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Simultaneous nitrification and denitrification using an autotrophic membrane-immobilized biofilm reactor

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Aims: To develop a laboratory-scale autotrophic membrane-immobilized biofilm reactor to remove nitrogen from drinking water.
Methods and Results: A polyvinyl alcohol (PVA) immobilized biofilm, attached to the surface of a silicone tube, was used as the basis of a bioreactor for simultaneous nitrification and denitrification of water. The bioreactor was aerated with air to supply oxygen for nitrification. Pure hydrogen was supplied to the silicone tube and diffused through the membrane wall to feed the biofilm for autotrophic denitrification. The bioreactor was effective for the simultaneous nitrification and denitrification of water after a short period of acclimation, while the biofilm exhibited good resistance to the inhibition of denitrification by dissolved oxygen; the denitrification rate decreased by only 8% as the dissolved oxygen increased from 2 mg l^{-1} to saturation.
Conclusions: By using PVA crosslinked with sodium nitrate to entrap nitrifying and denitrifying sludge on the surface of a silicone tube, a novel bioreactor for simultaneous nitrification and denitrification was developed. In addition to performing as an immobilizing agent to strengthen the biofilm, PVA protected the denitrifying microorganisms to reduce the inhibition by dissolved oxygen under aerobic condition. Therefore, nitrification and denitrification occurred simultaneously within the biofilm. Furthermore, the immobilization technique shortened the acclimation period of the bioreactor.
Significance and Impact of the Study: The described space saving and simple to operate bioreactor for nitrogen removal performed autotrophic denitrification to solve the problem of residual carbon in heterotrophic denitrification, and thus is suitable for removing nitrogen from drinking water.

INTRODUCTION

Rising nitrogen levels in drinking water have become a serious environmental problem. Besides causing eutrophication of lakes or reservoirs, higher nitrogen levels also threaten public health (Mirvish 1985), making the development of a water treatment system for nitrogen removal a high priority.

Given their high efficiency and low cost, biological treatment systems are frequently selected for the removal of nitrogen from wastewater. Such systems accomplish nitrogen removal through two steps: nitrification, the transformation of ammonium to nitrite or nitrate, and denitrification, the transformation of nitrite or nitrate to nitrogen gas. Nitrification requires an aerobic, inorganic environment, while denitrification requires an anoxic, organic-rich environment. Consequently, conventional nitrogen removal systems required separate nitrification and denitrification units.

To achieve an effective, one-step biological nitrogen removal system, many researchers have examined the possibilities of combining nitrification and denitrification in a single bioreactor (Timberlake *et al.* 1988; Bertanza 1997; Zhao *et al.* 1999; Uemoto and Saiki 2000; and Lee

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et al. 2001). Our previous work developed a double membrane bioreactor for simultaneous nitrification and denitrification (Chang and Tseng 1999). This bioreactor contained two silicone membrane modules. Both of the modules were fed with substrates in the lumen side of the silicone tubes by diffusion them to the biofilms formed on the surface of the tubes. One module was fed with methanol for nitrification and the other was fed with pure oxygen for nitrification. However, this bioreactor was not suitable for treating drinking water, as the use of methanol is potential harmful for human health and as it also penetrates the biofilm when the denitrification is completed.

The present study applies a combination of autotrophic denitrification, cell immobilization and membrane bioreactor approaches to design an autotrophic membrane-immobilized biofilm reactor for the removal of nitrogen from drinking water. A cell immobilization technique, using polyvinyl alcohol (PVA) crosslinked with sodium nitrate (Chang and Tseng 1998), was used to form a biofilm on the surface of a silicone tube for simultaneous nitrification and denitrification. The biofilm was aerated with air for nitrification, and pure hydrogen instead of methanol was supplied to the silicone tube for denitrification, solving the problem of residual carbon from heterotrophic denitrification. The effects of dissolved oxygen on the nitrification and denitrification were evaluated.

