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How to ascertain the importance of autotrophic denitrification process in a bioelectrochemical system



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HIGHLIGHTS

- A method for evaluating the role of autotrophic denitrification was established.
- It would be helpful to further study the nitrate removal mechanism in the BESs.
- The possible electron transfer ways in the BES were presented.

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ABSTRACT

The ratio of autotrophic denitrification (R_{auto}) is proposed as a metric to better evaluate the nitrate removal efficiency of bioelectrochemical systems (BESs). Two methods for computing R_{auto} were compared, one based on electron charge transfer and the other on a controlled reaction. The experiment was conducted in a greenhouse with a temperature of 30 ± 2 °C. A reaction with a constant voltage supply was used as a BES and open circuit control was used for the controlled reaction. The results indicated that calculating the R_{auto} based on electron charge transfer was more suitable for evaluating the performance of BESs. Further discussion about the factors influencing the result of this computing method is presented.

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1. Introduction

Bioelectrochemical systems (BESs) have been developed and used in contaminant removal and energy generation during the past few decades (Oh and Logan, 2005; You et al., 2010; Mu et al., 2009; She et al., 2006; Lu et al., 2010). The principle involved is that at a high electrochemical potential, microorganisms can transfer electron charge from a reduced substance to an electron charge acceptor (Virdis et al., 2008). In the BES, electron charge transfer between the soluble with insoluble electron charge donor and acceptor was achieved. During the process of organic oxidation, the microorganisms function as a catalyst. The operation of the BES is determined using the Gibbs free energy change for the overall reaction (Rozendal et al., 2008). For the cathode, an oxide, such as a nitrate, can be used as an electron charge acceptor. A variety of reports have shown that the BES is capable of removing nitrate in the cathode (Rezania et al., 2005; Shahnaz Islam, 1998; Virdis et al., 2009). If organics are added to the anode, the electric-

ity can be generated with nitrate removal. It is promising to treat wastewater, which contains nitrate and organics, and generate electricity.

Recently, there has been much research on applying BESs to nitrate removal (Kondaveeti and Min, 2013; Rezania et al., 2005; Zhou et al., 2007). The specific mechanism of denitrification in the cathode may differ depending on whether hydrogen is produced or not (Clauwaert et al., 2007; Sakakibara and Kuroda, 1993). However, it is generally recognized that the autotrophic denitrification process mainly contributes to nitrate removal in the cathode.

The efficiency of current use has been adopted by researchers to evaluate the performance of the nitrate removal effect in BESs. According to several studies, 100–227% of current use efficiency has been achieved (Prosnansky et al., 2002; Sakakibara and Nakayama, 2001; Zhou et al., 2007). From an energy transfer perspective, even 100% use efficiency is unreasonable. The implication is the computing method may not be valid. In addition, when organics exist in the system, the denitrification process is complicated because it is possible for both the autotrophic and heterotrophic denitrifying bacteria to reduce nitrates.

When applying a BES to treat nitrate-contaminated wastewater that contains some organics, several electron charge transfer

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pathways may exist (Feng et al., 2013). The co-existence of autotrophic and heterotrophic denitrification processes prevail in BESs. Our previous study (Feng et al., 2013; Huang et al., 2013) indicated that the factors of carbon source and C/N ratio are influential in nitrate removal. The biggest advantage for applying BES to treat nitrate-contaminated water is no need of external carbon sources supplying. By distinguishing the autotrophic denitrification from heterotrophic denitrification, the influence of organic on nitrogen removal can be much more clear. In addition, the simple consideration of total nitrogen removal in the BESs while ignoring the external organic input can be avoided. It is essential to determine the proportion of autotrophic and heterotrophic denitrification when systematically evaluating the performance of BESs. As a result, the objective of this study is to contrast the merits and demerits of the two methods used for calculating the ratio of autotrophic denitrification (R_{auto}). One is based on electron charge transfer and the other on setting the controlled reaction. The results can provide a useful reference for researchers further investigating BESs in treating nitrate-contaminated water.

2. Methods

2.1. Apparatus and microorganisms' media

The apparatus was similar to that used in our previous study (Feng et al., 2013). In brief, bottles with a total volume of 1 L were used in this study. Two rectangular graphite electrodes (15 cm long and 8 cm wide) were used as the anode and cathode. The electrodes were fixed in a bottle using glass cement at a distance of 4 cm. The effective volume of the reaction was 500 mL. Experimental apparatus can be seen in Fig. 1. The seed sludge was collected from the Qige wastewater treatment plant (Hangzhou, China). A biofilm was formed and, in our previous study, ran for about 150 days.

