



The effect of C/N ratio on nitrogen removal in a bioelectrochemical system



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HIGHLIGHTS

- Influence of C/N ratio on autotrophic and heterotrophic denitrification were investigated.
- Electron transform and utilization efficiency of anode and cathode were calculated.
- Effects of C/N ratio on microbial metabolism were discussed.

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ABSTRACT

The effect of C/N ratios of 2, 2.7, and 3.5 on nitrogen removal in a bioelectrochemical system (BES) was investigated. Starch was used as a carbon source for the electrogenesis phenomenon we observed in a previous study. The results showed that an increased C/N ratio helped the BES to remove nitrate and depress nitrite accumulation but did not increase autotrophic denitrification. Nitrate and total nitrogen removal were increased from $0.69 \pm 0.02 \text{ g m}^{-3} \text{ h}^{-1}$ to $1.09 \pm 0.16 \text{ g m}^{-3} \text{ h}^{-1}$, and from $0.52 \pm 0.08 \text{ g m}^{-3} \text{ h}^{-1}$ to $0.97 \pm 0.06 \text{ g m}^{-3} \text{ h}^{-1}$, respectively, when the C/N ratio was increased from 2.0 to 3.5. However, the autotrophic denitrification ratio decreased from 72.74% to 50.23% with the same increase in the C/N ratio. High C/N ratios postponed the excretion of soluble microbial products and increased electrogenesis, but did not improve the anode transformation efficiency.

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1. Introduction

Bioelectrochemical systems (BESs) have recently emerged as potentially interesting technologies for producing energy from wastewater (Rozendal et al., 2008; Call and Logan, 2008). In BESs, microorganisms catalyze electrochemical reactions through interactions with the electrodes (Pham et al., 2009). In the past few years, several novel and cost-effective BESs, such as a microbial fuel cell (MFC) (Liu et al., 2005), a microbial electrolysis cell (MEC) (Call and Logan, 2008), and a biofilm-electrode reactor (Sakakibara and Nakayama, 2001), have been developed to treat wastewater and have achieved good carbon and nitrogen removal and energy generation (Min et al., 2005; Pant et al., 2010). BESs are promising technologies for treating wastewater because the wastewater can be used as a fuel source as part of its treatment (Logan, 2010).

In BESs, microorganisms are combined with the electrodes, and a circuit is formed with the electrodes using wires. Bacteria obtain energy from the oxidation of organic compounds or inorganic species by mediating chemical reactions that typically involve inter-compound electron transfer (Rivett et al., 2008). In a BES, electron exchange remains at equilibrium because the anode loses electrons and the cathode accepts them. Either nitrate or oxygen can be used as an electron acceptor by microorganisms in the cathode, and the bacteria in the anode can use organic chemicals to produce either biomass or a current. Generally, the lower the biomass yield the better the cell functions (Rabaey et al., 2003). High electron transformation and utilization is a key to an efficient BES, and this parameter is used to evaluate the efficiency of the system.

Although BESs show good performance in wastewater treatment, the specific nitrate removal pathway is unclear. Electrons can be transferred to the anode by direct membrane-associated electron transfer (Bond and Lovley, 2003), electron mediators (Rabaey et al., 2005), or nanowires (Reguera et al., 2005). However, when using BESs for nitrate removal, organic chemicals could be used by the microorganisms for either denitrification or

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electrogenesis. The electron transfer process is complicated and remains unexplored for the case that the organics are added to the system.

In a previous study (Feng et al., 2013), we investigated the effects of different carbon sources on nitrogen removal by BESs. The results showed that a BES fed with starch gave the highest accumulation of nitrite and production of soluble microbial products (SMPs) of the three organic carbon sources tested. We also found that the BESs were capable of using organic chemicals to generate electricity, and a maximum current of 11.0 mA was achieved in the BES fed with starch at a C/N ratio of 3.5 and 3.8–4.0 V constant voltage. We achieved the coupling of nitrate removal and organic electrogenesis in the BESs. In BESs fed with carbon sources, the electrons can be provided by organic chemicals or an external power supply. The influence of different C/N ratios on nitrogen removal is still unknown.

In the study presented here, we used starch as a carbon source and aimed to further investigate the effect of the chemical oxygen demand/nitrate (i.e., C/N) ratio on nitrogen removal performance in a BES. C/N ratios of 2.0, 2.7, and 3.5 were tested, which are below the theoretical heterotrophic ratio but allow heterotrophic microbes to grow unrestrictedly. NaHCO_3 was used as an inorganic carbon source.

