Bio-electrochemical denitrification by a novel proton-exchange membrane electrodialysis system – a batch mode study

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Abstract

BACKGROUND: Contamination of nitrate in ground and surface water has become an ever-increasing and serious environmental problem. Biological methods hold the promise of converting nitrate into harmless nitrogen. A novel denitrification system which combines proton-exchange membrane electrodialysis with simultaneous bio-electrochemical autotrophic denitrification has been developed. The proton-exchange membrane was used to transfer current and to exclude oxygen or other oxidative chemicals generated in the anode reaction. The H2 generated by the cathode was utilized by autotrophic denitrifying microorganisms in the cathode cell to reduce nitrate. In this study, the transport of H+, a denitrification kinetics model and factors influencing the denitrification rate were explored in batch mode.

RESULTS: The addition of 0.03 mol L−1 H2SO4 into the anode cell enhanced proton transport and maintained the pH of the cathode cell in an appropriate range for biological denitrification. The denitrification rate was affected by applied current and biomass. Under adequate current conditions, the kinetics of the denitrification process followed a zero-order kinetics model; the average denitrification rate for unit biomass was calculated to be 9.36 mg NO3−-N VSS g−1 h−1.

CONCLUSIONS: Results indicate that the system is suitable for denitrification. Owing to its simple structure and operation, it has the potential for use as a system to reduce nitrate in water.

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Keywords: bio-electrochemical; denitrification; proton-exchange membrane; electrodialysis; kinetics; nitrite

INTRODUCTION

Contamination of groundwater and surface water by nitrate is a world-wide and serious problem.1 Sources of nitrate in groundwater include fertilizers, industrial, food processing, animal and human wastes. Nitrate has to be removed because nitrate in high concentrations has a detrimental effect on human health and the environment.2 The current methods for nitrate removal include ion exchange (IE),3 reverse osmosis (RO), catalytic4 and biological processes.5–24 However, both IE and RO processes cannot transfer nitrate into harmless compounds; they concentrate nitrate from water to brine and require further treatment. High costs and deactivation of catalysts limit the application of catalytic processes. Biological denitrification is an attractive way to convert nitrate into harmless nitrogen with low operating and capital costs.5

Hydrogen gas is an ideal electron donor for biological autotrophic denitrification because of its clean nature and low biomass yield, but it has a poor solubility in water (1.6 mg L−1 at 20 °C) and is dangerous to control.5–7 To overcome the above mentioned problems, the biofilm-electrode reactor (BER), an electrochemical and biological reactor, was first developed by Sakakibara et al. in 1993.5 In this system, hydrogen gas as an electron donor is generated by electrolysis of water. Considerable effort has been made to improve designs for the efficient and economical removal of nitrate from water by a bioelectrochemical method.6–12 As anoxic conditions are required for the biodenitrification process, carbon material is usually used as sacrificial anode because of its low oxidative electrode potential; CO2 is formed prior to O2 at low applied voltage.7 However, at higher applied voltage, O2 is generated at the anode restricting the denitrification rate.6 From this aspect, a multi-electrode system containing an electrolysis cell and a packed-bed column.26 The anode cell and cathode cell are separated by a proton-exchange membrane. When a direct current potential is applied between the two electrodes, the positively charged cations move to the cathode, passing through the cation exchange membrane. In 2000, Kiss et al. developed a two-reactor denitrification system containing an electrolysis cell and a packed-bed column.26 The
Denitrification by proton-exchange membrane electrodialysis system

EXPERIMENTAL MATERIALS AND METHODS

Experimental setup

The bio-electrochemical denitrification system used in this study is represented schematically in Fig. 1. The anode cell (300 mL volume) and cathode cell (400 mL volume) were separated by a proton-exchange membrane (GEFC-107, GEFC, China); the membrane was located at 5 mm and 10 mm distance from anode and cathode, respectively. The cathode was Cu plate with 36 cm² (6 × 6 cm) effective surface area and the anode was a DSA (dimensionally stable anode) electrode. The suspended autotrophic denitrifying microorganisms were continuously mixed by a recirculation pump in the cathode cell. Direct current was supplied by silicon rectifier (Model DH1718E-4, Beijing Dahua Electronic Instruments Group, Beijing, China). All experiments were conducted at an applied current of 15–100 mA and factors influencing the system in batch mode.