Synthetic wastewater, consisting of nutrients and trace elements, was used to simulate nitrate-contaminated water. The nutrient medium consisted of the following reagents dissolved in 1 L of water, according to Prosnansky et al. (2002): 0.176 g NaNO_3 , 4 mg $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2.08 mg KH_2PO_4 , 1.76 mg K_2HPO_4 , 0.96 mg NaCl , 1.12 mg CaCl_2 and 1.92 mg $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. A carbon source and 1 mL of a trace element solution were added to the medium for each experiment. The trace metal solution contained (L^{-1}): 30 mg $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 30 mg $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 10 mg $\text{CuCl}_2 \cdot \text{H}_2\text{O}$, 70 mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 300 mg H_3BO_3 , 600 mg $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 20 mg $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ and 1000 mg tetrasodium EDTA.

2.2. Experiments

The C/N ratio of synthetic wastewater was controlled to a value of 2 in this study. Initially, glucose was used as a carbon source when investigating the feasibility of both computing methods. During the experiment, the current was collected and recorded by a multimeter every 30 s, for about 30 h. After data collection, the nitrate, nitrite, COD, pH of influent and effluent were measured. In addition, the soluble microbial products (SMPs) in the effluent were quantified. Furthermore, the excretion of SMPs in the BES fed with glucose was assessed. The sodium acetate (NaAc) and sodium carboxymethylcellulose (CMC) were then used as simple and complex carbon sources to test and verify the accuracy of both methods under the same operating conditions. In addition, the drawbacks were discussed. All the experiments were conducted in a greenhouse with an ambient temperature of $30 \pm 2^\circ\text{C}$. A constant voltage of 3.5–4.0 V was applied to the system to ensure the initial current of the circuit was 5 mA. The controlled reaction was achieved in the control reactor which was with an open circuit.

2.3. Calculations

To ascertain the best method for calculating the R_{auto} , two calculation processes were conducted as follows. All the calculations presented here were on the hypothesis that the nitrate was reduced to nitrogen gas and the removal of nitrate through cell assimilation was ignored.

(1) R_{auto} based on electron charge transfer

The R_{auto} based on electron charge transfer (expressed as R_{auto1}) was calculated using Eq. (1) according to Liu and Logan (2004) and Liu et al. (2005) by some modification:

$$R_{\text{auto1}} = \left(1 - \frac{Q_{\text{H}}}{(\text{mb}_{\text{nitrate}} \times 5 - \text{mb}_{\text{nitrite}} \times 3) \times F} \right) \times 100\% \quad (1)$$

where $\text{mb}_{\text{nitrate}}$ (mol) and $\text{mb}_{\text{nitrite}}$ (mol) are the removal amounts of nitrate and effluent nitrite amount in the BES, and the numbers 5 and 3 are used to convert moles of nitrate and nitrite to moles of nitrogen gas. Q_{H} (mol) is the total amount of electron charge that is used as a heterotrophic denitrification donor. For the R_{auto1} calculations, it was hypothesized that the organic material was only for the electron charge generated and heterotrophic denitrification, and no other pathways existed. The Q_{H} is given by Eq. (2):

$$Q_{\text{H}} = \frac{4\Delta\text{COD}}{M_{\text{O}_2}} \times F + \int_{t=0}^t I_{\text{b}} dt - \int_{t=0}^t I dt \quad (2)$$

