

# Simultaneous nitrification and denitrification in a single reactor using bio-electrochemical process

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**Abstract** Feasibility of a bio-electrochemical process for simultaneous nitrification and denitrification in a single reactor was experimentally investigated. The reactor consisted of anodic and cathodic electrodes, on which nitrifying and denitrifying biofilms, respectively, were fixed. Effects of the applied electric current and DO concentration in the bulk solution were examined. The TN removal could be achieved through the occurrence of nitrification and denitrification in anodic and cathodic biofilms, respectively. Both nitrification and denitrification rates increased with an increase in the applied electric current. Even at low DO concentration in the bulk solution, nitrification proceeded at a high rate by utilizing oxygen generated on the anode. Denitrification rate remained relatively high at high DO concentration due to supplying hydrogen gas to the inner side of the cathodic biofilm. The higher TN removal rate tended to be obtained at lower DO concentration and higher current density. From these results, it was concluded that the bio-electrochemical process was applicable to simultaneous nitrification and denitrification due to stable formation of aerobic and anoxic regions in the single reactor.

**Keywords** Bio-electrochemical process; denitrification; electrolysis; nitrification

## Introduction

Biological nitrification and denitrification have been the most available processes for removal of ammonium from wastewater. In general, nitrification requires an aerobic environment for oxidation of ammonium to nitrite and then to nitrate, whereas, denitrification occurs by utilizing electron donors under an anoxic condition. Therefore, the conventional treatment processes are achieved either by separate aerobic and anoxic reactors or by temporal division of the conditions in case of a one-sludge system such as the sequencing batch reactor (Wiesmann, 1994). However, the phenomenon of simultaneous nitrification and denitrification (SND) under aerobic conditions was also observed in several treatment processes (Rittmann and Langeland, 1985; Masuda *et al.*, 1991). The comprehensive explanations for this phenomenon were the diffusional limitation of oxygen into microbial floc and biofilm and the contributions of aerobic denitrifiers and heterotrophic nitrifiers.

The SND in a single reactor under identical overall conditions has been attractive as it may have advantages over the separated process with respect to the reduction of reactor volume and time. As a method to facilitate the occurrences of aerobic denitrification and heterotrophic nitrification, the control strategies of the reactor have been investigated (Munch *et al.*, 1996; Zhao *et al.*, 1999). More recently, the microorganism capable of anaerobic ammonium oxidation to nitrogen gas by utilizing nitrite as an electron acceptor was discovered and was tried for ammonium treatment (Gaaf *et al.*, 1995; Strous *et al.*, 1997). On the other hand, an intentional formation of aerobic and anoxic zones in biofilm and cell aggregates was applied to enhance the SND. The occurrence of the SND was observed in the beads co-immobilized nitrifier and denitrifier with inert support under aerobic conditions in the bulk liquid (Kokufuta *et al.*, 1987; dos Santos *et al.*, 1996). Uemoto and Saiki (2000) used a tubular polymeric gel immobilized ammonia oxidizer and

denitrifier and supplied hydrogen gas in the lumen of the gel. They reported that nitrogen removal occurred effectively.

A bio-electrochemical reactor process for denitrification of nitrate contaminated water has been proposed (Sakakibara and Kuroda, 1993). This process involved direct fixation of denitrifiers on the cathode surface and generation of hydrogen gas, as an electron donor, by electrolysis of water. The denitrification proceeded effectively (Kuroda *et al.*, 1996; Feleke *et al.*, 1998). In the electrolysis of water, it is well known that oxygen generates on the surface of the anode when anodic material is suitable for oxygen generation. This implies that SND might be possible in a single reactor with the aid of stable aerobic and anoxic regions formed by electrochemical process. Kuroda *et al.* (1997) observed nitrification which utilized oxygen generated at the anode, on which nitrifiers were attached.

