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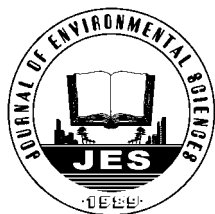
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## Effects of cathode potentials and nitrate concentrations on dissimilatory nitrate reductions by *Pseudomonas alcaliphila* in bioelectrochemical systems

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### ABSTRACT

The effects of cathode potentials and initial nitrate concentrations on nitrate reduction in bioelectrochemical systems (BESs) were reported. These factors could partition nitrate reduction between denitrification and dissimilatory nitrate reduction to ammonium (DNRA). *Pseudomonas alcaliphila* strain MBR utilized an electrode as the sole electron donor and nitrate as the sole electron acceptor. When the cathode potential was set from  $-0.3$  to  $-1.1$  V (vs. Ag/AgCl) at an initial nitrate concentration of  $100$  mg  $\text{NO}_3^-$ -N/L, the DNRA electron recovery increased from  $(10.76 \pm 1.6)\%$  to  $(35.06 \pm 0.99)\%$ ; the denitrification electron recovery decreased from  $(63.42 \pm 1.32)\%$  to  $(44.33 \pm 1.92)\%$ . When the initial nitrate concentration increased from  $(29.09 \pm 0.24)$  to  $(490.97 \pm 3.49)$  mg  $\text{NO}_3^-$ -N/L at the same potential ( $-0.9$  V), denitrification electron recovery increased from  $(5.88 \pm 1.08)\%$  to  $(50.19 \pm 2.59)\%$ ; the DNRA electron recovery declined from  $(48.79 \pm 1.32)\%$  to  $(16.02 \pm 1.41)\%$ . The prevalence of DNRA occurred at high ratios of electron donors to acceptors in the BESs and denitrification prevailed against DNRA under a lower ratio of electron donors to acceptors. These results had a potential application value of regulating the transformation of nitrate to  $\text{N}_2$  or ammonium in BESs for nitrate removal.

## Introduction

In the biological nitrogen (N) cycle, both denitrification and dissimilatory nitrate reduction to ammonium (DNRA) are the processes of dissimilatory nitrate reduction. Denitrification process gradually reduces nitrate ( $\text{NO}_3^-$ ) to nitrite ( $\text{NO}_2^-$ ), nitric oxide (NO), nitrous oxide ( $\text{N}_2\text{O}$ ) and nitrogen gas ( $\text{N}_2$ ); while DNRA is the reduction of  $\text{NO}_3^-$  into ammonium (Knowles, 1982). In contrast to denitrification, DNRA is a N retention process in which nitrate is transformed into another inorganic N form which is available to biological organisms (Buresh and Patrick, 1978; Tiedje et al., 1982).

Traditional studies of regulating nitrate reduction in

mixed culture focused on principal factors, such as C/ $\text{NO}_3^-$  ratio (Kelso et al., 1997; Tugtast and Pavlostathis, 2007; Yin et al., 1998), oxidation status (Brunel et al., 1992; Matheson et al., 2002) and pH (Stevens et al., 1998). Among these factors, C/ $\text{NO}_3^-$  ratio was considered as an important factor that influenced the occurrence of denitrification and DNRA (Rütting et al., 2011). The C/ $\text{NO}_3^-$  ratio could function as electron donor/electron acceptor ratios (Tiedje et al., 1982) in the partition process. While C/ $\text{NO}_3^-$  ratio also influenced other factors in the experiments, such as  $\text{O}_2$  consumption (Matheson et al., 2002), the change of DNRA bacteria population (Tiedje, 1988) and the character of the microflora (Yin et al., 2002). The change of the carbon source would also effect the nitrate reduction type (Yin et al., 1998). These diverging findings questioned the true function of C/ $\text{NO}_3^-$  in traditional researches.

