



# The effect of carbon sources on nitrogen removal performance in bioelectrochemical systems

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## HIGHLIGHTS

- Coupling the electrogenesis and hydrogen production of electrode to nitrogen removal.
- Contribution rate of autotrophic and heterotrophic denitrification was investigated.
- Influence of organic carbon sources on coulombic efficiency of BES was studied.

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## ABSTRACT

In order to ascertain the effects of different carbon sources (methanol, glucose, starch and  $\text{NaHCO}_3$ ) on denitrification in BESs, the experiment was conducted in a constant current, 3.5 of chemical oxygen demand to nitrate ratio in a greenhouse. Among the four carbon sources investigated in BESs,  $\text{NaHCO}_3$  showed the highest nitrite accumulation and the ratio of soluble microbial products to soluble chemical oxygen demand (SMP/SCOD) with a value of  $3.68 \pm 0.68$  mg/L and 94%, respectively. And the addition of organic substrates could reduce SMP production and enhance the denitrification process. In the constant voltage experiment, it was observed that the organics could be used by microbes to generate electrons at the anode. And a maximal current value of 11.0 mA in the BESs fed with starch indicated that the complex carbon source was easier to be used by microorganisms to generate electricity than the simple carbon source.

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## 1. Introduction

Traditional biological processes are widely used in treating wastewater because of their low cost and high treatment efficiency. However, as a result of efforts to improve environmental quality and the emergence of novel contaminants, conventional biological processes can be no longer relied on to meet the required environmental standards. In recent decades, electric current has been used in wastewater treatment to enhance the biological degradation of pollutants. The combination of biology and electrochemistry is thus a novel approach to wastewater treatment and bioelectrochemical systems (BESs) are now being widely investigated by researchers. BESs are unique systems capable of converting the chemical energy of organic waste, including low-strength

wastewaters and lignocellulosic biomass, into electricity or hydrogen/chemical products (Pant et al., 2012).

The microbial electrolysis cell (MEC) is one kind of BES; it was developed on the basis of the microbial fuel cell by applying a small voltage ( $>0.2$  V in practice) between the anode and the cathode (Logan et al., 2008). The intrinsic mechanism of the MEC is the generation and transmission of electrons. MECs are efficient for organic degradation and hydrogen production. The focus of research has changed from easily degraded wastewater to refractory wastewater. In the MEC, the key microbial degrading enzyme system may be activated in the presence of an electric current, and some recalcitrant wastes can be effectively removed due to the simultaneous and cooperative roles of biological treatment, electrolytic dissociation and electrochemical oxidation/reduction (Huang et al., 2011). When MECs are applied to protein or cellulose wastewater treatment, the chemical oxygen demand (COD) removal efficiency can reach 85%, and the rate of hydrogen production is about 470–980 mL/g COD (Lalaurette et al., 2009; Lu et al., 2010; Wang et al., 2010). These results indicate that the MEC is promising in terms of contaminant degradation and energy production.

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Another type of BES is the biofilm-electrode reactor (BER). It operates in a similar way to the MEC and has been developed to treat nitrate-contaminated water. BERs reduce nitrate to nitrogen gas by using hydrogen that is produced in situ as the electron donor, nitrate as the electron acceptor, and carbon dioxide as the carbon source. The process has small biomass yield and does not need organic substrates (in contrast to heterotrophic denitrification). In addition, BERs are relatively easy to target to the exclusion of undesirable processes (Smith et al., 2005; Zhang and Lampe, 1996). Research into BERs has mostly concentrated on nitrate removal at the cathode; however, the degradation of organic substrates most likely occurs at the anode, but this aspect of BERs has not been considered in depth.

Conventional BER systems achieve a high utilization rate by microorganisms of the hydrogen produced by electrolysis of water. According to several studies, the current utilization efficiency is higher than 100%, and values have been reported as high as 227% (Zhou et al., 2007). From the point of view of energy transfer, 100% energy utilization is unreasonable. Furthermore, it has been reported that 1 mol of electrons can reduce 0.2 mol of nitrate to nitrogen gas (Shahnaz Islam, 1998). It is clear that, in addition to autotrophic denitrification, some other pathways such as assimilation of cell synthesis and heterotrophic denitrification as a result of cell autolysis probably contribute to the high levels of nitrate removal efficiency.