In this study, the feasibility of a bio-electrochemical process for simultaneous nitrification and denitrification in a single reactor was experimentally investigated. The reactor consisted of anodic and cathodic electrodes on which nitrifying and denitrifying biofilms, respectively, were attached. The effects of applied electric current and DO concentration in the bulk solution on nitrification and denitrification were examined by separate batch experiments. The performance of the simultaneous removal was investigated through the continuous experiments under different operating conditions.

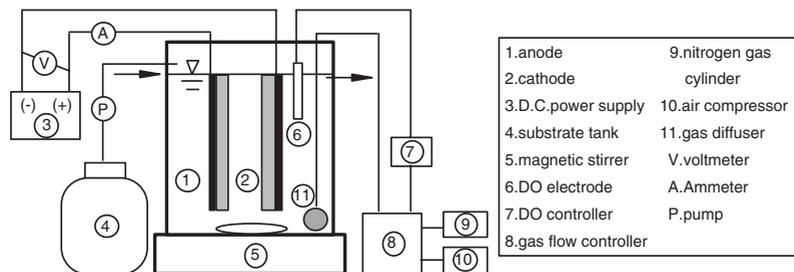
## Materials and methods

### Apparatus

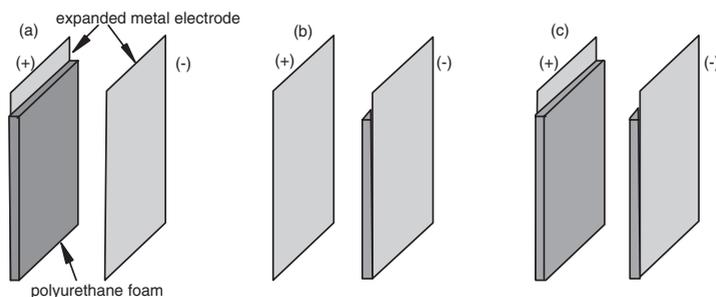
The schematic diagram of the experimental apparatus is shown in Figure 1. A vessel made of polyvinyl chloride resin was used as the reactor. The working volume of liquid was 3.8 L. A pair of plate type expanded metal electrodes, 12 cm in width and 15 cm in height, was placed in parallel configuration into the reactor and was connected to a DC power supply. The distance between the electrodes was kept at 2 cm. The effective area of each electrode immersed into liquid was about 170 cm<sup>2</sup>. A polyurethane foam 5 mm thick was fixed on the surface of the electrode for supporting biofilm. Prior to the experiments, the anodic and cathodic electrodes were immersed into nitrifying and denitrifying cultures, respectively, cultivated in our laboratory, for about 3 months to fix biofilm on the surface. The DO concentration in bulk liquid was regulated by means of a DO controller (EYELA, FM-2000) connected electrically to a gas flow control unit (EYELA, FM-130). The reactor liquid was mixed sufficiently by means of a magnetic stirrer. The temperature was kept at 30°C by immersing the reactor into a water bath.

### Batch experiment

The fundamental characteristics of nitrification and denitrification in the reactor were investigated by separate batch experiments. The anodic electrode, on which nitrifying biofilm was attached and the cathodic electrode without biofilm fixation (abiotic) were placed into the reactor for nitrification experiments as shown in Figure 2(a). A solution was prepared by dissolving ammonium sulfate, sodium bicarbonate and other trace nutrients in tap water. The initial concentration of ammonium was adjusted to 50 mg-N/L. For the denitrification experiments, the cathodic electrode where denitrifying biofilm was attached, and the abiotic anode were placed into the reactor as shown in Figure 2(b). A solution containing potassium nitrate, sodium bicarbonate, trace nutrients and no organic matter was used. The initial nitrate concentration was 50 mg-N/L. The solutions used in both batch experiments were buffered with 10 mM phosphate buffer to minimize the change in pH during experiments. The experiments were carried out at different DO concentration in bulk solution and electric current density ranging from 0 to 0.3 mA/cm<sup>2</sup>.



