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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 $0.69 \pm 0.02\ g\ m^{-3}\ h^{-1}$ to $1.09 \pm 0.16\ g\ m^{-3}\ h^{-1}$, and from $0.52 \pm 0.08\ g\ m^{-3}\ h^{-1}$ to $0.97 \pm 0.06\ g\ m^{-3}\ h^{-1}$, 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.

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 intercompound 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 $MgSO_4 \cdot 7H_2O$; 2.08 mg KH_2PO_4; 1.76 mg K_2HPO_4; 0.96 mg NaCl; 1.12 mg $CaCl_2$; 1.92 mg $FeCl_3 \cdot 6H_2O$. 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 $MnCl_2 \cdot 4H_2O$; 30 mg $Na_2MO_4 \cdot 2H_2O$; 10 mg $CuCl_2 \cdot H_2O$; 70 mg $ZnSO_4 \cdot 7H_2O$; 300 mg H_3BO_3; 600 mg $CoCl_2 \cdot 6H_2O$; 20 mg $NiCl_2 \cdot 6H_2O$; 1000 mg tetrasodium EDTA.

The synthetic wastewater in the reactor was replaced every 24 h and the temperature was maintained at 30 ± 2 °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 mg NO_3^-—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 (μs/cm) is the measured conductivity of the solution, and κ_0 (μs/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

$$Q_C = \frac{4\Delta COD}{MO_2} \times F, \quad (5)$$

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

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

where $mb_{nitrate}$ (mol) is the amount of nitrate removed from the BES system, $mb_{nitrite}$ 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:

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

2.3. Analytical method

All samples were passed through a 0.45 μm 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 method. Standard methods (APHA, 1998) were used. COD was measured using a DR2800 spectrophotometer (HACH Co., Loveland, CO, USA). The current was measured using an intelligent digital multimeter (UNI-T Co., Shanghai, China). Conductivity was measured using a FiveEasy Plus conductivity meter (Mettler Toledo, Greifensee, Switzerland).

Glucose was measured using a method published by Miller (1959). Volatile fatty acids were measured in a 4 μL sample withdrawn from the headspace with a gas-tight syringe, using a gas chromatograph (GC 7890 II, Shanghai Tianmei Science Instrument Co., Ltd., Shanghai, China) equipped with a flame ionization director. The GC carrier gas was nitrogen, and the detector, injection port, and column temperatures were 250, 230, and 180 °C, respectively. SMPs were measured according to Barker and Stuckey (1999), and SMP was defined as: 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.

2.4. Statistical analysis

All data are expressed as mean ± standard error. Statistical analyses (one-way analysis of variance) were conducted using SPSS software (version 19.0; IBM Co., USA).

3. Results and discussion

3.1. The effect of C/N ratio on nitrogen removal in BESs

3.1.1. Nitrate and total nitrogen removal

The effect of the C/N ratio on nitrogen removal in the BES is shown in Fig. 1. Both the nitrate and TN treatment efficiency was improved as C/N increased. The BES fed with $NaHCO_3$ showed worse nitrate and TN removal, with values of 0.513 ± 0.088 and 0.379 ± 0.077 g m^{-3} h^{-1}, respectively, than the three other BESs. Increased C/N ratios clearly helped the BES to remove nitrate. Nitrate and total nitrogen removal was increased from 0.69 ± 0.02 to 1.09 ± 0.16 g m^{-3} h^{-1}, and from 0.52 ± 0.08 to 0.97 ± 0.06 g m^{-3} h^{-1}, respectively, when the C/N ratio was increased from 2.0 to 3.5. Nitrate reduction in bioelectrochemical systems is a complex process. There are several possible nitrate removal pathways in the BES, including biological denitrification, dissimilatory nitrate reduction to ammonia (DNRA) (Shen and Wang, 2011), and electrochemical inorganic reduction (Li et al., 2009). In this study, there was no significant production of ammonia (data shown in Fig. 2b), so biological denitrification appeared to be the primary nitrate removal process. Adding starch to the BES system allowed the possibility of either, or both, heterotrophic and autotrophic denitrification processes to remove nitrate. It can be seen from Fig. 1 that the maximum difference in nitrate removal between the BESs and the control reactors was when the C/N ratio was 2.0. This may be because a low C/N ratio was not conducive to heterotrophic denitrification. Thus, autotrophic denitrification appears to play an important role in reducing nitrate at low C/N ratios. It also was found that in the constant voltage condition, the ratio of autotrophic denitrification was the highest at the C/N ratio of 2.0 (data was shown in Table 1).