In BESs, the micro-mechanism of electron transfer between organisms and the electrode is still unclear. Electrons can be transferred to the anode by electron mediators or shuttles (Rabaey et al., 2005), by direct membrane-associated electron transfer (Bond and Lovley, 2003), by so-called nanowires produced by bacteria (Reguera et al., 2005) or perhaps by other as yet undiscovered means. But at the cathode, the way in which electrons are conveyed to the bacteria has not yet been studied (Rabaey et al., 2007).

MECs and BERs are not significantly different in structure. In MECs, the emphasis is placed on organic degradation and hydrogen production by applying a low voltage. But in BERs, the emphasis is placed on nitrate removal through an autotrophic denitrification process. The coupling of MECs and BERs in a reactor is possible because of the similarity of their structures. Organic substrates are degraded at the anode, and this fact is possibly beneficial to the production of hydrogen at the cathode and the autotrophic denitrification process is probably strengthened as a result. In addition, heterotrophic bacteria can utilize organic substrates to remove nitrate. It is considered likely, therefore, that BESs can achieve relatively high nitrate removal efficiency.

Most researchers have considered the treatment of nitrate-contaminated wastewater by pure autotrophic denitrification in BESs. To the best of our knowledge, few researchers have investigated cooperative heterotrophic and autotrophic denitrification. In BESs, if organic substrates were added, the electron-transfer process could be strengthened and thereby promote nitrate removal. Rabaey et al. (2003) discovered that glucose can improve the efficiency of electricity generation in microbial fuel cells. This fact is likely the result of electron transfer by the bacteria to the electrode. In the presence of organic substrates, several pathways of electron current transfer probably exist: (1) organic substrates are decomposed through respiration and the electrons are transferred to nitrate directly; (2) some organisms grow on the surface of the electrode utilizing a mesostate and then transfer electrons to the nitrate; (3) the electrons produced through respiration are primarily delivered to the electrode and then are transferred to the nitrate through conductive materials. Zhao et al. (2011) used an intensified biofilm-electrode reactor (IBER), which uses heterotrophic and autotrophic denitrification to treat nitrate-contaminated water. In their further study, they found that heterotrophic and autotrophic denitrification processes occurred more

effectively compared with single heterotrophic denitrification or autotrophic denitrification in the IBER (Zhao et al., 2012). It can be deduced from these studies that some organic substrates may be beneficial to nitrate removal in BER systems. However, the specific factors influencing the effects of organic substrates remain unknown.

The objective of this study was to investigate the effects of different carbon sources (methanol, glucose, starch and  $\text{NaHCO}_3$ ) on denitrification in BESs and to evaluate the effectiveness of BESs in the treatment of nitrate-contaminated wastewater. The current utilization efficiency is discussed to shed light on the mechanism of nitrate removal in BESs.

## 2. Methods

### 2.1. Experimental apparatus

The experimental configuration is shown in Fig. 1. Rectangular graphite electrodes (length 15 cm, width 8 cm) were used in this study. The electrodes were fixed in a cylindroid reactor with an inter-electrode distance of 4 cm. The effective volume of the reactor was 450 mL. Current was supplied by DC power supply. Control reactors were set up in an identical way but with the electrical circuit open.

### 2.2. Experimental set up

Synthetic wastewater consisting of nutrients and trace elements was used to simulate nitrate-contaminated water in this study. The nitrogen was provided by  $\text{NaNO}_3$ ; the  $\text{NO}_3^-$ -N concentration was maintained at  $30 \text{ mg L}^{-1}$ . Methanol, glucose and starch were used as organic carbon sources and  $\text{NaHCO}_3$  was used as an inorganic carbon source. Seed sludge was collected from Qige wastewater treatment plant (Hangzhou, China). The COD to  $\text{NO}_3^-$ -N ratio and the current were maintained at 3.5 and 5 mA, respectively. The synthetic wastewater was replaced every 24 h and the temperature was maintained at  $30 \pm 2^\circ\text{C}$  in a greenhouse. When the total nitrogen (TN) removal efficiency was higher than 60% for more than 5 days, the BES was considered to be stable and experiments were conducted. The nitrogen removal performance was investigated and the effluent components were measured. After the constant-current experiments were completed, a constant voltage of 4.0 V was applied to the BESs with added glucose and with added starch, and the changes in current were investigated.