MATERIALS AND METHODS

Synthetic wastewater

This study used two types of synthetic wastewater: ammonium solution containing 100 mg l^{-1} NH_4-N; and nitrate solution containing 100 mg l^{-1} NO_3-N. The ammonium solution contained $(NH_4)_2SO_4$ (0·472 g l^{-1}), $NaHCO_3$ (1 g l^{-1}), Na_2HPO_4 (9 g l^{-1}), KH_2PO_4 (1·5 g l^{-1}), $MgSO_4 \cdot 7H_2O$ (0·2 g l^{-1}) and trace element solution (1 ml l^{-1}), the latter comprising $ZnSO_4 \cdot 7H_2O$ (2·2 g l^{-1}), $CaCl_2 \cdot 2H_2O$ (7·3 g l^{-1}), $MnCl_2 \cdot 4H_2O$ (2·5 g l^{-1}), $CoCl_2 \cdot 6H_2O$ (0·5 g l^{-1}), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0·5 g l^{-1}), $FeSO_4 \cdot 7H_2O$ (5·0 g l^{-1}), and $CuSO_4 \cdot 5H_2O$ (0·2 g l^{-1}). The nitrate solution was identical to the ammonia solution, except that $(NH_4)_2SO_4$ (0·472 g l^{-1}) was replaced with KNO_3 (0·722 g l^{-1}).

Immobilization nitrifying and denitrifying sludge by PVA

Nitrifying and denitrifying sludge from a municipal wastewater treatment plant was initially cultivated by synthetic wastewater in two 10-litre flasks at 30 °C, with a pH of 7·0 for one week. One flask had ammonium solution added and was aerated with air for nitrification, while the other flask had nitrate solution added and was aerated with hydrogen gas for denitrification. Following cultivation, the sludges were mixed prior to immobilization. Polyvinyl alcohol (16 g) and alginic acid (1·2 g) were dissolved in 100 ml deionized water and then mixed with 100 ml concentrated sludge. The mixture was used to coat the surface of a 5-m long silicone membrane tube (1·5 mm inner and 2·5 mm outer diameter; Fuji System Co., Tokyo, Japan), which was then immersed in a solution containing 50% sodium nitrate and 2% calcium chloride for 1 h to form an immobilized biofilm on the silicone tube.

Bioreactor

The bioreactor was constructed of Perspex and had a working volume of 1 l. The silicone membrane tube covered with immobilized biofilm was wound around the pillars in the bioreactor. A dissolved oxygen control system aerated the wastewater to supply oxygen for nitrification and regulated the level of dissolved oxygen in the bioreactor. Pure hydrogen gas was supplied to the lumen of the silicone tube for denitrification. Finally, the contents of the bioreactor were mixed with a magnetic stirrer and the temperature of the bioreactor maintained at 30 °C (Fig. 1).

Operation

The ammonium solution was pumped into the bioreactor at 2 ml min^{-1} for initial acclimation of the biofilm, and simultaneously the dissolved oxygen level in the bioreactor was maintained at 4 mg l^{-1} and the hydrogen gas was supplied through the lumen of the silicone tube at 20 ml min^{-1}. The concentrations of ammonium, nitrate and nitrite in the effluent were monitored daily to determine the nitrogen removal rate of the bioreactor.

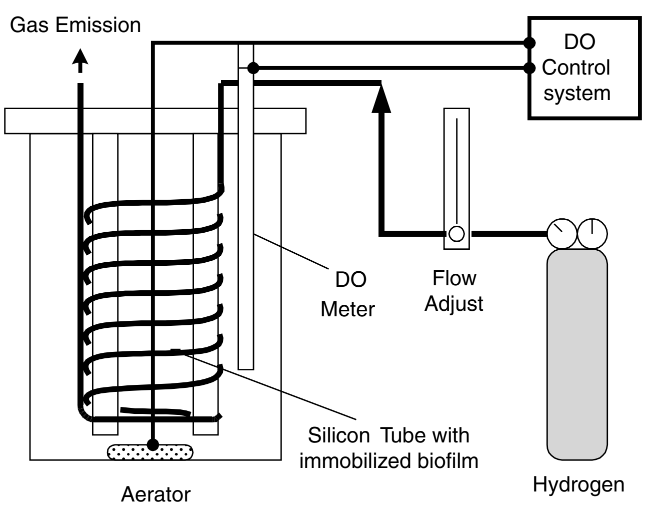

Fig. 1 Schematics of the bioreactor

Table 1 Initial conditions for batch experiments

Run no.	The synthetic wastewater	Dissolved oxygen maintained in the bioreactor (mg l^{-1})	Supply hydrogen gas to the lumen of the silicone tube
Simultaneous nitrification and denitrification assessment of the biofilm			
Run A	Ammonia solution	4	Yes
Run B	Ammonia solution	4	Yes
Run C	Nitrate solution	4	No
Run D	Nitrate solution	4	No
Dissolved oxygen effects on nitrification and denitrification			
Run 1	Ammonia solution	Saturated	Yes
Run 2	Ammonia solution	4	Yes
Run 3	Ammonia solution	2	Yes
Run 4	Nitrate solution	Saturated	Yes
Run 5	Nitrate solution	4	Yes
Run 6	Nitrate solution	2	Yes