3.1.2. By-product formation

Nitrite and ammonia were the main by-products of the electrochemical reduction of nitrate in previous studies (Kerstin et al., 1998; Li et al., 2009). Nitrite was the main by-product in our study, and it greatly inhibited nitrogen removal. Fig. 2b shows that the amount of ammonia nitrogen formed in the control reactors was negligible. A maximum of 2.60 ± 0.71 mg/L of ammonia nitrogen in the BES was formed at a C/N ratio of 2.0. We inferred that DNRA possibly contributed to the production of ammonia. It can be seen from Fig. 2a that 6.71 ± 0.80 mg/L nitrite accumulated in the BES fed with $NaHCO_3$. The presence of organic chemicals caused less nitrite to be accumulated. When the C/N ratio was increased from 2.0 to 2.7, the amount of accumulated nitrite decreased. Zhou et al. (2007) used a three-dimensional biofilm-electrode reactor to remove nitrate and found that nitrite accumulation was increased significantly when the C/N ratio decreased from 2.5 to 0.97. Nitrite accumulation was not observed in the control reactor. Biological denitrification can be divided into two steps, (1) conversion of nitrate to nitrite and (2) conversion of nitrite to gaseous nitrogen. It can be inferred from Fig. 1 that, for the heterotrophic denitrification process in our study, step (1) was the rate-limiting step because no significant nitrite accumulation was observed in the control reactor. However, step (2) could be the rate-limiting step for autotrophic denitrification because a large amount of nitrite was accumulated at a C/N ratio

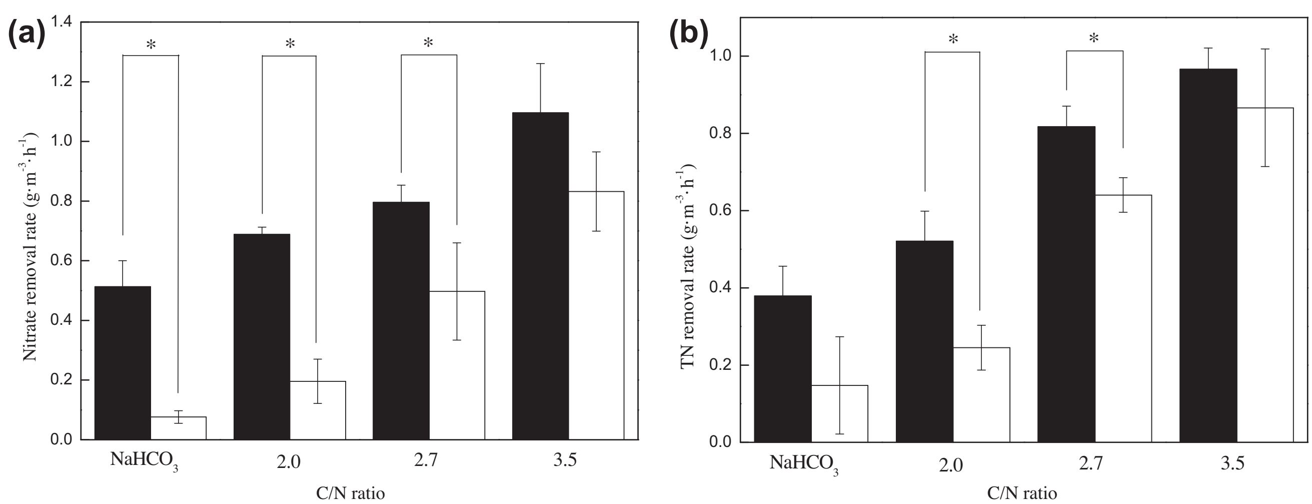

Fig. 1. (a) Nitrate and (b) total nitrogen (TN) removal performance at different C/N ratios and a constant current of 5 mA. Filled bars represent the BESs, unfilled bars represent control reactors. The asterisk (*) indicates that the difference was significant ($p < 0.05$).

