Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 2010, 22(2) 257–262
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Bio-reduction of nitrate from groundwater using a hydrogen-based membrane biofilm reactor

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Received 05 January 2009; revised 31 March 2009; accepted 18 May 2009

Abstract

A hydrogen-based membrane biofilm reactor (MBfR) using H_2 as electron donor was investigated to remove nitrate from groundwater. When nitrate was first introduced to the MBfR, denitrification took place on the shell side of the membranes immediately, and the effluent concentration of nitrate continuously decreased with 100% removal rate on day 45 under the influent nitrate concentration of 5 mg NO_3^--N/L, which described the acclimating and enriching process of autohydrogenotrophic denitrification bacteria. A series of short-term experiments were applied to investigate the effects of hydrogen pressures and nitrate loadings on denitrification. The results showed that nitrate reduction rate improved as H_2 pressure increasing, and over 97% of total nitrogen removal rate was achieved when the nitrate loading increased from 0.17 to 0.34 g NO_3^--N/(m^2·day) without nitrite accumulation. The maximum denitrification rate was 384 g N/(m^3·day). Partial sulfate reduction, which occurred in parallel to nitrate reduction, was inhibited by denitrifiфcation due to the competition for H_2. This research showed that MBfR is effective for removing nitrate from the contaminated groundwater.

Key words: hydrogen-based membrane biofilm reactor; autotrophic denitrification; nitrate; groundwater
DOI: 10.1016/S1001-0742(09)60102-9

Introduction

Removing nitrate (NO_3^-) and nitrite (NO_2^-) from drinking water is becoming increasingly important because of the risk posed to human health. In general, nitrate was mainly from agricultural fertilizers, septic systems, landfills, and wastewater treatment plants, which would not be absorbed by soil while can exist in groundwater for a long time (Cordy et al., 2000). The standards of nitrate and nitrite set by USEPA are 10 mg NO_3^--N/L and 1 mg NO_2^--N/L, respectively. However, a significant fraction of groundwater used or drinking water exceeds the maximum contaminant limits, and exhibits an increasing trend year by year (Jiang et al., 2001).

There are several conventional physical-chemical methods to remove nitrate from water, including ion exchange, reverse osmosis and electro-dialysis, which are effective for nitrate reduction, whereas these technologies were limited in application, because they are expensive and will produce the concentrated waste brines, requiring further treatment (Sarina and David, 2004). Biological denitrification included autotrophic and heterotrophic ways, which occur under anaerobic conditions (Soares, 2000). However, due to a very low organic carbon sources in groundwater, which were normally used as electron donors, nitrate can not be removed effectively through heterotrophic biological methods.

Autotrophic denitrification with H_2 was extensively investigated to remove nitrate from polluted groundwater or surface water. Kurt et al. (1987) studied autotrophic denitrification in a cone-shaped fluidized sand-bed reactor using a mixed culture. H_2 was transferred to the reactor using a bubbling-absorption. The nitrate elimination rate was 552 g NO_3^--N/(m^3·day) under the initial NO_3^--N concentration of 25 mg/L and the residence time of 4.5 hr. Dries et al. (1988) used a dual-column reactor, which was comprised of a downflow fixed bed for the first column and an upflow column for the second bed, to study the performance of autohydrogenotrophic denitrification. The H_2 was supplied to the reactor by direct bubbling of H_2 gas in the downflow column. Three types of polyurethane sponge matrixes were used as the biofilm carrier and the removal rate of 500 g NO_3^--N/(m^3·day) was achieved at 20°C. However, the hydrogen utility efficiency was low when bubbling H_2 gas in the reactors was used.

The hydrogen-based membrane biofilm reactor (MBfR), a new technical approach for bio-reduction of oxidized contaminants (Ergas and Reuss, 2001; Lee and Rittmann, 2002; Manem and Sanderson, 1996; Rittmann, 1998; Rittmann and McCarty, 2001), delivers hydrogen gas as

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the electron donor. H_2 is non-toxic, inexpensive compared to organic donors, and leaves no residuals that could cause bacterial re-growth. MBfR using hydrogen for denitrification has a very good H_2-utilization efficiency (more than 99%), which can avoid an air explosive hazard (Rittmann et al., 2004). Therefore, H_2 is used as a biologically available electron donor for the reduction of many oxidized contaminants in a hollow-fiber biofilm reactor, such as arsenate (AsO_4^{3-}), perchlorate (ClO_4^-), selenate (SeO_4^{2-}), chromate (CrO_4^-), trichloroethene (TCE), N-nitrosodimethylamine (NDMA) (Nerenberg et al., 2002; Chung et al., 2006a, 2006b, 2006c, 2008a, 2008b).

