Simultaneous removal of nitrate and pesticides from groundwater using a methane-fed membrane biofilm reactor

O. Modin, K. Fukushi and K. Yamamoto

ABSTRACT

Nitrate and pesticide contaminated ground- and surface-waters have been found around the world as a result of the use of these compounds in agricultural activities. In this study we investigated a biological treatment method to simultaneously remove nitrate and pesticides from contaminated water. Methane was supplied as the sole source of carbon to the microbial culture. A methane-fed membrane biofilm reactor (M-MBfR) was developed in which the methane was supplied through hollow-fiber membranes to a biofilm growing on the membrane surface. A methane-oxidizing culture enriched from activated sludge was used as inoculum for the experiments. Removal of nitrate and the four pesticides atrazine, aldicarb, alachlor, and malathion was examined both in suspended culture and in the M-MBfR. The maximum denitrification rate with suspended culture was 36.8 mg N $gVSS^{-1}$ d^{-1}. With the M-MBfR setup, a hydraulic retention time of approximately one hour was required to completely remove an incoming nitrate concentration of about 20 mg NO_3-N l^{-1}. The microbial culture could remove three of the pesticides (aldicarb, alachlor, and malathion). However, no atrazine removal was observed. The removal rates of both nitrate and pesticides were similar in suspended culture and in membrane-attached biofilm.

Key words | denitrification, membrane biofilm reactor, methane, pesticides

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INTRODUCTION

After the invention of the Haber-Bosch process in the early 20th century, synthetic nitrogen-fertilizers have been used to greatly increase agricultural yield. However, overuse of fertilizers and chemicals in agriculture has resulted in contamination of water bodies with nitrate (from nitrogen-fertilizers) and pesticides (chemicals to control pest, weed etc.). Nitrate concentrations exceeding the WHO guideline value of 50 mg l^{-1} (as NO_3^-) have been found around the world (Morris *et al.* 2003). Groundwater concentrations as high as 1,500 mg l^{-1} have been reported from India (Jacks & Sharma 1983). Pesticides are often detected together with nitrate as both originate from agricultural activities. A survey of 1,255 domestic drinking-water wells and 242 public supply wells in the United States detected pesticides in 38%, and anthropogenic nitrate in 28% of the wells. Many mixtures of the different chemicals were detected (Squillace *et al.* 2002). Porter *et al.* (1999) reported that mixtures of nitrate and pesticides in low concentrations may have greater health effects than each contaminant alone.

In this study, we investigate a biological treatment method for simultaneous removal of nitrate and pesticides from contaminated groundwater. Biological treatment has the advantages of (1) being relatively inexpensive and (2) having the ability to completely destroy the contaminants rather than producing a waste stream, which physical or chemical treatment methods tend to do. However, biological treatment of drinking water is challenging

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because the raw water generally does not contain enough organics to support a microbial population, which means a substrate must be added. Supplying a gaseous substrate is beneficial since gases are less likely to remain in solution and contaminate the effluent.

Methane is an inexpensive, widely available gas that can be utilized by microorganisms. Methane-oxidizing microorganisms (methanotrophs) are well-known for their ability to cometabolize a wide range of organic compounds (Hrsak 1995; Hanson & Hanson 1996; Hesselsoe *et al.* 2005). Much work has focused on halogenated aliphatics, primarily trichloroethylene (TCE). Thus far, no methanotrophic bacterium is known to denitrify (Knowles 2005); however, aerobic methane oxidation coupled to denitrification (AME-D) has been shown to occur by consortia of aerobic methanotrophs and coexisting denitrifiers able to utilize organic compounds released by the methanotrophs (Modin *et al.* 2007). Since methane is a greenhouse gas, it is important that no methane is lost to the atmosphere in the treatment process. By supplying the gas from the interior of hollow-fiber membranes to a biofilm growing on the membrane surface nearly 100% utilization efficiency can be achieved. This type of bioreactor setup is termed methane-fed membrane biofilm reactor (M-MBfR).

The aim of this study was to examine denitrification and pesticide removal by methane-oxidizing microbial consortia and to develop an M-MBfR for simultaneous removal of nitrate and pesticides from simulated contaminated water.

MATERIALS AND METHODS

Enrichment of methane-oxidizing culture

Environmental samples were collected from activated sludge, soil, groundwater, and pond sediments. The samples were diluted 1:10 in a nitrate mineral salts (NMS) medium of the following composition (modified from Costa *et al.* 2000) (mg l^{-1}): $MgSO_4 \cdot 7H_2O$ 1,000; $CaCl_2 \cdot 2H_2O$ 270; $FeSO_4 \cdot 7H_2O$ 9.1; and KNO_3 1,444. The medium also contained 2 ml/l of phosphate buffer and 1 ml/l of trace element solution. The phosphate buffer contained (g l^{-1}): KH_2PO_4 24.4; and Na_2HPO_4 10.2. The trace element solution contained (mg l^{-1}): $FeSO_4 \cdot 7H_2O$ 2,486; $MnCl_2 \cdot 4H_2O$ 500; $ZnCl_2$ 50; $NiSO_4 \cdot 6H_2O$ 101; $CoCl_2 \cdot 6H_2O$ 50; $Na_2MoO_4 \cdot 2H_2O$ 26; H_3BO_3 50; $CuSO_4 \cdot 5H_2O$ 310; and 5 ml 35% HCl. The final pH of the medium was adjusted to 7.0.