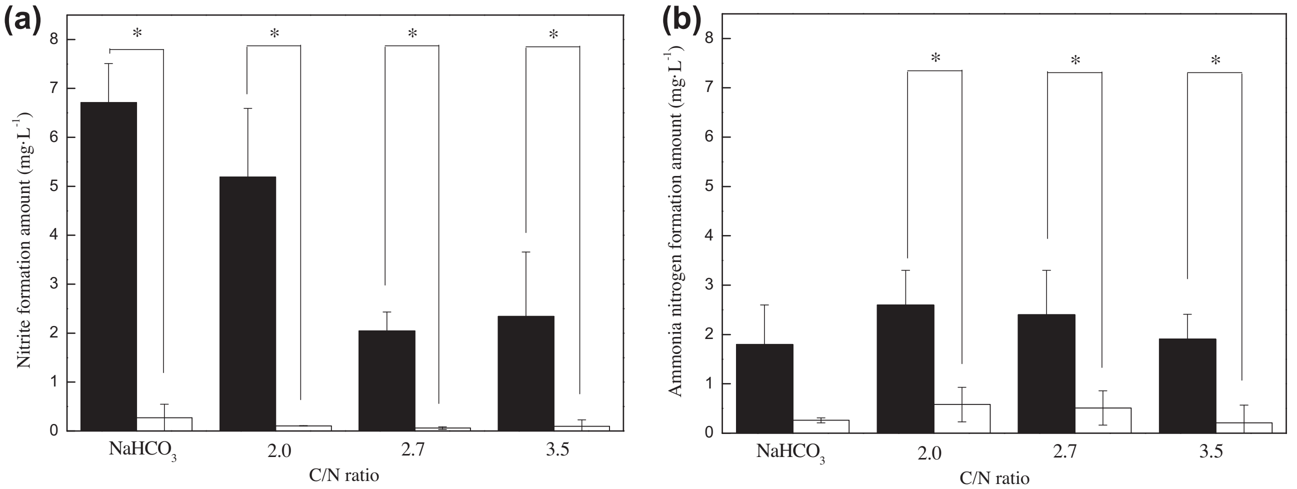

Fig. 2. The influence of C/N ratio on by-product formation, (a) nitrite and (b) nitrogen as ammonia, at a constant current of 5 mA. Filled bars represent the BESs, unfilled bars represent control reactors. The asterisk (*) indicates that the difference was significant ($p < 0.05$).

Table 1
BES performance evaluation parameters.

C/N ratio	Q_T (C)	Q_b (C)	Q_S (C)	Q_C (C)	E_A (%)	E_C (%)	R_{auto} (%)
0	1197.6	1141.5	56.1	128.33	43.72	0.958	13.71
2.0	683.6	536.3	147.3	206.9	71.19	23.27	72.74
2.7	770.4	610.7	159.7	283.2	56.39	26.45	62.28
3.5	750.8	579.6	171.2	317.51	53.92	19.67	50.23

of 2.0. The amount of nitrite accumulated dropped markedly when the C/N ratio was increased from 2.0 to 2.7. Both organic matter and external power could provide electrons to the BES. The total amount of electron donor present varied, and the biological denitrification process was affected when the concentration of organic chemicals was varied.

To fully understand the nitrite production process, the experiment was conducted at a constant voltage of 3.8–4.0 V, and the results are shown in Fig. 3. These data suggest that nitrate was removed gradually over time. However, the nitrate concentration in the effluent did not agree with that interpretation. The effluent nitrate concentration was 12.82 mg/L in the BES without added organic chemicals, and when the C/N ratio was increased from 2.0 to 3.5 the effluent nitrate concentration dropped from 7.75 mg/L to 2.96 mg/L. A similar change was observed for nitrite. The highest amount of nitrite accumulation (7.43 mg/L) was achieved in the BES without organic chemicals. Therefore, adding starch to the reactor could depress the formation of nitrite and the nitrite concentration first increased and then decreased. High C/N ratios resulted in little nitrite accumulation. These findings partially agreed with Zhao et al. (2011), who observed that a low C/N ratio led to a high amount of nitrite accumulated in the BES effluent. High C/N ratios accelerated the growth of heterotrophic denitrifying bacteria in the biofilm, increasing the total denitrification rate (Zhou et al., 2007). Taking biofilm growth and nitrogen removal into account, the heterotrophic denitrification process can be expressed as Eq. (9) (Henze, 1991).