In this article, the potential for using H_2-based MBfR for reducing nitrate to N_2 was evaluated detailedly. In particular, the objectives of this study were: (1) the acclimation of autohydrogenotrophic denitrification bacteria; (2) the effects of H_2 pressure and nitrate loadings on denitrification; (3) nitrite accumulation during the denitrification process.

1 Materials and methods

1.1 Experimental set-up

A schematic of the MBfR used in this study is shown in Fig. 1, and the characteristics of the reactor are listed in Table 1. The MBfR system consisted of two membrane modules connecting to a recirculation loop. A single peristaltic pump (Longer BT50-1J, Baoding, China) was used to keep a nitrate-medium-feed rate of 0.05–0.80 mL/min. Under the high recirculation rate (60 mL/min) and the high recirculation rate (more than 150:1), the system run as a completely mixed biofilm reactor to maintain a consistent biomass accumulation on the hollow fibers. The main membrane module contained a bundle of 32 hydrophobic hollow-fiber membranes (MHF 200TL, Mitsubishi Rayon, Japan) inside a glass pipe shell. The other module contained three fibers to collect biofilm samples. This allowed sample collection without disturbing the main bundle of fibers and without causing a significant change in total biofilm surface area in the reactor. Pure H_2 was supplied to the inside hollow fibers through the manifold at the base with the pressure ranged from 0.02 to 0.05 MPa.

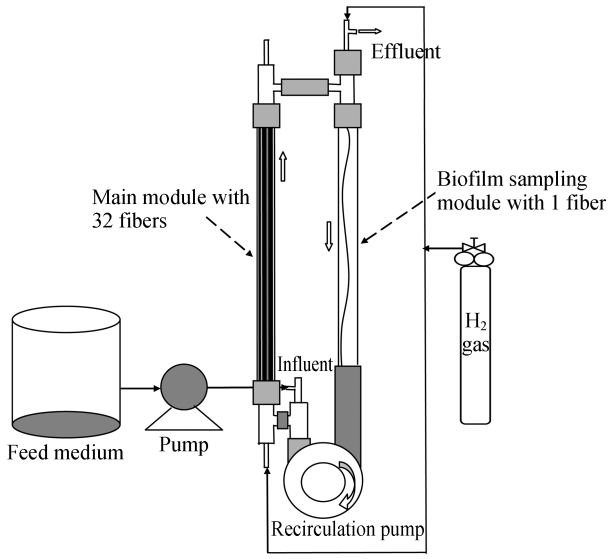

Fig. 1 Schematic of the hydrogen-based membrane biofilm reactor used to investigate nitrate reduction.

Table 1 Physical characteristics of the MBfR system

	Main tube	Coupon tube	Reactor total
Tube inside diameter (cm)	0.6	0.5	
Number of hollow fibers (cm)	32	1	33
Fiber inside diameter (cm)			0.0135
Fiber outside diameter (cm)			0.027
Fiber surface area (cm^2)	83.6	2.6	86.2
MBfR system volume (mL)			24

The stoichiometries of nitrate reduced to form nitrite and nitrite reduced to form N_2 with hydrogen as the electron donor are shown by Eqs. (1) and (2):

$$NO_3^- + H_2 \longrightarrow NO_2^- + H_2O \quad (1)$$

$$2NO_2^- + 2H^+ + 3H_2 \longrightarrow N_2 + 4H_2O \quad (2)$$

The mass consumption ratio of H_2 to N for nitrate reduction is 0.14 mg H_2/(mg N), while the ratio for nitrite reduction is 0.21 mg H_2/(mg N). The principle schematic of MBfR is shown in Fig. 2.