**Figure 1** Schematic diagram of the experimental apparatus



**Figure 2** Combinations of electrodes; (a) for nitrification experiments, (b) for denitrification experiments and (c) for SND experiments

### Continuous experiment

The anodic and cathodic electrodes, on which nitrifying and denitrifying biofilm, respectively, was fixed, were placed into the reactor for continuous experiments as shown in Figure 2(c). The same solution that was used in the batch experiment for nitrification was fed continuously to the reactor. The hydraulic retention time (HRT) was kept at 10 h during the experiments. The DO concentration in the bulk solution and the applied current density were changed from 0.1 to 3 mg/l and from 0 to 0.3 mA/cm<sup>2</sup>, respectively. The reactor was cleaned periodically to minimize the contribution of the biomass grown on the reactor wall to transformation of nitrogen compounds. After the steady state was attained, the experimental conditions were changed in stepwise manner.

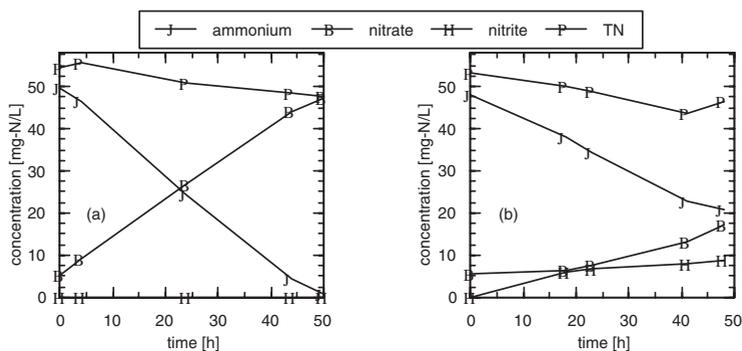
### Analytical methods

For the measurement of dissolved components, liquid samples were filtered with a membrane filter with 200 nm of nominal pore size and were subjected to analyses. Nitrate and nitrite were measured with an ion chromatograph (Yokogawa analytical systems, IC-7000). Ammonium concentration was analyzed by Nessler method by using a spectrophotometer (Hach, DR-2010).

## Results and discussion

### Nitrification in anodic biofilm

Preliminary experiments under abiotic conditions showed that the decrease in ammonium concentration was negligibly small (data not shown), although Marincic and Leitz (1978) reported that the transformation of ammonium to nitrogen gas was electrochemically possible with high current efficiency. The difference between their result and our observation was caused by the electrode system used, especially anode material. The small current density operated in our study may have also suppressed the electrochemical ammonium

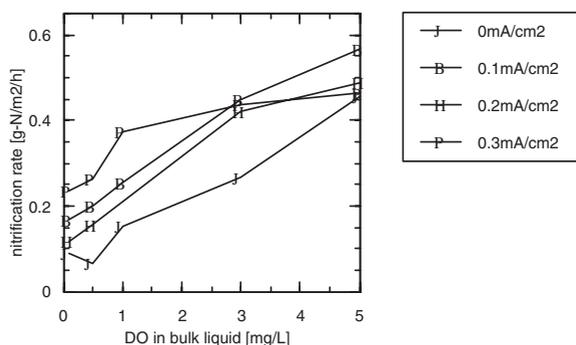


**Figure 3** Changes in ammonium, nitrite and nitrate; (a) at DO concentration 1 mg/L and current density 0.1 mA/cm<sup>2</sup> and (b) at DO concentration 0.1 mg/L and current density 0.1 mA/cm<sup>2</sup>

oxidation. Therefore, change in ammonium concentration in the bio-electrochemical system seemed to be derived from biological reactions.

Typical concentration changes in ammonium, nitrite and nitrate during the batch experiment are shown in Figure 3(a). The nitrate concentration increased with a decrease in ammonium, while no nitrite was observed. The total nitrogen (TN) balance in the reactor liquid was approximately kept during the experiments. However, in some experimental runs, nitrite as well as nitrate accumulated with time (Figure 3(b)). The reasons why nitrite accumulated were not clear.