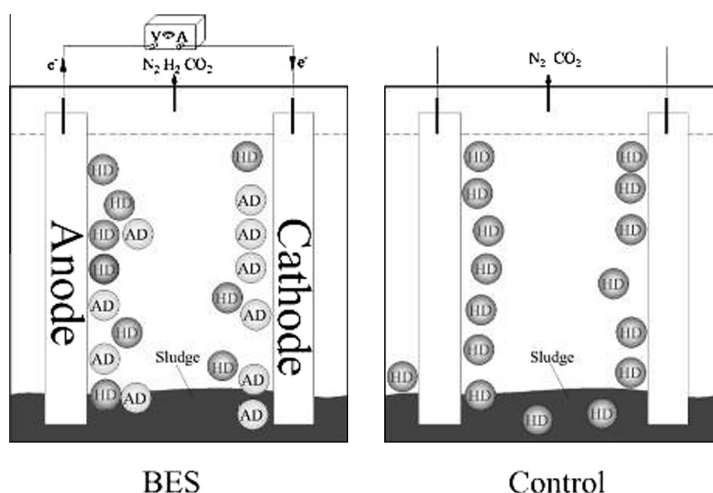


Fig. 1. Experimental setup for bioelectrochemical system (BES) and control reactors. AD, autotrophic denitrifier; HD, heterotrophic denitrifier.

where M_{O_2} (32 g/mol) is the molecular weight of oxygen, ΔCOD (g) is the amount of COD removed before and after the experiment, 4 is used to convert moles of COD to moles of electron charge, $F = 96485$ C/mol is Faraday's constant, I_b (mA) and I (mA) are the background and measured currents, and dt (s) is the interval (30 h) over which data were collected. I_b is calculated according to the conductivity of solution, as given by Eq. (3):

$$I_b = \frac{\kappa}{\kappa_0} I_0 \quad (3)$$

where κ_0 ($\mu S/cm$) and I_0 (mA) are the initial conductivity and current of the solution, and κ ($\mu S/cm$) is the measured conductivity of the solution.

(2) R_{auto} based on a controlled reaction

The R_{auto} based on nitrogen removal (expressed as R_{auto2}) is calculated using Eq. (4) according to Huang et al. (2013):

$$R_{auto2} = \frac{(mb_{nitrate} - mb_{nitrite}) - (mc_{nitrate} - mc_{nitrite})}{mb_{nitrate} - mb_{nitrite}} \times 100\% \quad (4)$$

where $mc_{nitrate}$ ($mmol L^{-1}$) and $mc_{nitrite}$ ($mmol L^{-1}$) are the removal amounts of nitrate and effluent nitrite concentrations in the controlled reactions.

2.4. Analytical method

All samples were filtrated with 0.45 μm filter membrane before analysis. The ultraviolet spectrophotometric screening method and colorimetric method were used to measure nitrate and nitrite, respectively, according to standard methods (APHA, 1998). The current was collected by an intelligent digital multimeter (UNI-T Company, Shanghai, China), which was connected to a computer. COD was measured using a DR2800 spectrophotometer (HACH Company, Loveland, CO, USA). The conductivity was measured using a FiveEasy Plus conductivity meter (Mettler Toledo, Greifensee, Switzerland).

The definition of SMPs according to Barker and Stuckey (1999) was adopted, $SMP = SCOD - 1.07 [HAc] - 1.51 [HPr] - 1.82 [Hbu] - 1.07 [Glu]$, where SCOD is the soluble chemical oxygen demand, concentrations are in mg/L, HAc is acetic acid, HPr is propionic acid, Hbu represents *iso*- and *n*-butyric acids, and Glu is glucose. The values 1.07, 1.51, and 1.82 are conversion factors assuming the complete oxidation of the volatile acids and glucose to CO_2 and H_2O . The detection of glucose and volatile fatty acids was the same as in our previous study (Feng et al., 2013). The colorimetric method and gas chromatograph were used to detect glucose and volatile fatty acids, respectively.

3. Results

3.1. The results of the two computing methods

The R_{auto} calculated using the two different methods is shown in the Table 1. The R_{auto1} and R_{auto2} were 107.1% and 79.7%. The total organic electron charge based on COD was 158.96 C. The total electron charge transformed through electrogenesis was 183.61 C according to the calculation of I_b and I . As a result, the value of Q_H was negative (−24.65 C). It was generally considered that the SMP could be produced during the biological treatment (Ichihashi

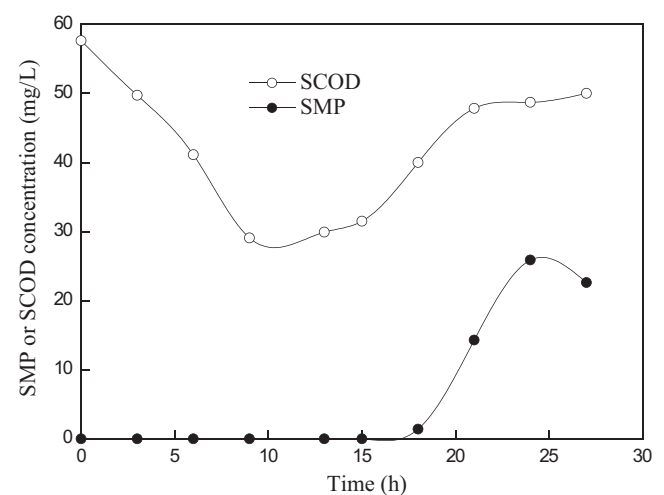


Fig. 2. The SMP excretion and SCOD variation in the BESs.