$$(C_6H_{12}O_6)_n + 2.8nNO_3^- + 0.5nNH_4^+ + 2.3nH^+ \rightarrow 0.5nC_5H_7NO_2 + 3.5nCO_2 + 6.4nH_2O + 1.4nN_2 \quad (9)$$

where $C_5H_7NO_2$ represents the molecular formula of microbes, suggested by Hoover and Porges (1952). The molecular formula of starch is expressed as $(C_6H_{12}O_6)_n$ in the equation because starch is a polymer of glucose.

The equation for the oxidization of starch is shown in Eq. (10).

$$(C_6H_{12}O_6)_n + 6nO_2 \rightarrow 6nCO_2 + 6nH_2O \quad (10)$$

Combining Eqs. (9) and (10), the stoichiometric C/N ratio for complete denitrification using starch as the organic carbon source is around 4.89. The C/N ratios we used in this study were lower than the theoretical ratio, so both heterotrophic and autotrophic denitrification processes were responsible for nitrogen removal.

3.2. The effect of C/N ratio on microbial metabolism

3.2.1. The effect of C/N ratio on microbial metabolites

Organic chemicals can be degraded and soluble microbial products (SMPs) can be excreted by the microorganisms in the BESs. The SCODs of the BES effluents are shown in Fig 4. These data revealed that the SCOD increased from 64 mg/L, at a C/N ratio of 2, to 84 mg/L at a C/N ratio of 2.7. The SCOD reached 127 mg/L when the C/N ratio was 3.5. The SCOD for the BES fed with $NaHCO_3$was similar to the SCOD for the C/N ratio of 2.0. It is known that BESs are capable of converting chemical energy from organic pollutants in wastewater directly into electricity or other energetic products such as hydrogen gas (Logan et al., 2008; Rozendal et al., 2008, 2009). In addition, some heterotrophic bacteria might also degrade organic chemicals to form inorganic carbon. In addition to residual organics, SMPs contribute to the effluent SCOD, and the SCOD alone cannot be used to express the level of microbial metabolism.

SMPs are produced by microorganisms as they remove organic pollutants, and it has been found that SMP concentrations can be negative to biological activities and was adverse to treatment effect (Ichihashi et al., 2006; Xie et al., 2012). The concentrations and compositions of SMPs have been found to be affected more by stressful conditions than by the species of bacteria present (Wang and Zhang, 2010). In our study, the changes in SMP concentrations were similar to the changes in SCOD, and the lowest SMP concentration, 61 mg/L, was found in the BES fed with $NaHCO_3$. The SMP concentrations were 64 and 74 mg/L at C/N ratios of 2 and 2.7, respectively, and the SMP concentration increased markedly, to 120 mg/L, at a C/N ratio of 3.5. A high SMP/SCOD ratio (as a percentage) was found for all four conditions, with SMP/SCOD percentages of 94%, 100%, 88%, and 94% found for the C/N ratios of 0, 2, 2.7, and 3.5, respectively. The effluent SCOD was primarily caused by SMPs. A number of factors influence the amount of SMPs produced, and Kuo (1993) cited several possible causes for SMP production. We believe that, in our study, SMPs were probably produced as a response to the current stimulation, which we