Following the stabilization of the nitrogen removal rate of the bioreactor, 10 runs of the batch experiment were performed to assess the simultaneous nitrification and denitrification ability of the bioreactor and the effects of dissolved oxygen on nitrification and denitrification. Table 1 summarizes the initial conditions of these batch experiments.

Analysis

Ion chromatography (Dionex 120) determined the concentrations of nitrate and nitrite. Ammonium determined colorimetrically (APHA *et al.* 1995), and a dissolved oxygen (DO) meter (WTW, pH 537; Wilhelm, Germany) was used to determine the dissolved oxygen concentration.

RESULTS

Acclimation of the biofilm

Figure 2 shows the changes of ammonia, nitrite and nitrate concentrations in effluent during the acclimation period. Within the first 14 days, ammonium concentration decreased gradually from 100 to < 0·1 mg l^{-1}, while nitrate concentration increased to 45 mg l^{-1}. They then remained stable during the next 16 days, with very little nitrite being detected. The data indicated that the biofilm performed a stable ability for nitrogen removal after a short period of acclimation. The average nitrogen removal rate was 2·5 g Nm^{-2} d^{-1} of surface area of silicone tube.

Simultaneous nitrification and denitrification of the bioreactor

The assessment of the bioreactors ability to achieve simultaneous nitrification and denitrification is shown in Fig. 3.

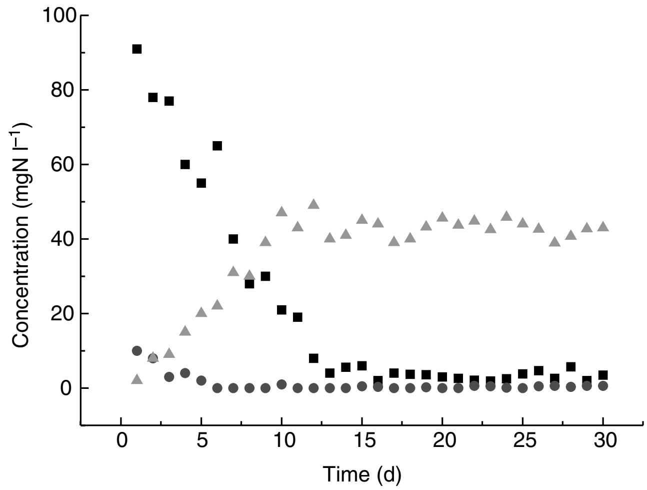

Fig. 2 Time-dependent changes of ammonia (■), nitrite (●) and nitrate (▲) concentrations in effluent during the acclimation period

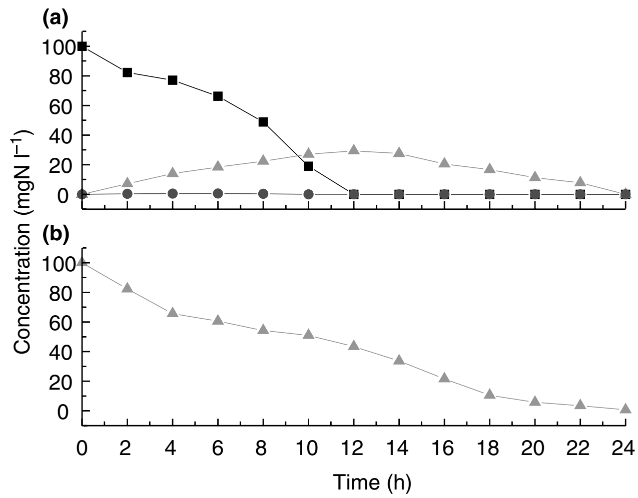

Fig. 3 Time-dependent changes of ammonia (■), nitrite (●) and nitrate (▲) concentrations in synthetic wastewater containing (a) 100 mg l^{-1} NH_4-N and (b) 100 mg l^{-1} NO_3-N during the batch experiment