1.2 Feed medium

In present study, the components of synthetic influent drinking water were (g/L): KH_2PO_4 0.128, Na_2HPO_4 0.434, $MgSO_4{\cdot}7H_2O$ 0.2, $CaCl_2{\cdot}2H_2O$ 0.001, $FeSO_4{\cdot}7H_2O$ 0.001, and $NaHCO_3$ 0.252, and 1 mL trace solution. Nitrate concentration ranged from 2.5 to 10 mg NO_3^--N/L. The components in 1 mL trace solution were (mg/L): $ZnSO_4{\cdot}7H_2O$ 100, $MnCl_2{\cdot}4H_2O$ 30, H_3BO_3 300, $CoCl_2{\cdot}6H_2O$ 200, $CuCl_2{\cdot}2H_2O$ 10, $NiCl_2{\cdot}2H_2O$ 10, $Na_2MoO_4{\cdot}2H_2O$ 30, and Na_2SeO_3 30. The trace solution was made in a 10.0-L (available volume) glass bottle under the purge by H_2 to eliminate dissolved oxygen initially exist in the influent (Chung et al., 2006b). $NaNO_3$ and $NaHCO_3$ were used as an inorganic nitrogen and carbon sources for the growth of autotrophic microorganisms, respectively, and phosphate buffer (KH_2PO_4 + Na_2HPO_4) was used to keep initial pH value of the influent around 7.5.

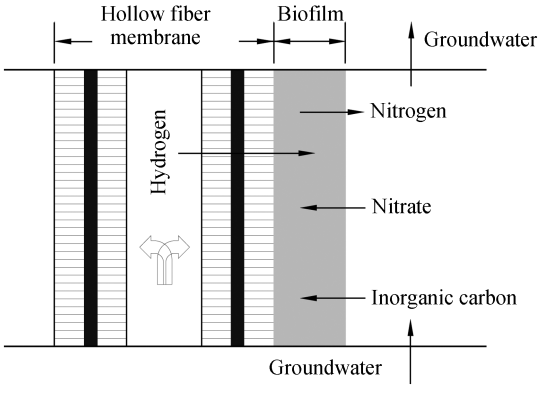

Fig. 2 Principle schematic diagram of MBfR.

1.3 Inoculation, start-up and stage experiments

Start-up of MBfR was initiated by seeding with 5 mL of anaerobic activated sludge from a municipal wastewater treatment plant in Shanghai, China. At the beginning of start-up, the synthetic drinking water containing the anaerobic sludge as bacterial seed was recirculated through MBfR for 2 days to establish a biofilm on the membrane surface.

Several short-term experiments were conducted to investigate systematically how H_2 pressure and nitrate loading affect nitrate reduction. The experiments were organized into two series, as listed in Table 2. In the first short-term experiment, the H_2 pressure was varied from 0.02 to 0.05 MPa to evaluate the effect of H_2 pressure on denitrification. In series 2, hydraulic retention time decreased from 4 to 0.5 hr to investigate the performance of MBfR for the denitrification.

1.4 Sampling and analysis

Influent and effluent samples were taken daily and filtered immediately through a 0.45-μm PVDF syringe filter. Nitrate, nitrite and sulfate were analyzed using ion chromatograph (ICS-1000, Dionex, USA) using an AS-11 column. Hollow fiber membrane surface and the bioflim were examined by scanning electron microscopy (XL-30, Philips, Netherlands). The samples were sputter coated with Au/Pd to enhance the quality of the image. Turbidity was determined by turbidmeter (2100P, HACH, USA). Chemical oxygen demand (COD) was measured using standard HACH COD vials (Loveland, CO) with a range of 0–40 mg COD/L. Total organic carbon (TOC) and dissolved organic carbon (DOC) concentrations were determined by TOC analyzer (TOCVCPH, Shimadzu, Japan). The titration method (SEPA, 2002) was used to determine the alkalinity.

Table 2 Conditions for stage experiments of MBfR

	Hydraulic retention time (hr)	H_2 pressure (MPa)	Nitrate loading (g NO_3^--N/(m^2·day))
Series 1	4	0.02, 0.03, 0.04, 0.05	0.17
Series 2	4	0.05	0.17
	2	0.05	0.34
	1	0.05	0.68
	0.5	0.05	1.36

The influent nitrate was 10 mg NO_3^--N/L and sulfate concentration was 78.5 mg SO_4^{2-}/L; the influent pH value was 7.5 in the experiment period.