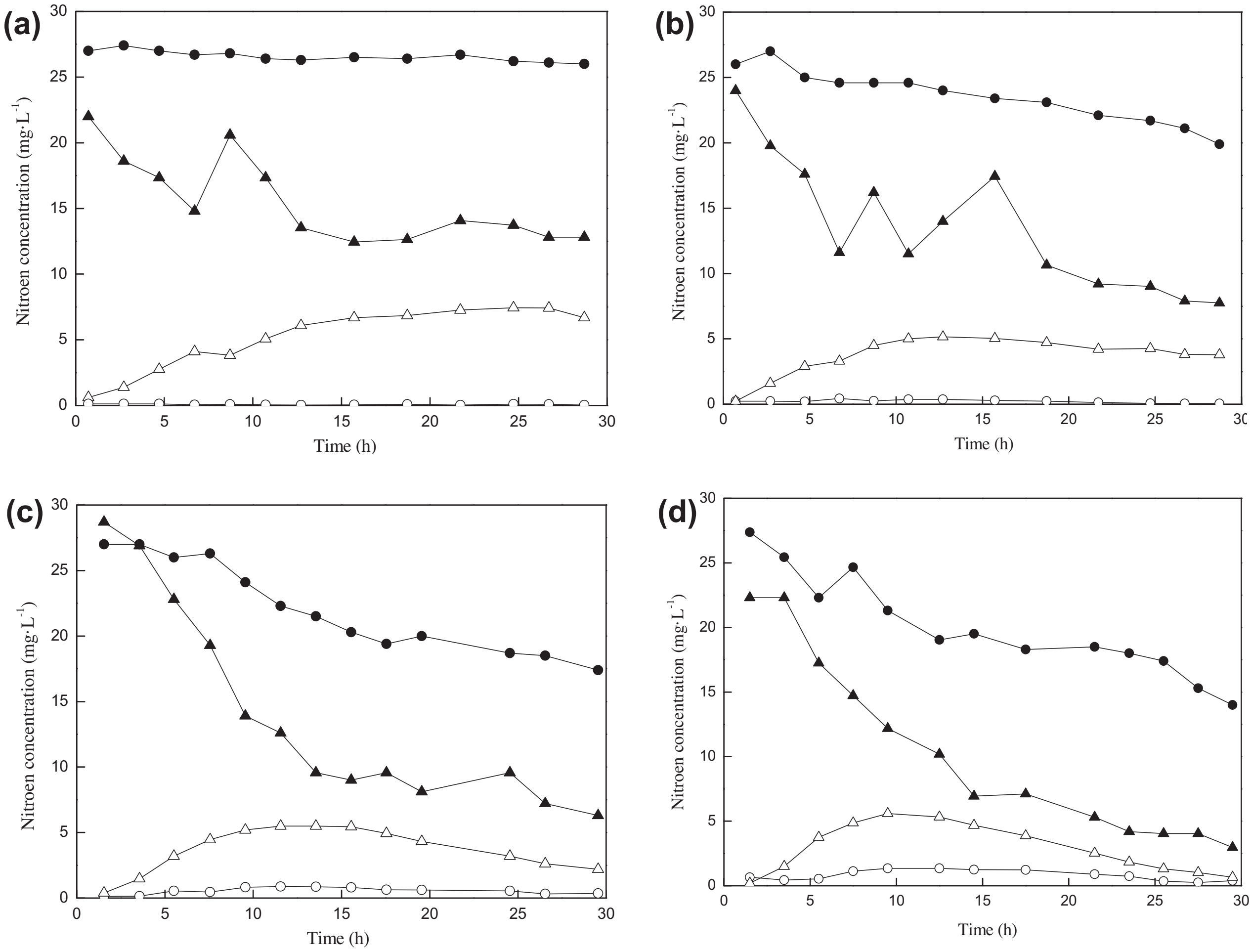

Fig. 3. Nitrogen transformation processes in the BESs at a constant voltage of 3.8–4.0 V. (a) The BES fed with $NaHCO_3$, (b–d) the BESs with C/N ratios of 2.0, 2.7, and 3.5, respectively. Triangles indicate the BES tests and the circles indicate the control tests. Solid and hollow represent the nitrate and nitrite, respectively.

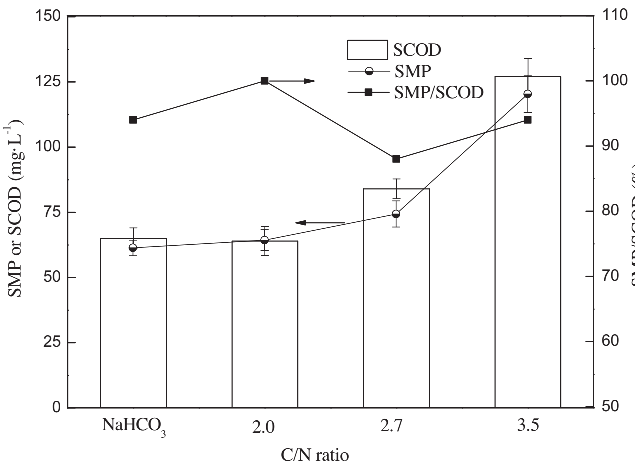

Fig. 4. The microbial metabolites generated by the microorganisms in the BESs at a constant current of 5 mA.