The effects of the DO concentration and the current density on nitrification rate per unit anodic area are shown in Figure 4. Under the identical condition of DO concentration in the bulk liquid, the nitrification rate was higher than that obtained from the control experiment, in which no electric current was applied. The nitrification rate tended to increase with increasing current density. At the lower DO concentration, the applied electric current contributed effectively to the increase in the nitrification rate. With the aid of electric current, high nitrification rate could be achieved even at low DO concentration. These results indicate that nitrification proceeded within the anodic biofilm by utilizing oxygen derived from the electrolysis of water as well as that served from the bulk liquid. In general, an anoxic environment forms in the deeper region of the aerobic biofilm due to limitation of oxygen penetration even at high DO concentration in the bulk liquid (Zhang *et al.*, 1995). In the bio-electrochemical system, oxygen was supplied from both sides of the biofilm, i.e. from anode surface as well as from the bulk solution. This might lead to the reduction of the anoxic zone in the biofilm and the increase in nitrification rate.



**Figure 4** Effects of DO concentration and current density on nitrification in anodic biofilm

### Denitrification in cathodic biofilm

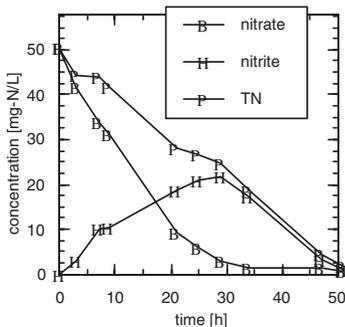
Typical concentration changes in nitrate and nitrite are shown in Figure 5. The nitrate concentration decreased with elapse of time and nitrite accumulated at the beginning. The TN concentration was gradually decreasing until it finally approached zero. When electric current was not applied, the TN concentration did not decrease (data not shown). These results indicate that denitrification proceeded by utilizing hydrogen gas derived from electrolysis.

The relationship between the denitrification rate per unit cathodic area, the DO concentration and the current density is shown in Figure 6. The denitrification rate was calculated on the basis of decrease in TN concentration. Within the experimental range, the denitrification rate increased with the increasing current density, whereas it decreased with the increasing DO concentration. Assuming that hydrogen gas was stoichiometrically generated and consumed for denitrification, the theoretical denitrification rates for 0.1, 0.2 and 0.3 mA/cm<sup>2</sup> were 0.11, 0.23 and 0.34 g-N/m<sup>2</sup>/h, respectively. Therefore, hydrogen utilization efficiency for denitrification was more than 80% under approximately anoxic condition. When DO concentration exceeded 3 mg/L, the denitrification rates were reduced to 40–70% of the anoxic rates. Thus, the dissolved oxygen entering the cathodic biofilm inhibited the denitrifying activity. Oh and Silverstein (1999) reported a significant decrease in the denitrification rate of activated sludge flocs with an increase in DO concentration. Compared to their results, the denitrification rate remained relatively high in the bio-electrochemical system at high DO concentration, because hydrogen gas derived from electrolysis was supplied to the inner side of the biofilm. Biological consumption and electrochemical reduction of dissolved oxygen may result in the formation of an anoxic region in the cathodic biofilm.

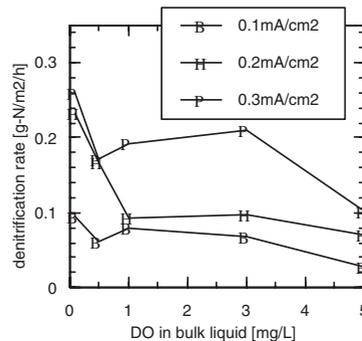
### TN removal under continuous operation

When the electric current was applied to the electrodes, ammonium concentration decreased and nitrate and nitrite were detected in the effluent. In addition, the difference in TN concentrations between the influent and the effluent was observed, depending on the DO concentration and the current density. The steady state with respect to the dissolved nitrogenous compounds was reached within 2 to 3 days of operation in every experimental run (data not shown).