### 2.3. Analytical method

All samples were filtrated using a  $0.45 \mu\text{m}$  filter membrane before analysis.  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N, TN and  $\text{NO}_2^-$ -N were measured according to standard methods (APHA, 1998). COD was measured using a DR2800 spectrophotometer (HACH Company, Loveland, CO, USA). pH was measured using a pH meter (Mettler Toledo, Greifensee, Switzerland). The current was measured by an intelligent digital multimeter (UNI-T Company, Shanghai, China).

Glucose was measured according to the standard method (Miller, 1959). Volatile fatty acid was measured by withdrawing a  $4\text{-}\mu\text{L}$  sample from the headspace using a gas-tight syringe followed by gas chromatography with a chromatograph (GC 7890, Shanghai Tianmei Science Instrument Co., Ltd., Shanghai, China) equipped with a flame ionization director. The carrier gas was nitrogen, and the detector, injection port and column temperatures were 250, 230 and  $180^\circ\text{C}$ , respectively. The measurement of soluble microbial products (SMPs) is difficult. Following the advice of Barker and Stuckey (1999), SMP was defined as:  $\text{SMP} = \text{SCOD} -$

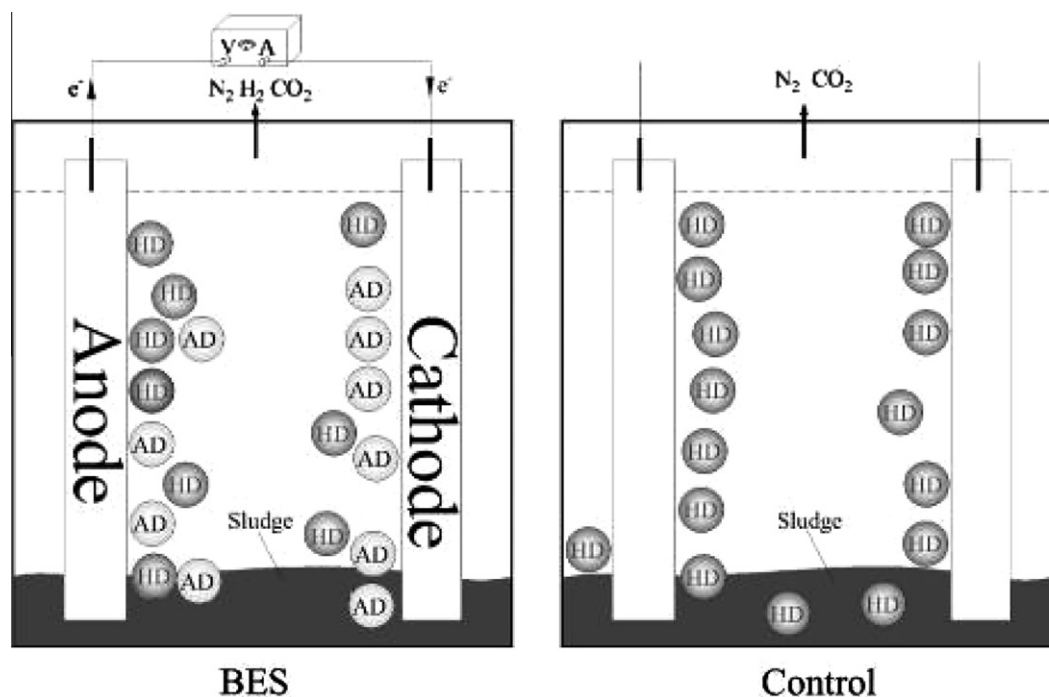


Fig. 1. Experimental setup for bioelectrochemical system (BES) and control reactors. AD, autotrophic denitrifier; HD, heterotrophic denitrifier.

1.07(HAc) – 1.51(HPr) – 1.82(Hbu) – 1.07(Glu), where SCOD is the soluble chemical oxygen demand, HAc (mg/L) is the acetic acid concentration, HPr (mg/L) is the propionic acid concentration, Hbu (mg/L) represents the concentration of *iso*- and *n*-butyric acids, and Glu (mg/L) is the glucose concentration. The values 1.07, 1.51 and 1.82 are conversion factors assuming the complete oxidation of volatile acids or glucose to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ .

#### 2.4. Statistical analysis

All data were expressed as the mean  $\pm$  standard error. Statistical analyses consisted of one-way analysis of variance conducted using SPSS software (version 19.0; IBM company, United States). Differences were considered significant when  $p < 0.05$ .

### 3. Results and discussion

#### 3.1. Nitrogen removal performance

##### 3.1.1. Nitrate removal

Fig. 2 illustrates the nitrate removal levels on the addition of methanol, glucose and starch as organic carbon sources and  $\text{NaHCO}_3$  as an inorganic carbon source. The four BESs with added carbon sources were more effective at nitrate removal than the control reactors with no current flow. The organic carbon sources were clearly more beneficial to nitrogen removal in BESs than the inorganic carbon source was. The  $\text{NO}_3^-$ -N volume removal rate in the  $\text{NaHCO}_3$  reactor was  $0.44 \pm 0.08 \text{ g m}^{-3} \text{ h}^{-1}$ , which was lower than that of the other three BESs that had been fed with organic substrates. In the  $\text{NaHCO}_3$  reactor, no external organic substrates were added, and denitrification was accomplished mainly by autotrophic bacteria. In the other three reactors fed with organic substrates, heterotrophic bacteria and autotrophic bacteria likely co-existed, greatly improving the denitrification efficiency. As shown in Fig. 2, the nitrate removal efficiency was not very high in any BES. In this study, the systems were  $\text{H}_2$  limited and the response of denitrification rate to the applied current was probably in the linear region (Skakibara et al., 1994). The low nitrate re-

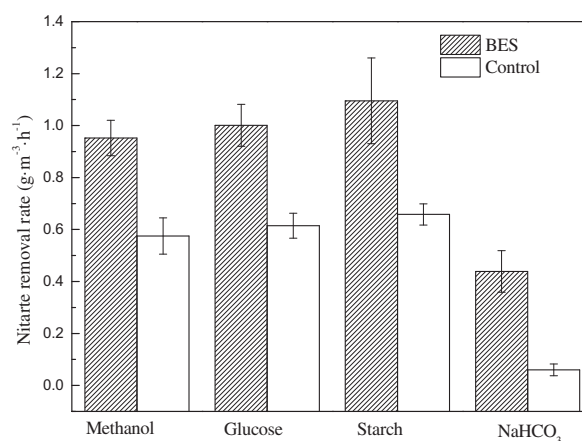


Fig. 2. Effect of different carbon sources on the nitrate removal rate.

moval efficiency was caused by the inadequate supply of electrons in the system.

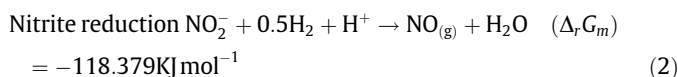
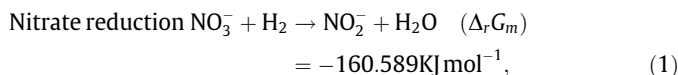
The nitrogen removal efficiencies of BESs fed with methanol, glucose and starch were no big differences. The  $\text{NO}_3^-$ -N removal rates of BESs fed with methanol, glucose and starch were  $0.95 \pm 0.06$ ,  $1.00 \pm 0.08$  and  $1.09 \pm 0.17 \text{ g m}^{-3} \text{ h}^{-1}$ , respectively. Heterotrophic denitrification played an important role in the nitrogen removal process. For most heterotrophic denitrifying bacteria, an organic carbon source is required for cell growth and nitrate reduction (Lee and Rittmann, 2003). The characteristics of the added carbon source have major effects on the denitrification process, such as on the denitrification rate, the kinetics and the COD (Obaja et al., 2005).

##### 3.1.2. Nitrite accumulation

The effluent nitrite concentrations in the BESs and control reactors were shown in Fig. 3. Significant nitrite accumulation was observed in all the BESs. In the four control reactors, the maximum effluent nitrite concentration was observed in the glucose reactor

with a value of  $0.65 \pm 0.12 \text{ mg L}^{-1}$ . Her et al., (1995) also found that using glucose as a carbon source resulted in greater accumulation of nitrite than did other carbon sources during heterotrophic denitrification. In this study, no significant nitrite accumulation was observed in the three control reactors that used carbon sources other than glucose. Conventional biological denitrification involves a series of biological reduction processes. Nitrate and nitrite compete for electrons in the denitrification system. The conversion rates of nitrate to nitrite and nitrite to nitric oxide vary in different systems. In the control reactors, nitrate removal efficiencies were low because of the low C/N ratio of 3.5, and the nitrite converted by microorganisms was depleted quickly during the denitrification process. The conversion of nitrate to nitrite became the rate-limiting step of denitrification in the control reactors.