Ammonium concentration decreased linearly from 100 to < 0·1 mgN l^{-1} during the first 12 h, while nitrate concentration gradually rose to 30 mgN l^{-1}. Thereafter the nitrate concentration decreased over the next 12 h to < 0·1 mgN l^{-1} (Fig. 3a). The data indicate that the ammonium was oxidized to nitrate, causing an increase in nitrate during the first 12 h, after which the nitrate was removed during the following 12 h. When the bioreactor was used to treat nitrate, the concentration decreased linearly from 100 to < 0·1 mgN l^{-1} within 24 h (Fig. 3b), indicating that, although the bioreactor was being used in an aerobic environment (DO level of 4 mg l^{-1}), denitrification occurred.

Confirmation of the use of hydrogen for denitrification

To confirm that hydrogen was used for autotrophic denitrification of the biofilm, two batch experiments were conducted in which hydrogen gas was not supplied to the silicone tube (Fig. 4). Under this condition, ammonium declined from 100 to < 0·1 mgN l^{-1}, while nitrate concentration increased to 93 mgN l^{-1} within the first 12 h, remaining stable during the subsequent 12 h (Fig. 4a). These data confirm that the denitrification of nitrate formed from ammonium by the bioreactor requires hydrogen. Additionally, most of the ammonium was oxidized to nitrate for energy, and very little was used for the synthesis of new cells. Following treatment of the nitrate solution, no decrease in nitrate was observed (Fig. 4b), confirming that denitrification was not possible in the absence of hydrogen.

Influence of dissolved oxygen on nitrification and denitrification

The influence of oxygen on nitrification (Fig. 5) showed that the ammonium concentration decreased linearly from 100 mgN l^{-1} to < 0·1, 3·3 or 54·6 mgN l^{-1} within 12 h when dissolved oxygen level was maintained at saturation (7·5 mg l^{-1}), 4 mg l^{-1} or 2 mg l^{-1}, respectively. The nitrification rates were 5·1, 4·9 and 2·3 gN d^{-1} m^2 of the silicone tube in 12 h. The data indicate that the level of dissolved oxygen significantly influenced the rate of nitrification by the bioreactor. The effect of dissolved oxygen on the rate of denitrification is shown in Fig. 6. At dissolved oxygen concentrations of saturation, 4 mg l^{-1}, or 2 mg l^{-1}, the nitrate concentration decreased linearly from 100 mgN l^{-1} to 8·3, 4·7 or < 0·1 mgN l^{-1}, respectively, within 24 h. The denitrification rates were 2·3, 2·4 or 2·5 g Nm^{-2} d^{-1} of the silicone tube over 24 h, indicating that dissolved oxygen did not significantly inhibit the denitrification activity of the biofilm.

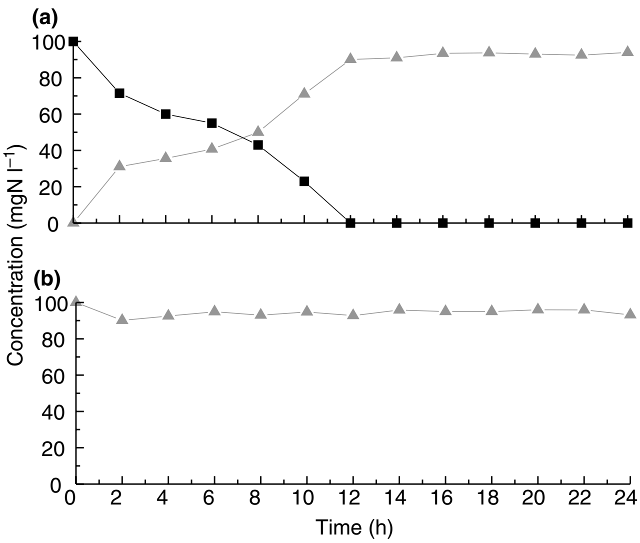

Fig. 4 Time-dependent changes of ammonia (■), nitrite (●) and nitrate (▲) concentrations in wastewater containing (a) 100 mg l^{-1} NH_4-N and (b) 100 mg l^{-1} NO_3-N during the batch experiment without hydrogen supply to the silicone tube