The effect of the current density on the removal rates for ammonium and TN is shown in Figure 7. Both ammonium and TN removals increased with an increase in the applied electric current, since the generation rates of oxygen and hydrogen increased. This result indicates that the SND could be achieved in a single reactor using the bio-electrochemical process. The oxygen generated on the surface of the anode accelerated the ammonium



**Figure 5** Typical changes in nitrate and nitrite in DO concentration 0.5 mg/L and current density 0.3 mA/cm<sup>2</sup>



**Figure 6** Effects of DO concentration and current density on denitrification in cathodic biofilm

oxidation and hydrogen generated on the cathode was utilized for reduction of nitrate and nitrite to nitrogen gas. At the DO concentration 0.1 mg/L and the current density 0.3 mA/cm<sup>2</sup>, the TN removal rate per unit electrode area attained about 5 g-N/m<sup>2</sup>/d.

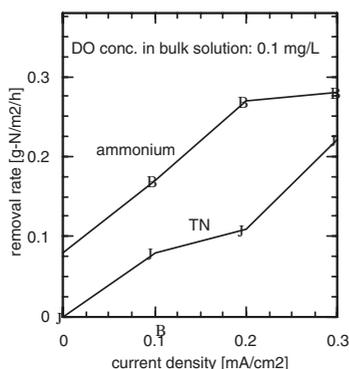
The removal rates for ammonium and TN as a function of DO concentration in the bulk solution are shown in Figure 8. As can be seen, the decrease in TN removal rate was observed with increasing the DO concentration, while the ammonium oxidation rate increased. The total nitrogen was not removed in the absence of the electric current. These results suggest that denitrification in the cathodic biofilm limited the TN removal. The dependency of the denitrification rate on the DO concentration as shown in Figure 6 seems to support these results. The anoxic condition in the cathodic biofilm seemed to be created due to the consumption of dissolved oxygen with hydrogen utilization by aerobic microorganisms that did not contribute to denitrification. In addition, an electrochemical reduction of the dissolved oxygen might occur on the surface of the cathode. However, these phenomena caused lowering of the current efficiency for hydrogen generation and limited the utilization of the generated hydrogen for denitrification. Consequently, the denitrification rate was restricted. Within the experimental range in this study, low DO concentration in the bulk solution was desirable for approaching the TN removal rate to the ammonium removal rate.

### Conclusions

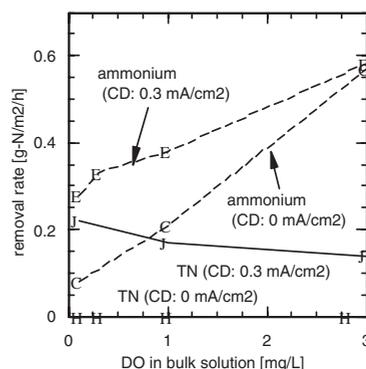
The TN removal from a synthetic wastewater containing ammonium in a single reactor using a bio-electrochemical process was demonstrated experimentally. The TN removal could be achieved through the occurrences of nitrification and denitrification in anodic and cathodic biofilms, respectively. Both nitrification and denitrification rates increased with an increase in the applied electric current. At low DO concentration in the bulk solution, nitrification proceeded with relatively high rate by utilizing oxygen generated on the anode. Denitrification rate remained relatively high at high DO concentration due to supplying hydrogen gas to the inner side of the cathodic biofilm. The higher TN removal rate tended to be obtained under lower DO concentration and higher current density. However, the TN removal in the reactor was limited by denitrification in the cathodic biofilm. Further research on operational methods and the optimum configuration of anode and cathode will be necessary to increase the removal efficiency.

### Acknowledgements

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**Figure 7** Relation between current density and TN removal



**Figure 8** Effect of DO concentration in the bulk solution on ammonium and TN removals

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