Bioelectrochemical technology, as a developing technol-

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ogy, has attracted great interest in pollutant removal from wastewater (Aelterman and Verstraete, 2009; Hamelers et al., 2010). This novel technology is also used for water and wastewater denitrification. One direction is to combine nitrogen removal from wastewater with simultaneous energy production in an anode chamber while using organic substances as the electron donor (Cai and Zheng, 2013; Cai et al., 2013). Another direction is to remove nitrate in the cathode chamber using nitrate as the electron acceptor and an electrode as an electron donor. Gregory (2004) first demonstrated that *Geobacter metallireducens* had the ability to reduce nitrate to nitrite using the electrode as a sole electron donor (Gregory et al., 2004). Recently, a sediment-type MFC based on two pieces of bio-electrodes was utilized for nitrogen removal as well as electricity production (Zhang and Angelidaki, 2012). In that research, a total nitrogen removal of 62% and 77% was accomplished, for nitrate and nitrite, respectively on biocathode. A neutral red modified electrode was also used as an electron donor for permeabilized *Ochrobactrum anthropi* SY509 to remove nitrate without carbon source feeding (Kim et al., 2007). More recently, some reviews had summarized the development and obstacles of bioelectrochemical technology for nitrate removal (Duca and Koper, 2012; Mousavi et al., 2012). The impact of different factors had been discussed, for example, configurations, hydraulic retention time, electric current, pH, and carbon source on the performance of the BESs. However, in this field, less research had focused on the competition between denitrification and DNRA in BESs which was important for the application of bioelectrochemical technology for nitrate removal.

Our previous research has shown that the *Pseudomonas alcaliphila* strain MBR has the ability of denitrification and DNRA simultaneously using a cathode as the sole electron donor (Su et al., 2012). Based on the previous result, this work directly proved that the electron donor/acceptor ratio substantially influenced the competition between denitrification and DNRA in BESs. There was no disturbance of organic carbon source and the nature of the microflora in the environment. After further progress, our work improved and completed the understanding of the nitrate reduction and nitrate removal in BESs. The result has the potential value for the development of bioelectrochemical technology for nitrate removal.

## 1 Method and materials

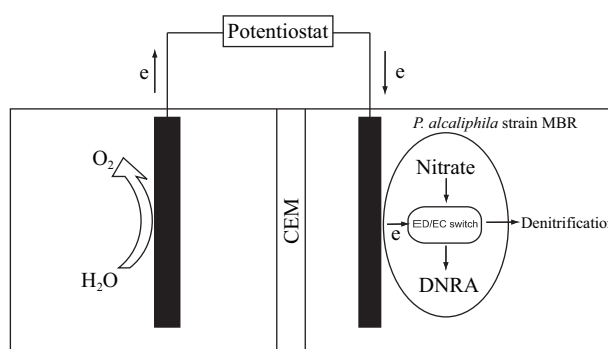
### 1.1 Source of microorganism and cultivation conditions

*P. alcaliphila* strain MBR (CGMCC2318) was obtained from Chengdu Institute of Biology, Chinese Academy of Science (Chengdu, China). The 16S rDNA sequence of strain MBR was submitted to the GenBank with accession

No. EU307111. The activation culture was grown at  $(30 \pm 1)^\circ\text{C}$  under aerobic conditions. Strain MBR was cultivated in the modified Giltay medium with 5.3 g/L of sodium citrate before inoculation (Su et al., 2012). Cathodic medium contained the following (per liter of distilled water): 0.1 g KCl, 0.04 g MgCl<sub>2</sub>, 10.9 g Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O, 3.0 g NaH<sub>2</sub>PO<sub>4</sub>, 2.0 g NaHCO<sub>3</sub>, 1.0 mL vitamin, and 1.0 mL trace mineral mix, with nitrate as the sole electron acceptor. The medium used in the anode chamber was the same as the cathodic medium without nitrate. All mediums were sterilized by autoclaving (121°C, 15 min) and the pH was 7.0.

### 1.2 Bioelectrochemical system

The configuration of the bioelectrochemical cell in this study was the same as the system described by Su et al. (2012) (Graph 1). The BES was separated into an anode chamber and cathode chamber by a cation exchange membrane (CEM, AMI7001, Membranes International Inc., USA). The two chambers were of equal volume (150 mL), and each chamber was filled with 100 mL of medium with a 50 mL headspace. The cathode (working electrode) was carbon felt of 4.0 cm × 4.0 cm and the anode (counter electrode) was a titanium plate of 4.0 cm × 4.0 cm. The carbon felt was dealt with 1.0 mol/L HCl and 1.0 mol/L NaOH, and rinsed with sterile water till neutral before use. The reference electrode (placed in the cathode chamber) was a Ag/AgCl electrode (sat. KCl, +0.197 V vs. SHE,) and disinfected with 75% alcohol. All potentials were reported versus Ag/AgCl. A potentiostat (CHI 1000B, ChenHua Instruments Co., Ltd., Shanghai, China) poised the cathode at a constant negative potential and monitored the current in experiments.