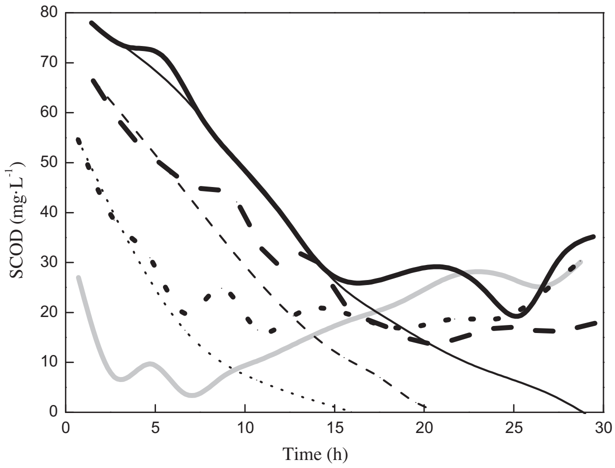

Fig. 5. The microbial metabolic processes in the BESs at a constant voltage of 3.8–4.0 V. The gray line represents the BESs fed with $NaHCO_3$. The solid, dash and dot lines represent the BESs at C/N ratios of 2.0, 2.7, and 3.5, respectively. Thick lines indicate the actual soluble chemical oxygen demand (SCOD) and the thin lines indicate the simulated organic concentration over time.

considered to be the most relevant environmental stressor. The SMP/SCOD ratio was higher at the low C/N of 2.0 than at high C/N ratios, so we concluded that the organic chemical concentrations were insufficient for the bacteria to express their full function at the low C/N ratio, promoting SMP secretion.

SMPs were produced over time while the organic chemicals were degraded. Therefore, a minimum (valley point) SCOD may have existed at some time during the experiment. The data shown in Fig. 5 show that SCOD could be detected even in the BES that did not have organic chemicals added at the beginning of the experiment. This