The sample suspensions (30 ml) were enriched in 120 ml vials with either air/methane or pure methane headspace. In the vials with air/methane headspace, oxygen was kept as the limiting substrate to promote growth of denitrifiers. The headspace was exchanged when all oxygen had been consumed. Methane and oxygen consumption, nitrate removal, microbial growth (measured as optical density), and removal of certain pesticides were monitored in the vials. Subculturing was carried out after microbial growth had been observed.

No growth was observed in vials with pure methane headspace. In vials with air/methane headspace, the activated sludge enrichment culture showed the highest growth rate and was thus easiest to subculture. It also compared favourably to the other cultures in terms of nitrate and pesticide removal and was selected as inoculum for subsequent experiments.

Batch experiments

Nitrate removal was investigated in a 5-litre batch reactor (liquid volume 4 litres). Methane (30 ml min^{-1}) and air (60, 100, 140, or 280 ml min^{-1}) were continuously sparged through the liquid. At each time of sampling 70 ml of the mixed culture was withdrawn for analysis and replaced with 70 ml fresh NMS medium. Changes in nitrate, nitrite, dissolved organic carbon, and biomass concentration were monitored. Ammonia was measured on one occasion but could not be detected.

Pesticide removal was measured in 120 ml vials over a period of 6 days. Atrazine and aldicarb, alachlor, or malathion was supplied to each vial. Pesticides dissolved in NMS medium without inoculum were used as controls.

Methane-fed membrane biofilm reactor (M-MBfR) experiments

The membranes used in this study were Mitsubishi-Rayon MHF200TL. Each membrane module (test samples supplied by Mitsubishi-Rayon) contained 192 fibers with an outer diameter of 280 μm. The membrane surface area was

0.014 m^2 per module. This type of membrane fibers are composed of a 1-μm thick nonporous polyurethane layer enclosed on both sides by microporous polyethylene membranes. The polyurethane layer provides some resistance to gas transfer and thus makes it possible to achieve bubblefree gas transfer even at elevated pressures (Ahmed *et al.* 2004).

The reactor setup (Figure 1) consisted of two parallel methane permeating membranes modules in series with two oxygen-permeating membrane modules. The gases were kept at elevated pressures within the membrane fibers. By separating the supply of methane and oxygen, flammable gas mixtures could be avoided. Water was recirculated through the membrane modules using a peristaltic pump. The recirculation flow rate was always much higher than the feed flow rate which meant the water inside the loop could be considered as completely mixed. The total volume of the system was 10 ml. A gas bubble was kept within the recirculation loop in order to measure the amount of methane and oxygen dissolved in the liquid and assuming equilibrium between the dissolved concentration and gas bubble partial pressure. The recirculation tubing was Masterflex precision tubing, Tygon 6409-16. When pesticide removal was examined, the recirculation tubing was changed to Tygon SE-200 inert tubing to minimize the amount of pesticide adsorbed to the tubing wall. Due to a larger tubing diameter, the total system volume increased to 25 ml when pesticide removal was examined.

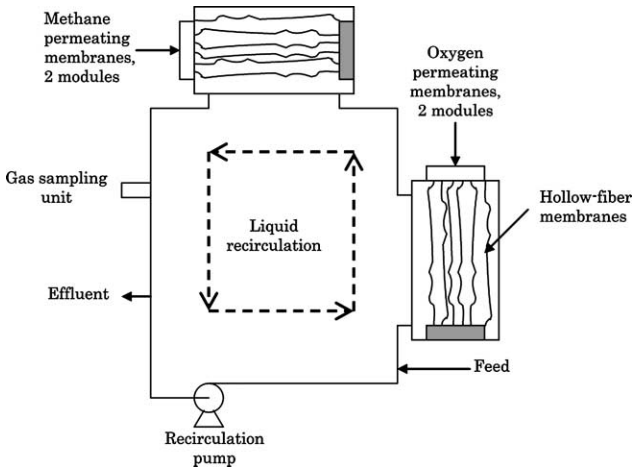

Figure 1 | Methane-fed membrane biofilm reactor setup. Water is recirculated over two methane permeating and two oxygen permeating membrane modules.