Table 2

The R_{auto1} calculated by amending the ΔCOD .

$\int_0^t Idt$ (C)	$\int_0^t I_b dt$ (C)	$\frac{4\Delta COD}{M_{O_2}} \times F$ (C)	Q_H (C)	R_{auto1} (%)
1035.54	851.93	273.47	89.86	73.0

et al., 2006; Laspidou and Rittmann, 2002). Microorganisms synthesized and excreted the SMPs for a purpose. They contributed to the effluent COD and biochemical oxygen demand during biological treatment processes (de Silva and Rittmann, 2000a,b). As a result, the “ ΔCOD ” in the formula should be amended.

The R_{auto2} based on a controlled reaction was calculated by setting a controlled reactor. With the co-existence of autotrophic and heterotrophic denitrification processes in the BES, the contribution of heterotrophic denitrification was excluded by the reaction without a supply voltage. However, the microbial community could be changed after running the two reactions under different working conditions. Therefore, the influence of microorganisms on the computation needs to be further studied.

3.2. The amendment of ΔCOD in the R_{auto1} calculator formula

According to Xie et al. (2012), the SMPs refer to the products of microbial metabolisms and proteins, while carbohydrates and humic substances contribute to its composition. In our previous study, the SMPs make up the majority of the effluent SCOD in the BES fed with glucose (Feng et al., 2013). The variation of SCOD and excretion of SMPs in the BES fed with glucose is shown in Fig. 2. After running the system for a period of time, it can be seen that the concentration of SCOD in the solution decreased first and then increased. The SMPs could possibly contribute to the increase in the SCOD. The SMPs in the solution were detectable after 18 h. Therefore, the sampling time could be used to determine the concentration of SMPs in the effluent.

Table 1

The ratio of the calculated autotrophic denitrification based on different computing methods.

R_{auto1} based on electron charge transfer					R_{auto2} based on a controlled reaction		
$\int_0^t Idt$ (C)	$\int_0^t I_b dt$ (C)	$\frac{4\Delta COD}{M_{O_2}} \times F$ (C)	Q_H (C)	R_{auto1} (%)	$mb_{nitrate} - mb_{nitrite}$ (mol)	$mc_{nitrate} - mc_{nitrite}$ (mol)	R_{auto2} (%)
1035.54	851.93	158.96	−24.65	107.1	0.69	0.14	79.7

Table 3
The R_{auto} of the BES with different carbon sources.

Carbon source	R_{auto1} based on electron charge transfer					R_{auto2} based on a controlled reaction		
	$\int_0^t I dt$ (C)	$\int_0^t I_b dt$ (C)	$\frac{4\Delta\text{COD}}{\text{MO}_2} \times F$ (C)	Q_H (C)	R_{auto1} (%)	$mb_{\text{nitrate}} - mb_{\text{nitrite}}$ (mol)	$mc_{\text{nitrate}} - mc_{\text{nitrite}}$ (mol)	R_{auto2} (%)
NaAc	802.16	717.67	420.00	335.67	14.85	0.79	0.32	59.49
CMC	766.18	756.81	145.69	136.32	32.81	0.37	0.07	81.08

In Table 1, the ΔCOD used in calculating the R_{auto1} was the difference value between the influent and effluent SCOD. As the SMPs made up the major percentage of the effluent SCOD, it was not reasonable to calculate the ΔCOD without excluding the amount of SMPs in the effluent. It would clearly reduce the value of ΔCOD and lead to a high result. Consequently, the value of R_{auto1} was recalculated by amending the ΔCOD . The revised ΔCOD is as follows:

$\Delta\text{COD} = \text{SCOD}_{\text{in}} - (\text{SCOD}_{\text{eff}} - \text{SMPs})$, where SCOD_{in} (g) and SCOD_{eff} (g) were the amounts of COD in the influent and effluent; and the SMPs (g) was the amount of the SMP in the effluent. The calculated results are shown in Table 2. R_{auto1} was reduced to 73.0%, which was the same level as for R_{auto2} .