However, in the four BESs, the concentrations of nitrite were notable. The maximum nitrite accumulation ( $3.68 \pm 0.48 \text{ mg L}^{-1}$ ) was observed in the BES with added  $\text{NaHCO}_3$ . In the BESs with added methanol, glucose and starch, nitrite accumulation was lower at  $1.65 \pm 0.66$ ,  $1.27 \pm 0.27$  and  $3.38 \pm 0.40 \text{ mg L}^{-1}$ , respectively. The nitrite concentration in the BES with added starch was significantly higher than that for the BESs with added methanol and glucose. In BESs with added organic substrates, heterotrophic and autotrophic denitrifiers co-exist, and both contributed to nitrogen removal. The combined effects of organic substrates and electric current likely resulted in nitrite accumulation. The current (5 mA) supplied in this study was too low to allow complete nitrate and nitrite removal. During the hydrogenotrophic denitrification process, nitrate reduction proceeds in the following two steps (Karanasios et al., 2010):



Of the two reactions, nitrate reduction is more likely to occur as a result of the lower Gibbs free energy. In the BESs, conditions were hydrogen limited, and so nitrate reduction occurred preferentially. Zhou et al. (2007) observed nitrite accumulation in BESs with low current and a hydraulic retention time of 13 h.

In the BES without an organic substrate, significant nitrite accumulation was observed; this fact implies that the reaction of nitrite to form NO gas was inhibited. By adding organic sources to the BESs, the denitrification process was enhanced. In BES systems, electrons (from either organic or inorganic substrates) and the current field likely influence nitrate removal and nitrite accumulation.

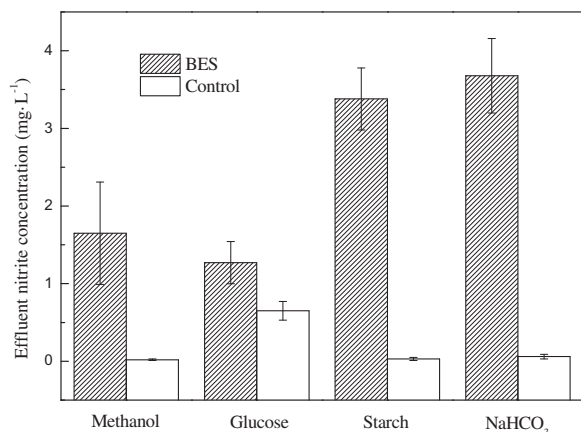


Fig. 3. Effect of different carbon sources on nitrite accumulation.

The activities of heterotrophic bacteria in the electric field were not investigated, and the activities of nitrate reductase and nitrite reductase of the denitrifiers on the electrode surface were not clarified in this study. These activities might promote or inhibit nitrate and nitrite removal and it was needed to be further studied.

### 3.2. The coulombic efficiency (CE) and the ratio of autotrophic denitrification

The ratio of autotrophic denitrification ( $R_{\text{auto}}$ ) and coulombic efficiency (CE) were calculated as follows:

$$R_{\text{auto}} = \frac{(\text{mb}_{\text{nitrate}} - \text{mb}_{\text{nitrite}}) - (\text{mc}_{\text{nitrate}} - \text{mc}_{\text{nitrite}})}{\text{mb}_{\text{nitrate}} - \text{mb}_{\text{nitrite}}} \times 100\% \quad (3)$$

$$\text{CE} = ((\text{mb}_{\text{nitrate}} - \text{mc}_{\text{nitrate}}) \times 5 - \text{mb}_{\text{nitrite}} \times 3) \times \frac{V}{T} \times \frac{F}{I} \times 100\%, \quad (4)$$

where  $\text{mb}_{\text{nitrate}}$  and  $\text{mc}_{\text{nitrate}}$  are the removal amounts of nitrate in BES systems and control reactors ( $\text{mmol L}^{-1}$ ), respectively;  $\text{mb}_{\text{nitrite}}$  and  $\text{mc}_{\text{nitrite}}$  are the effluent nitrite concentration in BES systems and control reactors ( $\text{mmol L}^{-1}$ ), respectively;  $V$  is the reactor volume (L);  $T$  is the hydraulic retention time (s);  $F$  is the Faraday constant ( $\text{C mol}^{-1}$ ) and  $I$  is the constant current applied to the reactor (mA); 5 and 3 are used to convert moles of nitrate and nitrite to moles of nitrogen gas. The calculated results are shown in Fig. 4.

