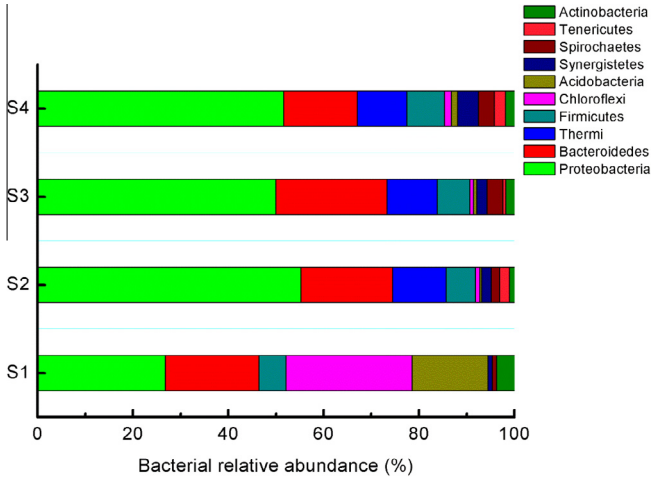

Fig. 6. A comparison of the relative bacterial community abundance in terms of phylum level among four samples.

29.77% in S3, 53.41 and 29.58% in S4. A previous study had proven that β-*Proteobacteria* and α-*Proteobacteria* belonged to nitrate-reducing organisms [28,29]. These community analyses demonstrated that the dominant species on biocathodes belonged to autotrophic denitrifying bacteria, which had the capacity of perchlorate reduction. In addition, *Firmicutes* also existed in the biocathode samples (S2, S3 and S4). Huang and co-workers have confirmed that the *Firmicutes* had the ability to transfer extracellular electrons, which just made a decisive role in electricity generation [21].

Although three types of biocathode performed a similar diversity based on the phyla, but there were some distinctions among them from views of genus. Nozawa-Inoue et al. found that *Azospirillum* were capable of oxidizing hydrogen microaerobically or anaerobically under denitrifying conditions and the members of this genus could directly couple perchlorate reduction [30]. Borole et al. also thought that several members of the *Azospirillum* genus were capable of perchlorate, selenate and arsenate reduction [28]. Through the experiments, it was observed that the presence of *Azospirillum* in biocathode samples and its proportion achieved 13.75% in S2, 29.04% in S3 and 22.44% in S4. *Thauera* related to β-*Proteobacteria* was the major genus in biocathodes with the

proportion of 40.92%, 38.10%, and 33.87%, respectively, in S2, S3 and S4. *Thauera* species known as nitrate-reducing microorganisms were exclusively found on the nitrate biocathode and preferably grew with nitrate over perchlorate [19]. However, Gao and co-workers have already demonstrated that most of denitrifying bacteria could simultaneously reduce perchlorate, nitrate and other oxygenous inorganic salts [10]. Therefore, further researches should be conducted to investigate the minor difference in microorganisms.

4. Conclusions

An autotrophic denitrifying biocathode was constructed to realize the reduction of nitrate or/and perchlorate with electricity generation. When the concentration of sole perchlorate and sole nitrate were 0.40 mmol/L and 0.32 mmol/L respectively, both MFCs showed an excellent performance on removal efficiency and stable current. As the influent molar ratio of NO_3^-/ClO_4^- was 1:1, the holistic substrate reduction (40.97% for perchlorate, 86.03% for nitrate) and electricity generation performance (3.10 A/m^3) reached the optimum. Compared with the bioreduction, the adsorption and electrochemical reduction of nitrate or/and perchlorate exerted the dinky influence on the removal and electricity. Microbiology community analysis showed that autotrophic denitrifiers (*Thauera* and *Thiobacillus*) were dominated bacteria in biocathodes but there were remarkable distinctions between different types of biocathode in genus.

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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.cej.2016.09.121.

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