2 Results and discussion

2.1 Start-up

The results for the start-up and steady-state phase are shown in Fig. 3. In the first few days of operation, denitrification began almost immediately when nitrate added to the feed medium. Nitrite was non-detected within 10 days, mainly because nitrate reduction was limited. On the other hand, biofilm accumulation on the surface of the hollow fiber was exiguous and uniform. Therefore, the HRT was increased to 4 hr on day 10 to further promote biomass enrichment. As a result, nitrate was partially converted to nitrite with the denitrification rate at 14 g/(m^3·day), which caused a nitrite accumulation of 1 mg N/L. On day 45, both nitrate and nitrite were not detected in the effluent, and the denitrification rate increased to 30 g/(m^3·day), indicating that nitrate was completely denitrified at the hydrogen pressure of 0.04 MPa. After approximately 50 days, a thin layer of biofilm covering the surface of the membranes was visible.

The increase of pH value in effluent was concomitant with nitrate reduction under denitrifying condition, and steady-state pH value (9.4) was observed on day 60. Previous research showed that the optimum pH for autotrophic denitrification was in the range 7.2–8.2, with the maximum efficiency at pH 7.7. Increasing the pH above 9.0 caused a significant decrease in nitrate removal rate and a dramatic increase in nitrite accumulation (Lee and Rittmann, 2003). The metal ions in aqueous solution would also deposit and then attach to the membrane surface due to the increase of alkalinity and pH, which adversely affect the H_2 transfer and denitrification. Thereby, phosphate buffer concentration was increased (15 mmol/L) to prevent the pH from exceeding 9.0 during denitrification process.

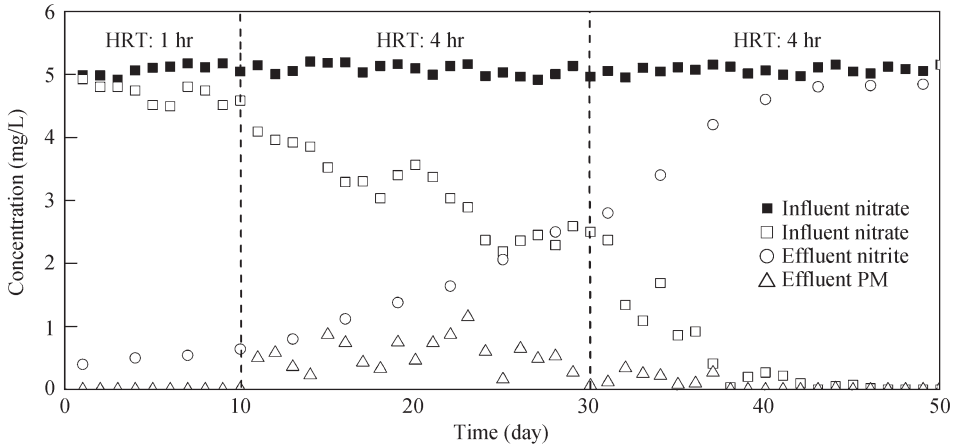

Fig. 3 Concentrations of nitrate and nitrite and pH in the effluent. Influent NO_3^--N concentration: 5 mg/L; influent sulfate concentration: 78.5 mg/L; influent pH: 7.2; H_2 pressure: 0.04 MPa.

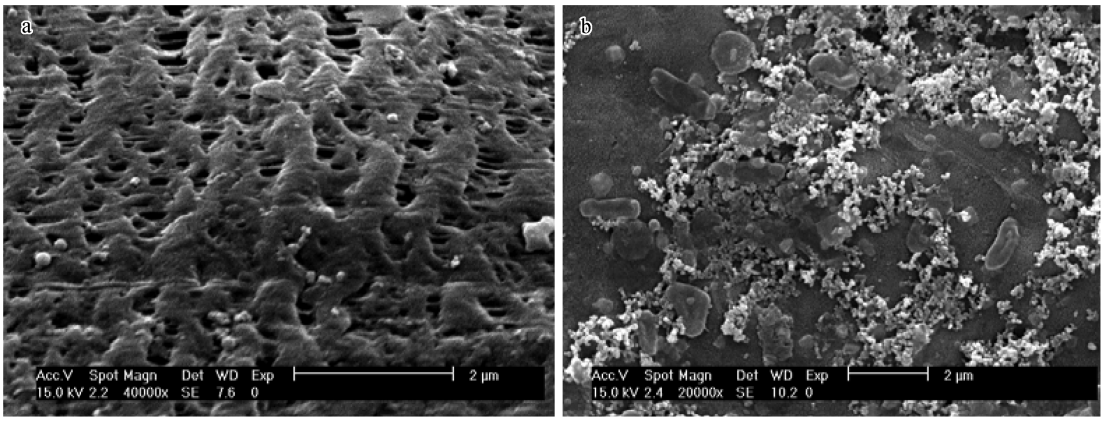

Fig. 4 Scanning electron micrograph of control hollow fiber membrane surface (a) and biofilm microorganisms from MBfR (b).