Experimental procedure

First, the acid concentration added to the anode cell to keep the cathode cell pH neutral was determined through a set of experiments. pH variations in the cathode cell under 0.01–0.03 mol L⁻¹ H₂SO₄ supplements in the anode cell were measured during a 3.5 h electrodialysis process. Experimental details were: applied current 50 mA; electrolyte synthesized by tap water. Microorganisms solution was not added to the cathode cell.

The effect of acid added to the anode cell on the denitrification rate of biomass in the cathode cell was explored. The effect of biomass, applied current and initial nitrate concentration was investigated through batch experiments.

Analytical methods

The concentrations of NO₃⁻–N and NO₂⁻–N were determined by ion chromatograph (Metrohm 861, Switzerland). The pH and ORP (oxidation reduction potential) were measured by multiparameters portable instruments (HACH Sension, USA). VSS (volatile suspended solids) were measured according to standard methods for the examination of water and wastewater.

Kinetics model and principle of the system

The denitrification process can be simplified into two steps including the consecutive reduction of nitrates to nitrites and then to nitrogen gas, while hydrogen is used as electron donor for denitrification:

\[
\begin{align*}
NO_3^- + H_2 &\rightarrow NO_2^- + H_2O \\
NO_2^- + 1.5H_2 + H^+ &\rightarrow 0.5N_2 + 2H_2O
\end{align*}
\]
The overall reaction is:

$$\text{NO}_3^- + 2.5\text{H}_2 + \text{H}^+ \rightarrow 0.5\text{N}_2 + 3\text{H}_2\text{O}$$ (3)

The kinetics of autotrophic denitrification were expressed in a double Michaelis–Menten (Monod) form and NO$_3^-$, NO$_2^-$ and H$_2$ were assumed to be the limiting substrates.

The zero-order kinetics based on the Monod model was suitable for non-substrate (H$_2$) limiting and low biomass growth yield conditions. The rate at which nitrate is converted to nitrite can be calculated as the first-order overall function of nitrate (zero-order kinetics):

$$\frac{d[\text{NO}_3^- - N]}{dt} = -k_1[\text{NO}_3^- - N]^d[\text{X}]^1$$ (4)

and its integration form:

$$[\text{NO}_3^- - N]_t - [\text{NO}_3^- - N]_0 = -k_1[\text{X}]t$$ (5)

where $[\text{NO}_3^- - N]_0$ and $[\text{NO}_3^- - N]_t$ are the initial concentration of nitrate and that at time t (mg-N/NO$_3^-$ N L$^{-1}$), $[\text{X}]$ is the biomass concentration (VSS g L$^{-1}$) and $k_1$ is the reaction rate constant, specific denitrification rate (mg NO$_3^-$ N VSS g$^{-1}$ h$^{-1}$).

In previous studies on hydrogenotrophic denitrification, a saturation coefficient of 0.18 mg-N L$^{-1}$ was reported for nitrate.$^{13}$ In another study, the reported hydrogen saturation coefficient ranged from 0.0009 to 0.0066 mg-H$_2$ L$^{-1}$. With such low saturation coefficients, it can be assumed that the kinetics of nitrate reduction is independent of nitrate, nitrite and hydrogen concentrations. Nitrate, nitrite and hydrogen concentrations in water treatment systems exceed saturation coefficients, and biomass changes little in batch mode experiment, thus zero-order kinetics can be used in this study.

Figure 2 illustrates the proton transport and bioreduction of nitrate in the proton-exchange membrane electrodialysis system. In this novel system, the proton-exchange membrane was installed between anode and cathode, thus the reactor was divided into two parts: anode cell and cathode cell. The current was carried by protons through the membrane from the anode cell to cathode cell. The oxygen and other oxidative chemicals generated in the anode reaction were excluded from the cathode cell and therefore could not prohibit hydrogenotrophic denitrification.