**Graph 1** Configuration and mechanism of the BESs. ED: electron donor, EC: electron acceptor. CEM: cation exchange membrane, DNRA: dissimilatory nitrate reduction to ammonium.

### 1.3 Bioelectrochemical experiments

Prior to the bioelectrochemical experiments, *P. alcaliphila* strain MBR was inoculated into the cathodic chamber while the reactor was flushed with ultrapure CO<sub>2</sub> (purity >

99.999%). In order to achieve a stable nitrate reducing biocathode, strain MBR was enriched on the surface of carbon felt with the addition of nitrate (0.8 g/L) and sodium citrate (5.3 g/L) into cathodic medium at 30°C for approximately 3 weeks, and the growth of a biofilm on the cathode surface was accelerated via slow magnetic stirring. Subsequently, the cathode chamber was rinsed with cathodic medium without sodium citrate three times and the bacteria was starved for 24 hr. Finally, the medium was replaced with cathodic medium without residual organic compounds. Meanwhile, a series of batch bioelectrochemical experiments were operated and the electrode was served as the sole electron donor. The effect of various cathode potential levels, such as -0.1, -0.3, -0.5, -0.7, -0.9 and -1.1 V (vs. Ag/AgCl), on partitioning nitrate reduction between denitrification and DNRA, was investigated with 100 mg NO<sub>3</sub><sup>-</sup>-N/L. The effect of various nitrate concentrations (29.09 ± 0.24, 90.48 ± 0.65, 190.98 ± 1.49, 276.93 ± 3.27, 350.68 ± 2.72 and 490.97 ± 3.49 mg NO<sub>3</sub><sup>-</sup>-N/L) was observed at -0.9 V. Each batch experiment was repeated three times. In parallel, several control experiments were performed under the same conditions above, in the absent of microbe. During the tests, magnetic stirrer was turned off. Liquid samples and gaseous samples were taken from the cathode chamber at regular intervals, respectively.

#### 1.4 Analysis and calculation

The current of working electrode was measured by multichannel potentiostat CHI 1000B (CHI Instrument) with a Power Laboratory 8SP unit connected to a computer. H<sub>2</sub> and N<sub>2</sub> gas were analyzed by gas chromatography (Agilent 7890A GC system; Agilent Technologies, Shanghai, China). NO<sub>3</sub><sup>-</sup>-N, NO<sub>2</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N were measured according to the Standard Methods for the Examination of Water and Wastewater. The nitrate concentration, nitrite concentration and ammonium concentration in this article were in the form of NO<sub>3</sub><sup>-</sup>-N, NO<sub>2</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N, respectively.

As previously described by Su et al. (2012), the

coulombic efficiency (CE) for reduced products (nitrite, ammonium and nitrogen) were calculated as Eq. (1):

$$CE = \frac{eq_p}{eq_i} \times 100\% \quad (1)$$

where, eq<sub>p</sub> (p stands for products) was cumulative reducing equivalents that were calculated from the measured amounts of NO<sub>2</sub><sup>-</sup>-N, NH<sub>4</sub><sup>+</sup>-N and N<sub>2</sub>, considering the corresponding molar conversion factor of 2, 8, and 10 eq/mol, respectively. eq<sub>i</sub> (i stands for intergrating) was a cumulative electric charge that was calculated by integrating current over the period of cathode polarization, by using the following conversion factor:

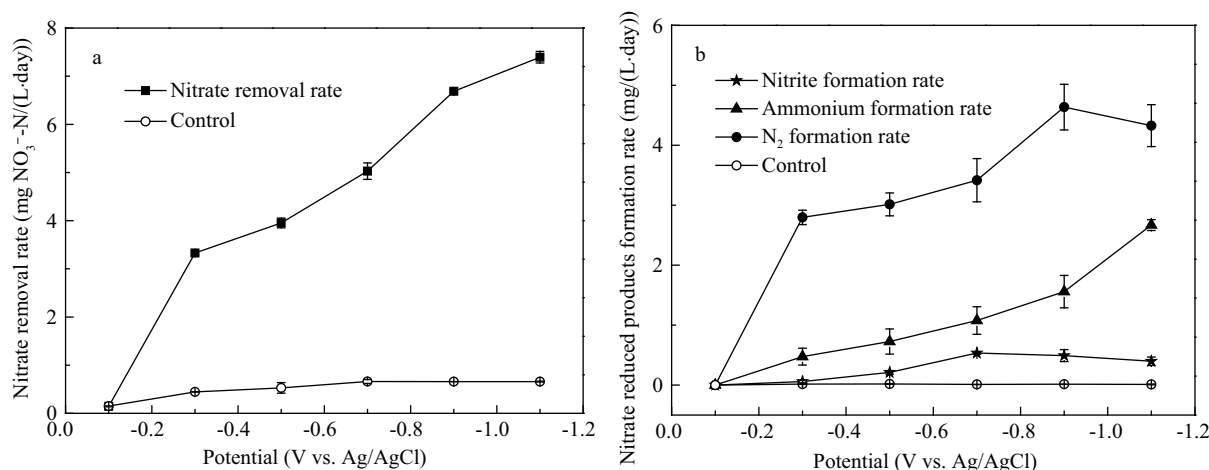
$$eq_i = C/F \quad (2)$$

where, *C* is the electric quantity; *F* is the Faraday constant (96485.4 C/mol e<sup>-</sup>).

## 2 Results and discussion

### 2.1 Effect of cathode potential for dissimilatory nitrate reduction in strain MBR

The cathode potential was set from -0.1 to -1.1 V with the same nitrate concentration (100 ± 0.15 mg NO<sub>3</sub><sup>-</sup>-N/L) as the sole electron acceptor, and the results of dissimilatory nitrate reduction are shown in Fig. 1. As the cathode potential descended, the nitrate removal rate obviously increased from (0.14 ± 0.01) to (7.39 ± 0.12) mg NO<sub>3</sub><sup>-</sup>-N/(L·day). However, the nitrate removal rate (0.14 ± 0.01) mg NO<sub>3</sub><sup>-</sup>-N/(L·day) at -0.1 V was close to the control case. Compared with the bio-cathode in the experiment, the nitrate removal rates of the abiotic cathode (control) were in the range of 0~(0.66 ± 0.01) mg NO<sub>3</sub><sup>-</sup>-N/(L·day) at different potentials (Fig. 1a). All indicated that the bacteria in the cathode chamber played an important role



**Fig. 1** Effect of different cathode potential on dissimilatory nitrate reduction by strain MBR using nitrate (100 mg NO<sub>3</sub><sup>-</sup>-N/L) as the sole electron acceptor. (a) nitrate reduced rate; (b) accumulation rate of nitrate reduced products.

of nitrate removal in BESs. The nitrate removal could not be contributed to direct electrocatalysis on the electrode.