Figure 4 shows the surface morphology of the control hollow fiber (Fig. 4a) and the biofilm surface from the nitrate-reducing MBfR (Fig. 4b). The clean control fiber showed the micro-porous structure of the outside polyethylene layer of fiber, whose surface was a flat surface with a relatively uniform pores of 0.1–0.3 μm. The biofilm taken from MBfR consisted of individual rod-shape bacteria about 1 μm in length.

2.2 H_2 pressure

Figure 5 shows the experimental results for the effect of H_2 pressure on nitrate reduction. The applied H_2 pressure was varied from 0.02 to 0.05 MPa with a fixed nitrate and sulfate influent concentrations of 10 mg NO_3^--N/L and 78.5 mg SO_4^{2-}/L, respectively. An increasing hydrogen pressure at a given nitrate loading caused a continuous decrease in effluent nitrate. At 0.02 MPa H_2 pressure, the effluent nitrate was 7.2 mg N/L (28% of total nitrogen removal). As the H_2 pressure increased to 0.03 MPa, the reduction rate of nitrate increased by 22% (50%) because of the increased H_2 availability, which increased the active depth of the biofilm and electron donor for nitrate reduction. Increasing the H_2 pressure to 0.04 MPa gave a further increase to nitrate reduction (95%). This result demonstrates that H_2 availability has a prominent effect on nitrate reduction. However, increasing the H_2 pressure to 0.05 MPa caused a little enhancement in nitrate reduction, suggesting that H_2 limitation in the biofilm was minimal for the highest H_2 pressure.

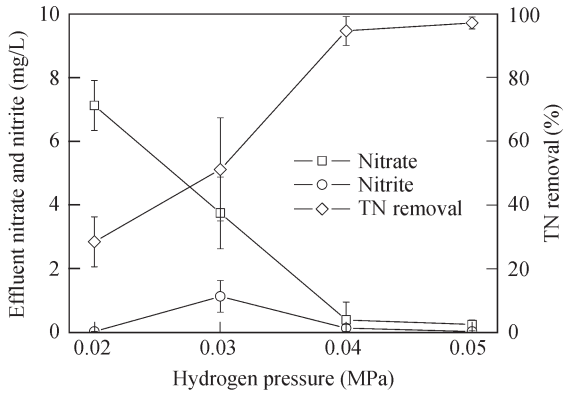

Fig. 5 Results for the first short-term series (H_2 pressure). Influent NO_3^--N concentration: 10 mg/L; influent SO_4^{2-} concentration: 78.5 mg/L; influent pH: 7.2; HRT: 4 hr.

2.3 Nitrate loading

In the second short-term series, the influent nitrate loading was varied from 0.17 to 1.36 g NO_3^--N/(m^2·day), with H_2 pressure, influent nitrate, and influent sulfate at the steady-state values of 0.05 MPa, 10 mg NO_3^-N/L, and 78.5 mg SO_4^{2-}/L, respectively. As shown in Fig. 6, under H_2 pressure 0.05 MPa, the nitrate concentration in the effluent increased as influent nitrate loading rising. Although the decrease percentage of TN removal was detected at the higher influent loading (0.34 g NO_3^--N/(m^2·day), over 97% of TN removal was achieved owing to H_2 availability. However, the TN removal decreased sharply to 80% in the period for further increasing nitrate loading to 1.36 g NO_3^--N/(m^2·day) and the maximum denitrification rate increased to 384 g N/(m^3·day). Nitrate seemed to inhibit sulfate reduction, because nitrate reduction competed more strongly for H_2. This can be verified by that sulfate removal rate sharply decreased as nitrate loading increased. In the hydrogen-based autotrophic denitrification process, sulfate and nitrate were the first electron acceptors, which were the largest consumers of electrons for reduction, together accounted for at least 94% of the total electron flux (Chung et al., 2008a). Therefore, nitrate reduction was significantly affected by nitrate loading.

2.4 Product water quality

Influent and effluent samples were taken from the reactor and analyzed for COD, TOC, DOC, alkalinity and pH at the end of each short-term test. The results are shown in Table 3. Turbidity, COD, TOC and DOC increased gradually as retention time shortened, mainly as a result of falling-off for aged biomass from the reactor (Lee and Rittmann, 2000). The biodegradable fraction of the DOC was more than 2 mg/L (> 75%), which is high enough to promote microbial growth during distribution. This suggests that the effluent from this process requires further treatment to remove the "biological instability" before being distributed.