2. Methods

2.1. Experimental setup

The experiment was conducted in a reactor with a total volume of 1 L. The apparatus was similar to that used in our previous study (Feng et al., 2013). Rectangular graphite electrodes (length 15 cm, width 8 cm) were fixed in the reactor with an inter-electrode distance of 4 cm. The effective volume of the reactor was 450 mL. The reactor was supplied with DC power. Control reactors were set up in an identical way but with open electrical circuits.

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 ; 1.92 mg $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. A carbon source (starch or NaHCO_3) 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 $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$; 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 $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$; 1000 mg tetrasodium EDTA.

The synthetic wastewater in the reactor was replaced every 24 h and the temperature was maintained at $30 \pm 2^\circ\text{C}$ in a greenhouse. The procedure of wastewater replacing was conducted three times by siphon in order to reduce the residual. The bacteria used were accumulated and cultured in our previous study. In brief, sludge collected from the secondary sedimentation tank of a wastewater treatment plant (Hangzhou, China) was anoxically cultivated for about one week. A $30 \text{ mg NO}_3^- \text{N L}^{-1}$ synthetic wastewater was added and the initial C/N ratio was set at 3.5. After another week, a current (5 mA) was applied to the reactor, to allow it to acclimatize.

The experiments presented here were conducted in two stages. First, the effect of C/N ratios of 2.0, 2.7, and 3.5 on nitrogen removal in the BESs and control reactors were investigated at a constant current of 5 mA. A BES fed with NaHCO_3 , as an inorganic carbon source, was also investigated. The reactors were run for 10 days at each stable C/N ratio and then samples were taken and analyzed for 3 consecutive days. The mean of the analytical results was used. Second, the nitrogen removal process in the BESs was studied by

applying a constant voltage of 3.8–4.0 V. This voltage ensured that the initial current in the circuit was 5 mA. The different C/N ratios were investigated by injecting the carbon and nitrogen sources into the BESs.

2.2. Calculations

The current, I , was measured using an intelligent digital multimeter. Excluding the current generated from starch, the background value of the current, I_b , was calculated according to the conductivity of the solution, as shown in Eq. (1),

$$I_b = \frac{\kappa}{\kappa_0} I_0 \quad (1)$$

where κ ($\mu\text{S/cm}$) is the measured conductivity of the solution, and κ_0 ($\mu\text{S/cm}$) and I_0 (mA) are the initial conductivity of, and current in, the solution, respectively. The total electrical quality, Q_T (C), was calculated according to Eq. (2), presented by Call and Logan (2008).

$$Q_T = \int_{t=0}^t I dt \quad (2)$$

where I (mA) is the current measured in the experiment, and dt (s) is the interval (30 h) over which data were collected. The total background electrical quality, Q_b , was given by

$$Q_b = \int_{t=0}^t I_b dt \quad (3)$$

and the electrical quality, Q_S (C), transformed from the starch was calculated using Eq. (4).

$$Q_S = Q_T - Q_b \quad (4)$$

According to Logan et al. (2008), on a chemical oxygen demand (COD) basis, the theoretical maximum amount of electrons produced from organic chemicals (Q_C) is given by

$$Q_C = \frac{4\Delta\text{COD}}{\text{MO}_2} \times F, \quad (5)$$

where MO_2 (32 g/mol) is the molecular weight of oxygen, ΔCOD (g) is the amount of COD removed, the value 4 is used to convert moles of COD to moles of e^- , and F is Faraday's constant (96,485 C/mol e^-). The efficiency of current generation at anode E_A (%) was calculated using Eq. (6).

$$E_A = \frac{Q_S}{Q_C} \times 100\% \quad (6)$$

The coulombic efficiency of the cathode, E_C , is given by

$$E_C = \frac{(\text{mb}_{\text{nitrate}} \times 5 - \text{mb}_{\text{nitrite}} \times 3) \times F + Q_S - Q_C}{Q_T} \times 100\% \quad (7)$$

where $\text{mb}_{\text{nitrate}}$ (mol) is the amount of nitrate removed from the BES system, $\text{mb}_{\text{nitrite}}$ is the amount of nitrite in the effluent (mol), and the values 5 and 3 are used to convert moles of nitrate and nitrite to moles of nitrogen, respectively. The autotrophic denitrification process ratio, R_{auto} , can be calculated as follows:

$$R_{\text{auto}} = \left(1 - \frac{Q_C - Q_S}{(\text{mb}_{\text{nitrate}} \times 5 - \text{mb}_{\text{nitrite}} \times 3) \times F} \right) \times 100\% \quad (8)$$

2.3. Analytical method

All samples were passed through a $0.45 \mu\text{m}$ membrane filter. The phenate method was used to measure nitrogen as NH_4^+ (called "ammonia nitrogen"). Total nitrogen (TN) and nitrogen as NO_3^- were measured using an ultraviolet spectrophotometric screening method. Nitrogen as NO_2^- was measured using a colorimetric

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