CE is often used to evaluate the performances of BESs. The four BESs in the current study yielded low CEs compared with the values found by previous reports (Sakakibara and Nakayama, 2001; Zhou et al., 2007). This difference might be caused by the use of different calculation processes. In this study, heterotrophic denitrification was excluded from the CE calculation. The CEs of BESs with glucose and  $\text{NaHCO}_3$  were higher than those of the other two reactors. The CE of the BES with  $\text{NaHCO}_3$  was  $32 \pm 0.5\%$ . This low value, compared with that of other researchers, probably resulted from the small surface area of the cathode, which meant that autotrophic bacteria could not utilize the hydrogen effectively. The BES with glucose achieved a CE of  $33 \pm 5\%$ , similar to that of the  $\text{NaHCO}_3$  reactor. It could be deduced from this result that glucose had only a small impact on autotrophic bacteria. In contrast, the TN and nitrate removal performance of the BES with glucose was superior to that of the BES with  $\text{NaHCO}_3$ . However, the BESs using methanol and starch as the organic carbon sources showed a negative effect on the autotrophic process with CE values of  $23 \pm 1.4\%$  and  $19 \pm 2.3\%$ , respectively.

The maximum ratio of autotrophic denitrification was achieved in the BES fed with  $\text{NaHCO}_3$ . Because no external organic sub-

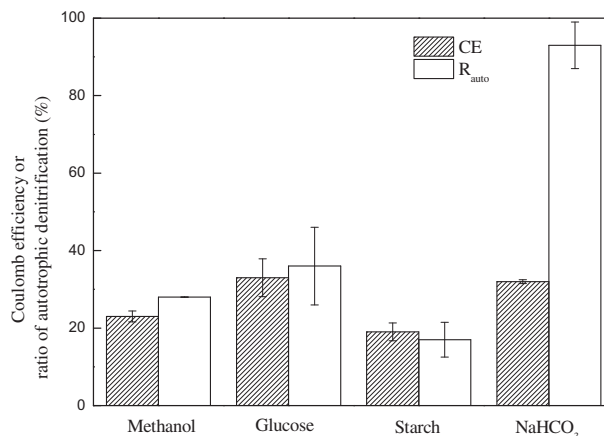


Fig. 4. Effect of different carbon sources on the coulombic efficiency (CE) and the ratio of autotrophic denitrification ( $R_{\text{auto}}$ ).



strates were added, nitrogen was removed through the autotrophic denitrification process using hydrogen as the electron donor and nitrate as the electron acceptor. By adding organic substrates, the microbial composition changes and heterotrophic processes start contributing to the removal of nitrogen. Fig. 4 indicates that the BES fed with glucose had a higher ratio of autotrophic denitrification than did the BESs with the other two carbon sources.

### 3.3. SMPs in the effluent

SMPs are composed of a variety of organic compounds released from microorganisms as a result of their metabolic activity. And researchers adopted the SMPs to characterize the organic compounds that are released into solution from substrate metabolism and biomass decay (Duncan & David, 1999). SMPs are considered to make up the majority of soluble organic matter in wastewater effluent (Jarusutthirak and Amy, 2007). The production of SMPs will undoubtedly influence the treatment efficiency in BESs. The SMP levels in the BESs are shown in Fig. 5. The SMP concentrations were 17, 39, 83 and 61 mg L<sup>-1</sup> in the presence of methanol, glucose, starch and NaHCO<sub>3</sub>, respectively. The BES fed with starch produced the highest level of SMPs. It indicated that even if no organic substrates are added in the influent, the SMP levels in the effluent was still be high. SMP is typically divided into two categories: biomass-associated products (BAP) (SMP associated with biomass decay) and utilization-associated products (UAP) (SMP associated with substrate metabolism and, typically, biomass growth) (Noguera et al., 1994). UAP mainly consists of low-molecular-weight carbonaceous compounds produced during the microbial growth process. In contrast, BAP mainly consists of cellular macromolecules generated indirectly via the hydrolysis of biomass components (Ni et al., 2011). The SMP produced in the NaHCO<sub>3</sub> reactor could possibly be BAP because there was inadequate nutrition in the reactor and the growth rate of autotrophs was slow.