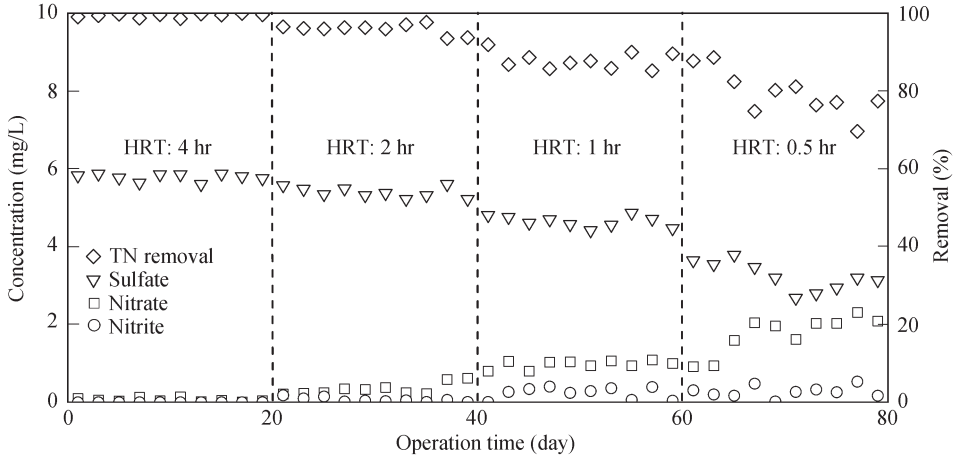

Fig. 6 Results for the second short-term series (nitrate loading). Influent NO_3^--N concentration: 10 mg/L; influent SO_4^{2-} concentration: 78.5 mg/L; influent pH: 7.5; H_2 pressure: 0.05 MPa.

Table 3 Influent and effluent water quality under different HRT

Parameter	Influent	Effluent at different HRTs			
		4 hr	2 hr	1 hr	0.5 hr
Turbidity (NTU)	0.87	1.37	1.58	2.72	4.19
COD (mg/L)	6	10	12	15	22
Total organic carbon (mg/L)	2.14	3.02	3.62	4.09	4.87
Dissolved organic carbon (mg/L)	0.24	2.24	2.34	3.63	4.57
Alkalinity (mg/L as $CaCO_3$)	480	533	528	515	498
pH	7.5	8.4	8.4	8.3	8.0

2.5 Nitrite accumulation analysis

Previous investigations had defined the reduction of nitrate to nitrogen gas (Eq. (3)) as a two-step mechanism in batch mode, which proceeded as follows: complete NO_3^--N reduction with simultaneous NO_2^--N accumulation, followed by nitrite conversion to dinitrogen gas (N_2) (Marazioti et al., 2003):

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO(g) \longrightarrow N_2O(g) \longrightarrow N_2(g) \qquad (3)$$

In this experiment, a nitrite accumulation (1.2 mg NO_2^--N/L), caused by nitrite reductase enzyme inhibition (Foglar and Briški, 2003), was detected during the MBfR start-up (Fig. 3) and can be explained by this mechanism. However, different proportion of denitrification bacteria and denitrification abilities in biological treatment system, which make nitrogen reduction rate discrepant, could also bring about nitrite accumulation (Drysdale et al., 2001). Therefore, the further research on microbiology analysis should be conducted.

3 Conclusions

The hydrogen-based membrane biofilm reactor successfully established a steady-state autohydrogenotrophic denitrifying biofilm that may remove nitrate using hydrogen as the electron donor. Short-term experiments confirmed that increasing H_2 pressure or reducing nitrate loading was favorable for denitrification, which indicates that the autohydrogenotrophic denitrification bacteria in the MBfR biofilms were highly efficient for the nitrate reduction, as long as they were not inhibited by H_2 availability.

Water quality test showed an increase in turbidity, COD, TOC and DOC, especially in a short retention time, which would result in a bacterial growth in the distribution system. This suggests that the MBfR system should be followed by a process that removes biological stability, such as rapid filtration or GAC adsorption.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 50978190) and the National High Technology Research and Development Program (863) of China (No. 2009AA062902).

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