DISCUSSION

While oxygen is essential for nitrification, it significantly inhibits denitrification (Oh & Silverstein 1999), making it difficult to combine nitrification and denitrification in a single bioreactor. However, this study indicates that a membrane-immobilized biofilm reactor can be applied for simultaneous nitrification and denitrification. By using a

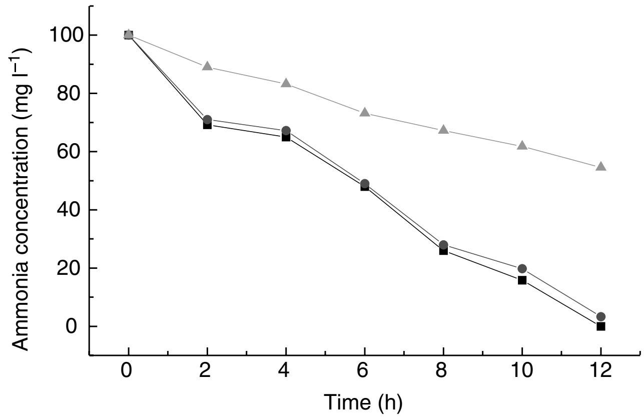

Fig. 5 Time-dependent changes in ammonia levels during the batch experiments when dissolved oxygen level was maintained at saturation (■), 4 mg l^{-1} (●) or 2 mg l^{-1} (▲)

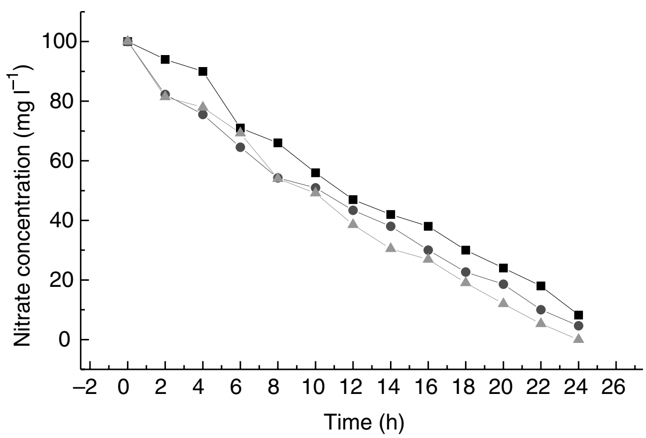

Fig. 6 Time-dependent changes in nitrate levels during the batch experiments when dissolved oxygen level was maintained at saturation (■), 4 mg l^{-1} (●) or 2 mg l^{-1} (▲)

new cell immobilization technique (PVA crosslinked with sodium nitrate) to entrap nitrifying and denitrifying sludge on the surface of a silicone tube, a novel bioreactor for the simultaneous nitrification and denitrification of water was designed. In addition to performing as an immobilizing agent to strengthen the biofilm on the silicone membrane (Hsieh *et al.* 2002), PVA also protected the denitrifying microorganisms to reduce the inhibition by dissoloved oxygen under aerobic conditions, thus allowing simultaneous nitrification and denitrification within the biofilm. The denitrification rate only decreased by 8% with the increase of dissolved oxygen from 2 mg l^{-1} to saturation level, and denitrification continued even when the bioreactor was operated under saturating dissolved oxygen levels. Moreover, this immobilization technique also shortened the acclimation period of the bioreactor. The bioreactor performed a stable nitrogen removal rate after about 14 days, while a long period of acclimation (about 60 days) was required for inoculating a pure culture (*Alcaligenes eutrophus*) to form a biofilm on the surface of the silicone tube for nitrogen removal (Ho *et al.* 2001).

This novel bioreactor was capable of simultaneous nitrification and denitrification, thus achieving a simpler and smaller nitrogen removal system than existing alternatives. Furthermore, the novel bioreactor used inorganic carbon source (sodium bicarbonate instead of methanol) and hydrogen gas for denitrification, which are clean and harmless to humans, thus avoiding the problems caused by using organic carbon sources for heterotrophic denitrification. Additionally, the novel bioreactor is stable and simple to operate, making it suitable for use in removing nitrogen from drinking water.

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