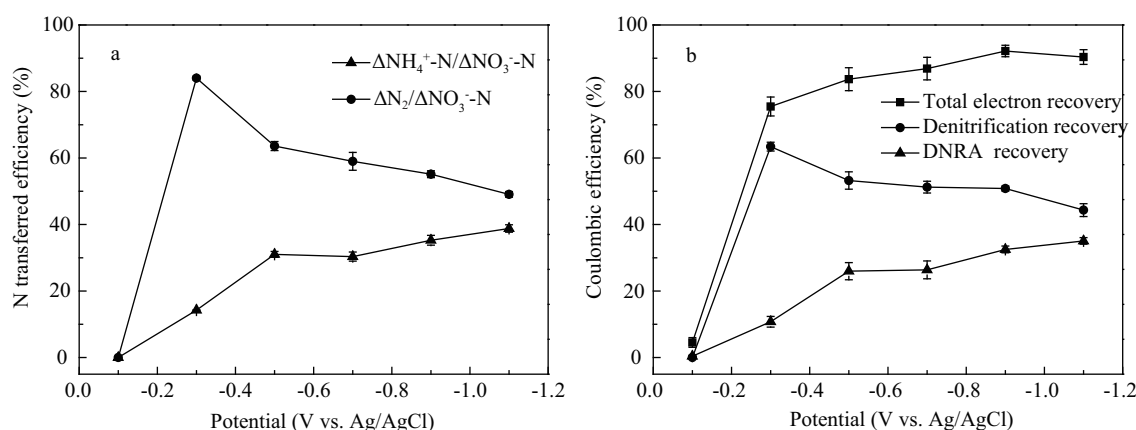
**Figure 1b** shows that the formation rate of nitrate reduction products, including nitrite, ammonium and  $N_2$  were measured at various cathode potentials. When the cathode potential was set at  $-0.1$  V, nitrate reduction products were not observed. However, nitrogen products gradually accumulated with the potential dropping. During the cathode potential decrease, the  $N_2$  generation rate was always higher than ammonium and nitrite. The maximum production rate of  $N_2$  ( $4.64 \pm 0.38$  mg/(L-day)) was obtained at  $-0.9$  V, but the rate decreased from ( $4.64 \pm 0.38$ ) to ( $4.33 \pm 0.35$ ) mg/(L-day) with the potential dropping from  $-0.9$  to  $-1.1$  V. The dropping may be caused by the increment of ammonium formation rate. The ammonium production rate by DNRA obviously increased with cathode potential dropping. The ratio of  $N_2$  production rate to ammonium, at different potentials, was 5.9 ( $-0.3$  V), 4.1 ( $-0.5$  V), 3.2 ( $-0.7$  V), 3.0 ( $-0.9$  V) and 1.6 ( $-1.1$  V), respectively. This ratio revealed that the strain MBR preferred to reduce nitrate through DNRA process at a lower potential. In addition, the formation rate of ammonium increased more obviously at  $-1.1$  V than other potentials (**Fig. 1b**). According to Butler-Volmer's equation, the current on the surface of electrode was controlled by the electrode potential (Bard and Faulkner 2000). The larger divergence between applied potential and stand potentials, the electrical current increased. In this experiment, the electrode supplied more electrons for the strain MBR under a lower potential which means the electrode supplied the most electrons to the strain MBR at  $-1.1$  V. In another words, DNRA process appeared at a higher electron donor/acceptor ratio. The change of nitrite production rate showed a slow-growth tendency along with the decrease of cathode potential and reached a maximum rate of ( $0.54 \pm 0.02$ ) mg  $NO_2^-$ -N/(L-day) at  $-0.7$  V. Subsequently, this production rate decreased from ( $0.54 \pm 0.02$ ) to ( $0.40 \pm 0.07$ ) mg  $NO_2^-$ -N/(L-day) as the

decrease of potential from  $-0.7$  to  $-1.1$  V. The nitrogen products were not observed in absence of microorganisms.

## 2.2 Effect of cathode potential on N transformation rate and coulombic efficiency in strain MBR

As shown in **Fig. 2**, the N transformation rate and coulombic efficiency of denitrification and DNRA in the BESs at different potential levels were observed, respectively. The highest transformation rate of nitrate to  $N_2$  was ( $84.02 \pm 0.57$ )% with  $-0.3$  V. When the potential dropped from  $-0.3$  to  $-1.1$  V, the transformation rate of denitrification decreased from ( $84.02 \pm 0.57$ )% to ( $49.06 \pm 0.86$ )%. By contrast, more ammonium was accumulated. The transformation rate of nitrate to ammonium at  $-0.3$ ,  $-0.5$ ,  $-0.9$  and  $-1.1$  V were ( $14.25 \pm 0.02$ )%, ( $31.03 \pm 0.89$ )%, ( $35.27 \pm 1.47$ )% and ( $38.80 \pm 1.12$ )%, respectively (**Fig. 2a**). Therefore, the DNRA activity of strain MBR was stimulated by a lower potential which supplied more electrons to the bacteria.