4. Discussion

4.1. The comparison of the two computing methods

The fundamental principle of the R_{auto2} calculation was to exclude the effect of heterotrophic denitrification in the BES through a controlled reaction. The influence of the electric field in heterotrophic denitrifying bacteria in this study was not considered. Once electricity was applied to the bioreaction, the bacteria activity or viability would change (Wei et al., 2011). Alshawabkeh et al. (2004) found that small DC fields (between 0.57 and 1.14 V/cm) would enhance the biological removal of COD. In addition, it was found that the electric field would stimulate the heterotrophic bacterial and increase the denitrification rate (Beschkov et al., 2004). In this study, the living environment of heterotrophic denitrifying in a controlled reaction was not the same as in the BES. The ratio of heterotrophic denitrification in the BES could be higher than that in the controlled reaction. As a result, the calculation ratio of autotrophic denitrification based on a controlled reaction was higher than the truth value. In Table 3, it can be seen that R_{auto2} was significantly higher than R_{auto1} . However, this method was simple and easy to operate.

The calculation method based on electron charge transfer was an in situ method. The deviations of different systems could be avoided. Hence, the R_{auto} based on electron charge transfer was further considered as it showed a more accurate result. In the BES, the organic material could be used to generate electron charge or reduce nitrate. The nitrate could be removed by autotrophic or heterotrophic denitrification. The electron charge transfer between the electrode and microbes was complex and it could influence R_{auto1} . As a result, it requires precise measurement.

4.2. Factors influencing the R_{auto1}

The whole calculation for the R_{auto1} was as follows, by combining Eqs. (1)–(3):

$$R_{\text{auto1}} = \left(1 - \frac{\frac{4\Delta\text{COD}}{\text{MO}_2} \times F + \int_{t=0}^t \frac{\kappa}{\kappa_0} I_0 dt - \int_{t=0}^t I dt}{(mb_{\text{nitrate}} \times 5 - mb_{\text{nitrite}} \times 3) \times F} \right) \times 100\% \quad (5)$$

From Eq. (5), the ΔCOD , κ and I clearly influence the result of R_{auto1} . ΔCOD was amended by considering the SMPs excretion in the system (details can be seen in Section 3.2). It was hypothesized

that there were only two organic use methods: electrogenesis and heterotrophic denitrification. Ignoring other pathways in the organic material would induce a lower R_{auto1} than the true value. In addition, the electron charge transfer efficiency between the microbes and anode was hypothesized to be 100%. Several electron charge transfer mechanisms, such as nanowires, direct membrane-associated electron transfer or electron mediators of shuttles, were proved to exist in BES (Bond and Lovley, 2003; Rabaey et al., 2005; Reguera et al., 2005). No matter which electron charge transfer mechanism is considered, the efficiency cannot reach 100%. It would also reduce R_{auto1} .

4.3. Applicability

Biological denitrification was a terminal nitrogen reduction method that contained autotrophic and heterotrophic processes. In recent years, the electric field has been applied in the biological denitrification. Compared with the traditional heterotrophic denitrification, the electric field could improve the autotrophic denitrification and fewer or even no external carbon sources are needed. In the BESs, two different types of denitrifying bacteria could possibly co-exist. The simple consideration of total nitrogen removal in the BESs while ignoring the external organic input was not appropriate because pure good nitrogen removal performance could not completely represent the role of BESs. High nitrogen removal efficiency could be achieved by adding adequate carbon sources. However, BESs would not be superior if plenty of organic materials existed. The index of R_{auto} could be used to better understand the role of heterotrophic and autotrophic denitrification in the BESs. Combining other parameters such as energy production and consumption would lead us to a more comprehensive understanding of BESs.

5. Conclusions

The ratio of autotrophic denitrification, which was calculated based on electron charge transfer, was suitable for evaluating BESs compared with the results that were calculated based on a controlled reaction. The efficiency of electron charge transfer and organic material use could influence the results. Other parameters such as energy production and consumption need to be studied to fully understand BESs.

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