The hydrogen for denitrification is provided by the cathode reaction:

$$10\text{H}_2\text{O} + 10\text{e} \rightarrow 5\text{H}_2 + 10\text{OH}^-$$ (6)

For the anode reaction:

$$5\text{H}_2\text{O} - 10\text{e} \rightarrow 5/2\text{O}_2 + 10\text{H}^+$$ (7)

pH was an important parameter for bio-denitrification. The reported suitable pH was in the range 7.0–8.0.$^{13,15–18}$ For this electrodialysis process, assuming that there is no separation between anode and cathode, based on H$^+$ and OH$^-$ balance (Equations (6) and (7)), the pH of the whole cell will not change. However, compared with the non-separation process, the separation of proton-exchange membrane between anode cell and cathode cell will restrict the proton transport to some extent. Proton transport from anode cell to cathode cell through the proton exchange membrane under the electrical force, and therefore the H$^+$ transport efficiency, was restricted by the proton-exchange membrane electric transport capacity. If the proton transport efficiency could not balance the H$^+$ consumption of electrical dialysis in the cathode cell, the water in the cathode cell will be alkalic, and the denitrification rate will be reduced. Accordingly, a suitable concentration of acid supplement in the anode cell to enhance proton transport is necessary.

**RESULTS AND DISCUSSION**

**Effect of acid supplement in anode cell on the pH balance of cathode cell**

As pH was an important parameter for bioreaction, the concentration of added H$_2$SO$_4$ in the anode cell to keep the cathode cell pH in a suitable range (7.0–8.0) was determined through a set of experiments. pH variation in the cathode cell under different concentrations of H$_2$SO$_4$ in the anode cell is shown in Fig. 3(a). Without acid supplement in the anode cell, the pH of the cathode cell increased rapidly during the electrodialysis process. After 2.5 h reaction, owing to inadequate transport of H$^+$, the pH of cathode cell exceeded 9.5. As shown in Fig. 3(a), acid supplement in the anode cell prevented pH increasing in the cathode cell effectively, indicating that the dialysis effect of the concentration gradient between the two separate cells could balance the proton transfer restriction of the proton-exchange membrane transport capacity. With the concentration of acid increased from 0.01 mol L$^{-1}$ to 0.03 mol L$^{-1}$, pH increase in the cathode cell was better restrained. It was concluded that 0.03 mol L$^{-1}$ was a suitable concentration for added H$_2$SO$_4$ in the anode cell to keep the pH of the cathode cell in an appropriate range.

The ORP of the cathode cell was below −150 mV after 0.5 h electrodialysis, which meant that the oxygen or other oxidative chemicals generated in the anode reaction were successfully excluded by the proton-exchange membrane and favorable anoxic conditions for denitrification were established in the cathode cell.

The effect of acid supplement in the anode cell on denitrification rate is shown in Fig. 3(b). It can be seen that without acid supplement, the pH of the cathode cell directly increased due to inadequate proton transport. After 1.5 h dialysis, the pH was higher than 9.0, and the denitrification rate was prohibited. From 1.5 h to 3.5 h, the pH increased from 9.0 to 10.7 and the removal efficiency increased only from 22.4% to 28.6%. This phenomena was in accordance with Lee et al.’s study, in which they found that increasing pH above 8.6 caused a significant decreased
in nitrate removal for autohydrogenotrophic denitrification.\textsuperscript{24} Comparatively, with the acid supplement (0.03 mol L\textsuperscript{-1} H\textsubscript{2}SO\textsubscript{4}) in the anode cell, the pH of the cathode cell remained neutral, indicating that it was favorable for bio-denitrification. After 3.5 h reaction, nitrate was almost completely reduced by the bio-electrochemical process. The pH of the cathode cell was 7.65 at the end of reaction, which was slightly higher than the initial pH 7.34. This was mainly attributed to the denitrification reaction (Equation (3)), NO\textsubscript{3}\textsuperscript{−} consumes an equal quantity of H\textsuperscript{+} for complete reduction, resulting in an increase of pH.