The nitrogen balance calculations based on total electrons flow were illustrated in **Fig. 2b**. The total electron recovery for nitrate reduction increased from ( $4.52 \pm 1.46$ )% to ( $90.36 \pm 2.21$ )% with the decrease of the cathode potential from  $-0.1$  to  $-1.1$  V. The highest total electrons recovery ( $92.17 \pm 1.73$ )% was obtained at  $-0.9$  V. This result demonstrated that the strain MBR could utilize electrons effectively from electrode for nitrate reduction at a low cathode potential. An optimal utilization of electrons by the strain MBR for nitrate reduction was attained at  $-0.9$  V. Denitrification electron recovery in the cultures decreased from ( $63.42 \pm 1.32$ )% to ( $44.33 \pm 1.92$ )% when the potential was set from  $-0.3$  to  $-1.1$  V. However, the DNRA electron recovery increased from ( $10.76 \pm 1.60$ )% to ( $35.06 \pm 0.99$ )% with the cathode potential dropping from  $-0.3$  to  $-1.1$  V. These results showed that more electrons flowed into ammonium at a lower cathode potential. For heterotrophic denitrifying bacteria, the electrons passed through electron transport



**Fig. 2** Effect of different cathode potential on dissimilatory nitrate reduction by strain MBR using nitrate ( $100$  mg  $NO_3^-$ -N/L) as the sole electron acceptor. (a) based on nitrogen balance calculations ( $\Delta Ni = Ni_{\text{effluent}} - Ni_{\text{influent}}$ ) ( $Ni$ :  $NO_3^-$ -N,  $NO_2^-$ -N,  $NH_4^+$ -N and  $N_2$ ); (b) based on final coulombic efficiency.

chains, which contained NADH, flavoproteins, iron-sulfur proteins, Coenzyme Q and Cytochrome, to the electron acceptors (nitrate, nitrite, nitric oxide and nitrous oxide) (Clark et al., 2009). According to the previous studies, *Pseudomonas* performed fermentative DNRA (Burgin and Hamilton, 2007). *Pseudomonas* contained a type of nitrite reductase (EC 1.7.2.2) which could accept 6 electrons from ferricytochrome *c* to reduce nitrite into ammonium in a single step (Zumft, 1997). When the working electrode potential dropped, the electron transport may be changed. More electrons flowed from electrodes into ferricytochrome *c* which provided these electrons for DNRA. From an energetic standpoint, denitrification should be theoretically favored over DNRA by comparing the potential free energy of total denitrification ( $\text{NO}_3^- \rightarrow \text{N}_2$ ,  $-2669$  kJ/mol glucose,  $\text{NO}_3^-/\text{N}_2$   $E^\circ = 0.73$  V, vs. NHE) and DNRA ( $-1796$  kJ/mol glucose) (Gottschalk, 1986). However, based on a comparison of electron-accepting capacity of denitrification ( $\text{NO}_3^- \rightarrow \text{N}_2$ , 5e per mole  $\text{NO}_3^-$ ) and DNRA ( $\text{NO}_3^- \rightarrow \text{NH}_4^+$ , 8e per mole  $\text{NO}_3^-$ ), DNRA could be favored over denitrification in lacking of electron acceptors (Tiedje et al., 1982). In this study, an electrode, whose power supply capacity could be regulated by controlling its potential, was served as electron donor. Electron donor/acceptor ratios could be easily controlled by potentials. Therefore, the findings of this study supported the hypothesis that the ratio of electron donors to acceptors was an important factor in partitioning of nitrate reduction between denitrification and DNRA (Tiedje et al., 1982).

### 2.3 Effect of initial nitrate concentration on dissimilatory nitrate reduction in strain MBR

As seen from Fig. 3, the rate of denitrification and DNRA at the same potential ( $-0.9$  V) in BES were tested. The data shown in Fig. 3a showed that the nitrate removal rate increased from  $(2.08 \pm 0.25)$  to  $(18.48 \pm 0.27)$  mg  $\text{NO}_3^-$ -N/(L·day) with an initial nitrate concentration varied from  $(29.09 \pm 0.24)$  to  $(490.97 \pm 3.49)$  mg  $\text{NO}_3^-$ -N/L. The nitrate

removal rate is higher than the reported value ( $17.85$  mg  $\text{NO}_3^-$ -N/(L·day) (Park et al., 2005). The increase of nitrate removal rate could be explained as the improvement of the mass transfer rate of the substrate. From the point of reaction kinetics, the relationship between nitrate reduction rate and nitrate concentration in the BER could be described by Monod equation (Park et al., 2005). The nitrate reduction rate increased with the nitrate concentration in a range of nitrate concentrations. While the nitrate removal rate in the abiotic cathode chamber (control) was  $0 \sim (1.19 \pm 0.01)$  mg  $\text{NO}_3^-$ -N/(L·day) which demonstrated that the strain MBR further involved of the nitrate reduction in the BES.