Diverse factors influence the amount of SMP produced. The absolute SMP content, however, is not sufficient to fully characterize the effluent. Consequently, the SMP/SCOD ratio was used to evaluate the effects of different carbon sources on BES performance. The effluent SCOD of the BES fed with NaHCO<sub>3</sub> was mostly accounted for by SMP, with a SMP/SCOD ratio of 94%, as shown in Fig. 5. In BESs with added methanol, glucose and starch, the ratios of SMP/SCOD were lower than that of BES fed with NaHCO<sub>3</sub>. For the BES fed with NaHCO<sub>3</sub>, hydrogenotrophic denitrifying bacteria were dominant, in which case it is more likely for SMP to be produced as a stress reaction to current than by heterotrophic denitrifying bacteria. Some researchers found that SMPs produced by microorganisms could be utilized slowly as secondary substrates (Barker et al.,

2000). Ni et al. (2011) demonstrated that the SMP released by autotrophs could be used by heterotrophs to remove nitrogen. In BESs without organics, autotrophic denitrifiers were dominant and the SMP could not be depleted by other bacteria, resulting in the greater accumulation of SMPs. However, in BESs fed with organic substrates, autotrophs and heterotrophs co-existed. Consequently, SMP might be utilized by the heterotrophs. The specific rates of SMP use varied as a result of the different chemical species present. In these experiments, the lowest SCOD concentration and SMP/SCOD ratio were achieved in the BES with added methanol, clearly suggesting that methanol was easily utilized by microorganisms and could reduce the production of SMPs.

### 3.4. The mechanism of nitrogen removal in BESs with different carbon sources

Nitrogen can be removed via autotrophic or heterotrophic denitrification processes. In BESs, both processes may exist, and the specific nitrogen removal pathway may vary as a result of the different carbon sources. The carbon sources used in this study can be divided into three categories: simple carbon sources (methanol and glucose), a complex carbon source (starch) and an inorganic carbon source (NaHCO<sub>3</sub>). The BES fed with NaHCO<sub>3</sub> showed the highest level of nitrite accumulation among the four BESs, and the nitrogen removal efficiency was low. This reactor was run as a bioelectrode reactor, and the denitrifying bacteria utilized the hydrogen generated via electrolysis of water at the cathode. The hydrogenotrophic denitrification process probably contributed to the nitrogen removal in this reactor.

When organic substrates were added, the mechanism of nitrogen removal changed. The BESs fed with organic substrates had higher nitrogen removal efficiencies than the BES fed with NaHCO<sub>3</sub>. But the specific nitrogen pathways of the two types of carbon sources are probably different. In the BES fed with starch, high levels of nitrite accumulation were observed, and SMP production was the highest of the four BESs. In addition, the SMP/SCOD ratio was 92%, compared with 94% in the BES fed with NaHCO<sub>3</sub>. Both the inorganic carbon source and the complex carbon source resulted in high nitrite accumulation and high SMP production. Similar outcomes were not evident in the BESs fed with the simple carbon sources methanol and glucose. We inferred that complex carbon sources might strengthen the hydrogenotrophic denitrification process, whereas simple carbon sources did not and the detail mechanisms were needed to be further studied.

By applying a steady potential of around 3.5 V, the current variation in BESs fed with glucose and starch was investigated, and the results were shown in Fig. 6. After adding the organic substrate and nitrate, the current passing through both reactors jumped abruptly. This was probably caused by the addition of electrolyte, thereby increasing the conductivity of solution. The current then fluctuated as the denitrification process proceeded. After the current initially peaked, the current in the two BESs gradually decreased. The reason for this pattern is not clear and requires further study. The peak current in the BES fed with starch was 11.0 mA compared with 8.3 mA in BES fed with glucose. The resistances of the BESs were measured before and after the experiment. The resistances of the systems showed a decreasing tendency. After the experiment, the resistances of the BESs fed with glucose and starch were 500 and 723 Ω, respectively. The theoretical current value could be calculated by Ohm's law. The theoretical currents of the BESs fed with glucose and starch were 6.8 and 4.7 mA, respectively. The actual currents at the end of the experiments of BESs fed with glucose and starch were 7.4 and 7.8 mA, respectively. The above results indicated that the complex carbon source could more easily be utilized by microbes at the anode to generate elec-