### Effect of biomass on nitrate removal

The influence of biomass concentration on nitrate removal was examined and a set of experiments was carried out to determine the kinetics of this system. The original biomass concentrations were 0.05–1.01 g L\textsuperscript{-1}, and the initial NO\textsubscript{3}\textsuperscript{−}-N was 22.13–23.13 mg L\textsuperscript{-1}. The results are presented in Fig. 4(a); the required time for complete denitrification was from 23.1 h to 2.5 h. In heterotrophic denitrification, nitrite accumulation may inhibit the bacterial denitrification rate,\textsuperscript{17} and the inhibitory effect of nitrite was also reported in the autotrophic denitrification process.\textsuperscript{18} In this study, nitrite inhibition was not observed: nitrite appeared as an intermediate product, it was reduced at the end of reaction, showing that nitrite appears favorable as an electron acceptor.

The zero-order kinetics model used for bio-electrochemical denitrification showed good correlation with the experimental results (Table 1); correlations throughout the experiments were from 0.9778 to 0.9980, indicating that the applied current (50 mA) was sufficient for denitrification. A strongly linear relationship (R\textsuperscript{2} = 0.9728) between biomass and denitrification rate was established in this study (Fig. 4(b)). In this process, the nitrate reduction rate was in the range 8.70–19.70 mg NO\textsubscript{3}\textsuperscript{−}-N g\textsuperscript{−1} VSS h\textsuperscript{−1} (0.21–0.47 g NO\textsubscript{3}\textsuperscript{−}-N g\textsuperscript{−1} VSS d\textsuperscript{−1}) for 20 ± 1 °C, and the average denitrification rate for unit biomass was calculated to be 9.36 mg NO\textsubscript{3}\textsuperscript{−}-N g\textsuperscript{−1} VSS h\textsuperscript{−1}. The kinetics of hydrogen autotrophic
Faraday’s constant (26.8 mA h mmol⁻¹) has provided denitrification rates. 13,19 A comparison between denitrification has been studied in recent years and some research has provided denitrification rates. 13,19 A comparison between denitrification rates was mainly due to mixed conditions for hydrogenotrophic microorganisms. The slight reduction. That is, this novel system could provide satisfactory that the microorganism in this novel system achieved the same complete reduction.

In the electrodialysis process, the rate of donor (H₂) supply was affected by the electrical current. The function of the electrical current in the denitrification process was explored in this study. The effect of electrical current on nitrate removal was modeled according to a zero-order kinetics model and the results are presented in Fig. 6 and Table 3. A highly linear relationship (R² > 0.97) between nitrate concentrations and time confirmed that the denitrification process could be well described by the Monod equation. It was indicated that the hydrogen generated by the cathode was enough for bioreduction. Nitrite also appears to be a favorable electron acceptor and it took about 0.5–1.0 h longer than nitrate for complete reduction.

**Table 2. Comparison between hydrogen autotrophic denitrification rates**

<table>
<thead>
<tr>
<th>Method of hydrogen supply</th>
<th>Biomass g L⁻¹</th>
<th>Temp. °C</th>
<th>pH</th>
<th>v_max g NO₃⁻N VSS g⁻¹ d⁻¹</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct</td>
<td>1.5–2.5</td>
<td>30</td>
<td>7.5</td>
<td>0.22–0.37</td>
<td>13</td>
</tr>
<tr>
<td>Direct</td>
<td>0.5</td>
<td>25 ± 1</td>
<td>7.5–9.5</td>
<td>0.38–0.74</td>
<td>19</td>
</tr>
<tr>
<td>Direct</td>
<td>0.5</td>
<td>12 ± 1</td>
<td>7.5–9.5</td>
<td>0.21–0.28</td>
<td>19</td>
</tr>
<tr>
<td>Electrodialysis</td>
<td>0.05–1.01</td>
<td>20 ± 1</td>
<td>7.05–7.20</td>
<td>0.21–0.47</td>
<td>This study</td>
</tr>
</tbody>
</table>