Figure 3b shows that the formation rates of nitrogen products increased with the initial nitrate concentration rising. The accumulated nitrite level increased as the initial nitrate concentration increased, which was consistent with previous studies (Park et al., 2005). The corresponding ratio of the  $\text{N}_2$  production rate to ammonium was 0.12, 0.57, 1.43, 2.18, 2.98 and 3.13 with an initial nitrate concentration of  $(29.09 \pm 0.24)$ ,  $(90.48 \pm 0.65)$ ,  $(190.98 \pm 1.49)$ ,  $(276.93 \pm 3.27)$ ,  $(350.68 \pm 2.72)$  and  $(490.97 \pm 3.49)$  mg  $\text{NO}_3^-$ -N/L, respectively. The findings suggested that DNRA had an advantage over denitrification under a low nitrate concentration with a certain cathode potential.

### 2.4 Effect of initial nitrate concentration on N transformation rate and coulombic efficiency in strain MBR

As seen from Fig. 4a, in batch tests, the conversion level of nitrate to  $\text{N}_2$  increased from  $(10.45 \pm 1.51)\%$  to  $(54.27 \pm 2.04)\%$  with the initial nitrate concentration varying from  $(29.09 \pm 0.24)$  to  $(490.97 \pm 3.49)$  mg  $\text{NO}_3^-$ -N/L at  $-0.9$  V. However, the transformation rate of nitrate to ammonium decreased from  $(86.75 \pm 1.16)\%$  to  $(17.32 \pm 1.48)\%$  as the initial nitrate concentration increasing. The transformation rate of DNRA was over 8-fold higher than denitrification at an initial nitrate concentration of  $(29.09 \pm 0.24)$  mg  $\text{NO}_3^-$ -N/L. The results indicated that denitrification was inclined

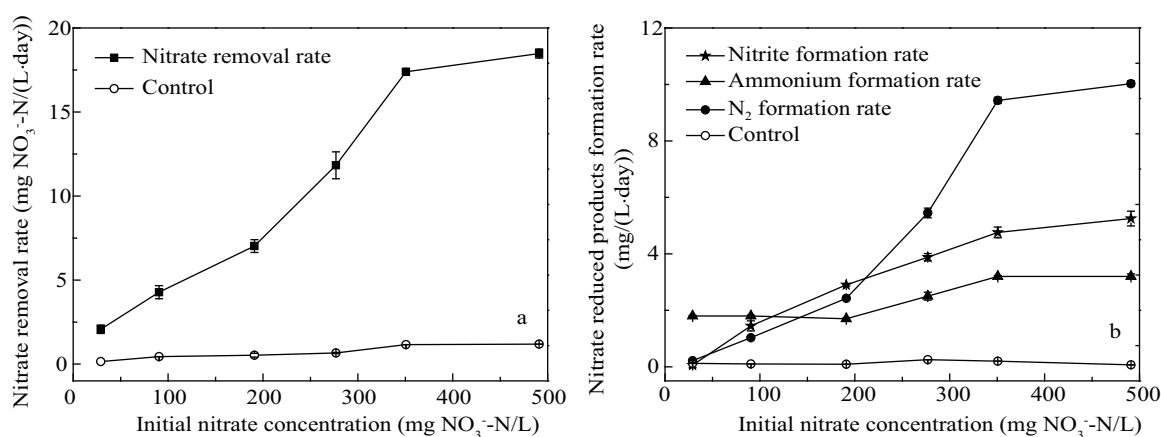
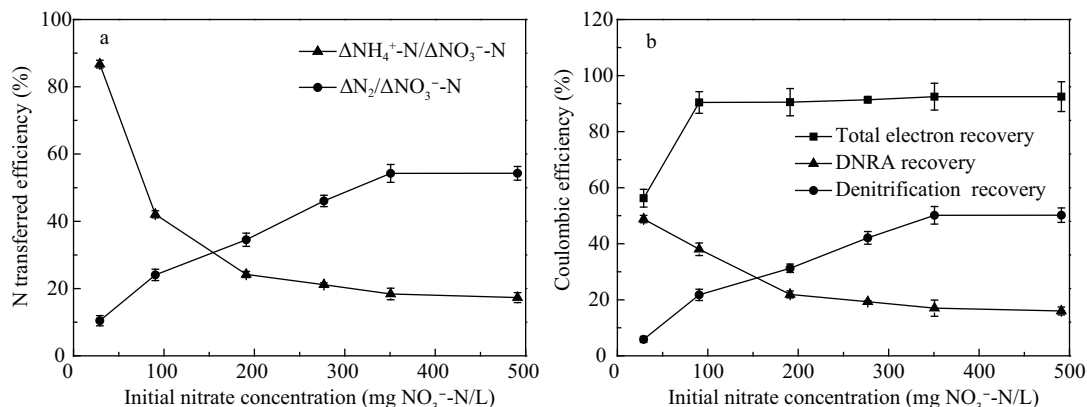