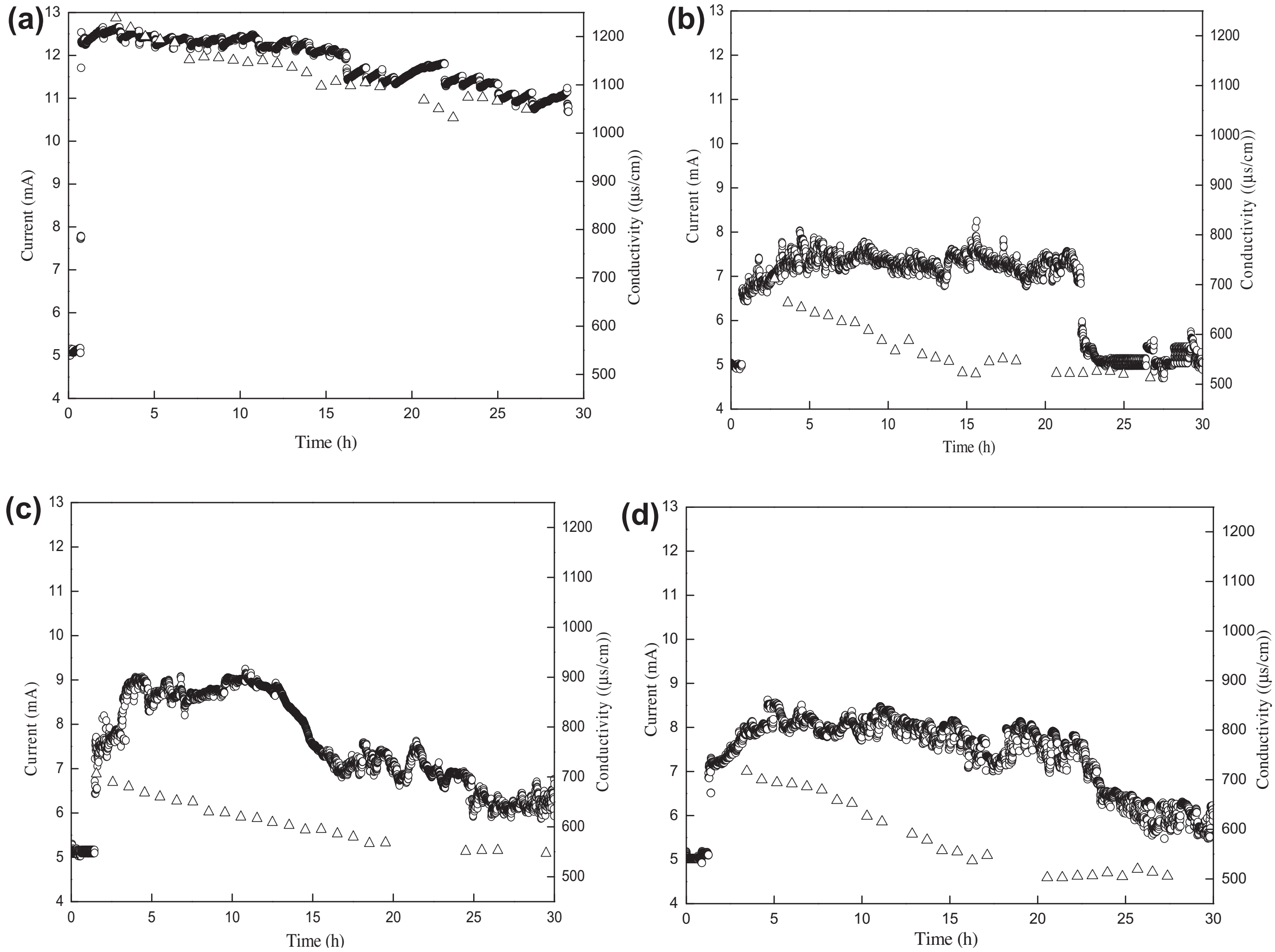

Fig. 6. Current generation and conductivity in the BESs at a constant voltage of 3.8–4.0 V. (a) The BES fed with $NaHCO_3$, (b–d) the BESs at C/N ratios of 2.0, 2.7, and 3.5, respectively. Circles (○) indicate the current, triangles (△) indicate the conductivity.

could be because the synthetic wastewater was prepared using tap water, which would have contained a low but measurable SCOD. The SCOD decreased gradually, and after about 6.7 h it reached its minimum value of 3.59 mg/L, before increasing again. 10.7, 19.9, and 25.5 hours were needed for the BESs with C/N ratios of 2.0, 2.7, and 3.5 to reach their SCOD valley points, respectively. The time needed to reach the SCOD valley point was, therefore, delayed with increasing C/N ratio. In the BES fed with $NaHCO_3$, the increased SCOD could have been from SMPs because no extra organic chemicals were added. We hypothesized that the SMPs produced in the BESs with starch added were the same as in the BES with $NaHCO_3$, and as a result, we could simulate the organic degradation that occurred, the details of which can be seen in Fig. 5. It can be seen that a longer time was needed to degrade the organic chemicals completely as the C/N ratio increased. The SCOD fluctuated with a shape similar to a "W" for each C/N ratio. The causes of the SCOD fluctuations are not known, and further studies are needed to investigate this phenomenon. From the above discussion, it is clear that the time taken for the microorganisms to secrete SMPs in the BESs increased with increasing C/N ratio.

3.2.2. The effect of C/N ratio on current generation by anode microorganisms

Many researchers have indicated that the bioanode has an important effect in BES systems (Logan and Rabaey, 2012; Pant et al., 2012; Pham et al., 2009). Changes in current and conductivity in our experiments are shown in Fig. 6. After adding carbon and nitrate, the current passing through the reactor increased abruptly. This might have been caused by the addition of electrolytes, increasing the conductivity of the solution. The current decreased gradually in the BES without added organic chemicals, caused by decreasing solution conductivity, shown in Fig. 6a. However, the current reached a peak in the BESs with added starch, which indicates that the starch was used by the microbes to generate electrons at the anode, increasing the current.