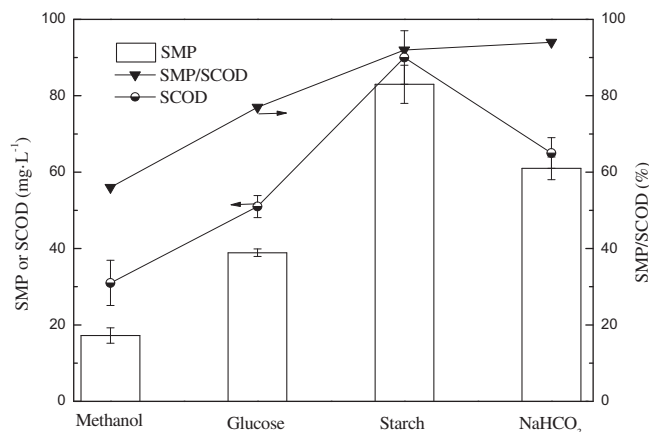
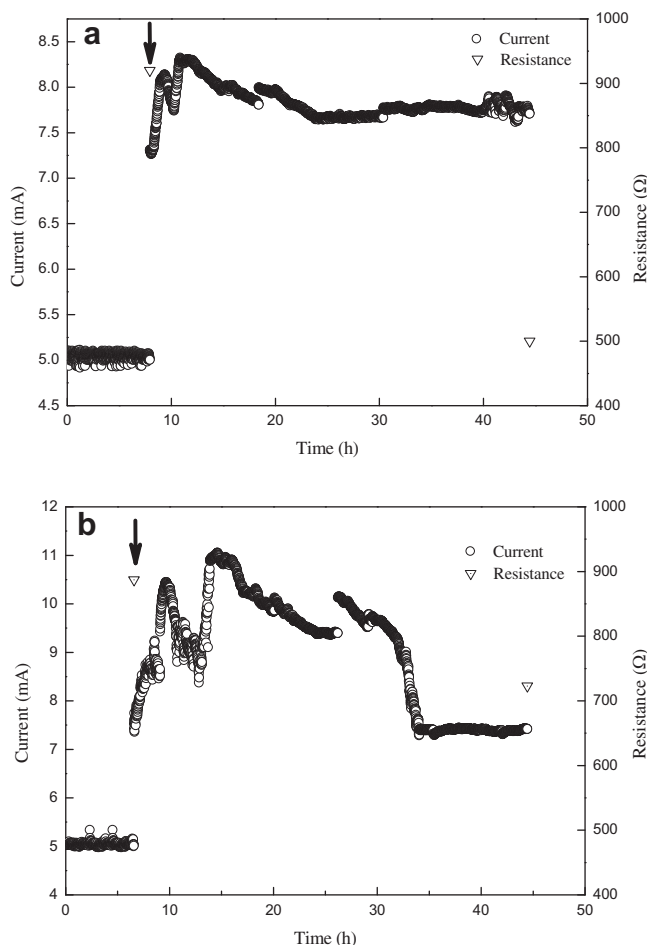


Fig. 5. SMP and SCOD concentrations in BES effluent.



**Fig. 6.** Variation in current at a constant voltage of 3.5 V in BESs with added glucose (a) and starch (b). The arrow indicates the addition of nitrate and organics.

tricity. The simple carbon source was depleted by microbes at the anode directly, which resulted in a low current, as shown in Fig. 6.

#### 4. Conclusions

Organic substrates were beneficial to nitrogen removal in BESs. Of the four carbon sources investigated in BESs, nitrite accumulation and the SMP/SCOD ratio were the highest in the BES fed with  $\text{NaHCO}_3$ . And the addition of organic substrates could reduce SMP production. Based on the above results, it is clear that simple carbon sources such as methanol and glucose are used directly by heterotrophic bacteria and promote the heterotrophic denitrification process. A complex carbon source such as starch could be utilized by microbes to generate electrons but with a high nitrite accumulation in the process.

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