Fig. 3 Effect of various initial nitrate concentrations on dissimilatory nitrate reduction by strain MBR using an electrode poised at  $-0.9$  V (vs. Ag/AgCl) as the sole electron donor. (a) nitrate reduced rate; (b) accumulation rate of nitrate reduced products.





**Fig. 4** Effect of various initial nitrate concentrations on dissimilatory nitrate reduction by strain MBR using an electrode poised at  $-0.9$  V (vs. Ag/AgCl) as the sole electron donor. (a) based on nitrogen balance calculations ( $\Delta Ni = Ni_{\text{effluent}} - Ni_{\text{influent}}$ ) (Ni:  $\text{NO}_3^-$ -N,  $\text{NO}_2^-$ -N,  $\text{NH}_4^+$ -N and  $\text{N}_2$ ), (b) based on final coulombic efficiency.

to appear with a high electron acceptor concentration, whereas DNRA dominated the nitrate reduction process under a lower electron acceptor ( $\text{NO}_3^-$ ) concentration.

The electron recovery for nitrate reduction in BESs was shown in **Fig. 4b**. The data also suggested that DNRA electron recovery decreased with the increase of the initial nitrate concentration, whereas both denitrification electron recovery and the total electron recovery shared the similar trends that increased with a higher initial nitrate concentration. The maximum ( $48.79 \pm 1.32\%$ ) and the minimum ( $16.02 \pm 1.41\%$ ) DNRA electron recovery were observed at an initial nitrate concentration of ( $29.09 \pm 0.24$ ) and ( $490.97 \pm 3.49$ ) mg  $\text{NO}_3^-$ -N/L, respectively. On the contrary, the maximum ( $50.19 \pm 2.59\%$ ) denitrification electron recovery was observed at an initial nitrate concentration of ( $490.97 \pm 3.49$ ) mg  $\text{NO}_3^-$ -N/L. These findings demonstrated that significant DNRA dominated the nitrate consumption process under higher ratios of electron donors to acceptors, but denitrification was favored under lower ratios of electron donors to acceptors (Kelso et al., 1997; Tugtas and Pavlostathis, 2007). In comparison with the traditional researches ( $\text{C}/\text{NO}_3^-$ , oxidation status and pH), this study directly proved the key factor of electron donor/acceptor ratios that substantially influenced the competition between denitrification and DNRA in BESs. There was no disturbance of organic carbon source and the nature of the microflora in the research environment. In addition, the results of this study supported the opinion that  $\text{C}/\text{NO}_3^-$  functioned as electron donor/acceptor ratios to regulate nitrate distribution between DNRA and denitrification (Tiedje et al., 1982).

### 3 Conclusions

This study systematically analyzed the effect of cathode potential and nitrate concentration on regulating nitrate partitioning between denitrification and DNRA in BESs.

The results revealed that strain MBR was more inclined to reduce nitrate through the DNRA process as cathode potential dropping. On the contrary, denitrification prevailed against DNRA under cathode potential limited or high initial nitrate concentration conditions. In addition, the findings of this study supported the opinion that  $\text{C}/\text{NO}_3^-$  ratio functioned as electron donor/electron acceptor ratios to regulate nitrate partitioning between denitrification and DNRA. Our work improves and completes the understanding of the nitrate reduction and nitrate removal in BESs. The result has potential value for the development of bioelectrochemical technology in nitrate removal.

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