The maximum current was different at different C/N ratios. As shown in Fig. 6, the C/N ratio of 2.7 gave the highest current peak, 9.1 mA, of the three C/N ratios tested. There were two possible organic pathways in the BES system, (1) heterotrophic microorganism degradation of organic molecules, and (2) anodic microorganism utilization of organic molecules. Organic chemical removal in this study was attributed to both pathways. Current generation could also be influenced by heterotrophic denitrification.

It can be seen from Fig. 6 that the current and conductivity fluctuated more at longer experimental times. This might be caused by the combined effects of denitrification and current generation. Nitrogen was removed as the experiment proceeded, which would reduce the conductivity of the solution. Conversely, starch was degraded by microbes at the anode to generate electrons, which would increase the system's current. The current went down until the starch was depleted, and the conductivity of the solution was lower at the end of the experiment. This proves that the increasing current was caused by the microbes using starch.

3.3. Evaluation of BESs

BESs are capable of converting energy. Oxidation and reduction processes can take place at the anode and the cathode, respectively. Organic chemicals can be used at the anode either to generate electrons or for denitrification, whereas electrons can be used at the cathode to remove nitrate. The efficiency of electron utilization can be used to evaluate the performance of the BES throughout the process, and the BES performance data are shown in Table 1.

From Table 1, it can be seen that the largest amount of electrons (171.2 C) was produced from the organic chemicals at a C/N ratio of 3.5 over 30 h of data collection. However, the highest anode efficiency was achieved at a C/N ratio of 2.0, which gave an E_A percentage of 71.19%. From the transformation efficiency perspective, a low C/N ratio favored the transformation of chemical energy to electric energy at the anode. Similar results were reported by Rabaey et al. (2003), who found that the efficiency of the transformation of organic chemicals to electricity was decreased from 89% to 10% by increasing the loading rate from 0.5 g COD L^{-1} d^{-1} to 5 g COD L^{-1} d^{-1}. This is probably because, at high C/N ratios, organic chemicals were used by microorganisms to remove nitrate or to increase biomass rather than generate electricity.

It can be seen from Table 1 that the E_C (0.958%) was extremely low in the BES fed with $NaHCO_3$. This was because $NaHCO_3$ is a strong electrolyte, and the current reached as high as 12.5 mA, meaning that the total number of electrons arriving at the cathode was higher than in the BES with starch added. The E_C was relatively low at the different C/N ratios tested. To the best of our knowledge, there are only two possible nitrate pathways at the cathode. Sakakibara and Kuroda (1993) suggested that, in a biofilm-electrode reactor, hydrogen provided by the electrolysis of water was used by microorganisms to remove nitrate at the cathode, giving an overall reaction of:

$$2NO_3^- + 2H^+ + 5H_2 \rightarrow N_2 + 6H_2O \quad (11)$$

However, Clauwaert et al. (2007) demonstrated that hydrogen was unnecessary for denitrification at the cathode in a MFC, and that the overall reaction is:

$$2NO_3^- + 12H^+ + 10e^- \rightarrow N_2 + 6H_2O \quad (12)$$

In our study, the voltage used, 3.9 V, was high enough to electrolyze water and provide hydrogen, according to the theoretical voltage required for the electrolysis of water (Rasten et al., 2003). From the energy transfer perspective, avoiding hydrogen production would enhance nitrate removal and give high coulombic efficiency. The different nitrate removal mechanisms need to be investigated in further studies.

R_{auto} can be used to evaluate the influence of the C/N ratio on the autotrophic denitrification process. It can be seen from Table 1 that R_{auto} decreased from 72.74% to 50.23% as the C/N ratio increased from 2.0 to 3.5. This indicates that a high C/N ratio did not improve the autotrophic denitrification percentage in the BESs, although it could improve the nitrogen removal effect. Further studies on how organic chemicals influence autotrophic denitrification in the BES are needed to fully understand the processes.

4. Conclusions

Increased C/N ratios improved the ability of the BES to remove nitrate and depress nitrite accumulation, but did not improve the autotrophic denitrification process. High SMP concentrations were formed because of a response to current stimulation, which was considered to be the environmental stressor. High C/N ratios postponed the excretion of SMPs and increased electrogenesis but did not improve the electric production efficiency at the anode. The autotrophic denitrification ratio decreased with increasing C/N ratio.

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