100%. As shown in Fig. 5, denitrification rate was not affected by currents between 30 and 100 mA. In the high current supply condition (I ≥ 30 mA), the bulk solution in the cathode cell was saturated with H₂ generated by the cathode, and the removal rate was not affected by the rate of donor supply. The removal rate reached 96.0%, 98.5% and 98.4% for 30 mA, 50 mA and 100 mA after 3.5 h electrodialysis, respectively. Meanwhile, the kinetics of denitrification attributed to zero-order reduction under this non-substrate (H₂) limiting condition. When the current became lower (15 mA), the denitrification rate was reduced because of inadequate H₂ supply, the removal rate was only 42.5% after 3.5 h electrodialysis, showing that the hydrogen generated by the electrodialysis process was not sufficient for bio-denitrification. Consequently, the kinetics did not fit the zero-order kinetics model and nitrite was accumulated under this substrate (H₂) limiting condition. At the end of the reaction, nitrite concentration increased to 1.27 mg-N L⁻¹. The corresponding current efficiency reached 70% at 5 mA, 80% at 30 mA, 48% at 50 mA and 24% at 100 mA. From both economic and removal efficiency aspects, 30 mA was the most suitable current for nitrate removal.

In the BER system of non-separation between anode and cathode, the reported maximum applied voltage was 5.0 V. 6 Higher applied voltages did not bring an increase of nitrate and nitrite removal. 5 The maximum nitrate removal rate was 5.68 mg-N L⁻¹ and the corresponding HRT was 1.2 h. In this study, this novel system removed 22.13 mg-N L⁻¹ NO₂⁻ in 3.5 h with biomass of 0.68 g L⁻¹; a high applied voltage of 6.5 V (corresponding current 100 mA) did not affect denitrification rate. The time required for complete denitrification could be made even shorter through increased biomass (Fig. 4). It can be concluded that this novel system could be operated under higher applied current and biomass concentration, and thus could provide a higher removal rate.

**Effect of initial nitrate concentration on nitrate removal**

The effect of initial nitrate concentration on nitrate removal was explored with initial nitrate concentration fixed at 22.13, 15.76 and 11.31 mg L⁻¹. Nitrate removal was modeled according to a zero-order kinetics model and the results are presented in Fig. 6 and Table 3. A highly linear relationship (R² > 0.97) between nitrate concentrations and time confirmed that the denitrification process could be well described by the Monod equation. It was indicated that the hydrogen generated by the cathode was enough for bioreduction. Nitrite also appears to be a favorable electron acceptor and it took about 0.5–1.0 h longer than nitrate for complete reduction.

**CONCLUSION**

The feasibility of bio-electrochemical denitrification by proton-exchange membrane electrodialysis was demonstrated in this
Denitrification by proton-exchange membrane electrodialysis system

**Figure 6.** Initial nitrate concentration effect on (a) nitrate removal, (b) nitrite generation; VSS=0.68 g L\(^{-1}\), applied current 50 mA.

<table>
<thead>
<tr>
<th>C(NO(_{3})(^{-})) (mg L(^{-1}))</th>
<th>NO(_{3}^{-})-N(_0) (mg L(^{-1}))</th>
<th>(K = k_1X) (mg NO(_{3}^{-}) g(^{-1}) VSS h(^{-1}))</th>
<th>(k_1) (mg NO(_{3}^{-}) -N VSS g(^{-1}) h(^{-1}))</th>
<th>(R^2)</th>
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</thead>
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<tr>
<td>22.13</td>
<td>6.52</td>
<td>9.58</td>
<td>0.9931</td>
<td></td>
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<tr>
<td>15.76</td>
<td>5.36</td>
<td>7.88</td>
<td>0.9721</td>
<td></td>
</tr>
<tr>
<td>11.31</td>
<td>5.08</td>
<td>7.47</td>
<td>0.9783</td>
<td></td>
</tr>
</tbody>
</table>

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**REFERENCES**