Analytical methods

Nitrate and nitrite were analyzed using ion chromatography (IC) on a Metrohm 761 Compact IC. Optical density was measured at 610 nm using a Colorimeter, ANA-18A^+, Tokyo Photoelectric Co. The optical density (OD) was correlated to volatile suspended solids (VSS) concentration, which had been measured according to Standard Methods (APHA 1998). The dissolved oxygen (DO) concentration was measured with a membrane electrode. The amount of biomass attached to the membranes was quantified according to the method by Park and Lee (2005). The biomass was suspended in water by vortexing the membranes at maximum speed three times, followed by sonication. The VSS concentration of the resuspended biomass was measured as above. Dissolved organic carbon was measured by filtering the sample through 0.45-μm PTFE filter and analysis with a Shimadzu TOC-5000A, total organic carbon analyzer. Ammonia concentrations were measured using the Cuvette-test LCK 304 (Dr. Lange, Dusseldorf, Germany). Pesticides were measured using High-Performance Liquid Chromatography (HPLC) with DAD-detection. The pesticides were separated on a NovaPak C18, 60 Å, 4 μm, 3.9 × 150 mm column, with an acetonitrile/water mobile phase. Atrazine was detected at 220 nm whereas aldicarb, alachlor and malathion were detected at 205 nm. Gaseous methane and oxygen were measured using a gas chromatograph (Shimadzu 8A) with thermal conductivity detector.

RESULTS AND DISCUSSION

Batch experiment – nitrate removal

The total inorganic nitrogen (TIN) concentration (nitrate + nitrite), dissolved organic carbon (DOC) concentration, and optical density (OD) of the batch culture over time are shown in Figure 2. The pH was controlled at 7.5 by adding diluted hydrochloric acid. From day 2, the DO concentration was consistently zero. The OD continuously increased indicating an accumulation of biomass in the batch. From day 54 and onwards, the effect of air sparging rate on nitrate removal was examined in four runs (Figure 3A–D). When all nitrate had been consumed, new nitrate was added and the air sparging rate was

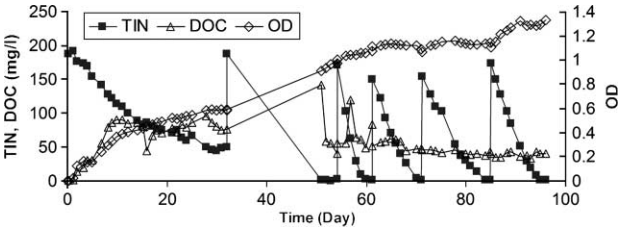

Figure 2 | Total inorganic nitrogen (TIN), dissolved organic carbon (DOC) concentrations, and optical density (OD) of the batch culture over time.

increased (see Table 1). The removal rate was compared during the linear period of TIN removal for each run. The TIN removal rates for run A–D were: 40.2; 19.8; 14.0; and 17.5 mg N gVSS^{-1} d^{-1}, respectively. Both assimilation and denitrification probably contributed to the total TIN removal since biomass production was observed in all runs. The denitrification rate was calculated assuming the produced biomass contained 12% nitrogen by weight ($C_5H_7O_2N$) (Metcalf & Eddy 1991). The denitrification rates for run A–D were: 36.8; 17.3; 12.0; and 13.6 mg N gVSS^{-1} d^{-1}, respectively.

Despite a measured DO concentration of zero in all runs, the denitrification rate decreased when the air sparging rate increased. Theoretically, a higher air sparging rate should be beneficial for AME-D, since it means the methanotrophs are able to oxidize more methane and thereby make more organic carbon available for coexisting denitrifiers (Modin *et al.* 2007). In this case, however, it appears the increase in air supply merely resulted in more of the present denitrifiers being able to utilize oxygen instead of nitrate as electron acceptor. Since the reactor was operated in batch mode, high concentrations of dissolved organic carbon accumulated in the reactor. Because of this, the denitrifiers were likely not limited by the amount of organic carbon and thus an increase in oxygen supply had a negative effect on the denitrification rate. Significant nitrite

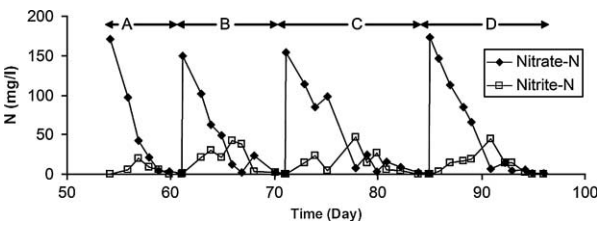

Figure 3 | Nitrate-N and nitrite-N concentrations in the batch from day 54 to day 96. The air sparging rate was stepwise increased. A: 60; B: 100; C: 140; and D: 280 ml air min^{-1}.

Table 1 | Air sparging rate during the batch experiment. The methane sparging rate was 30 ml min^{-1}

Run	Day	Air (ml min^{-1})
A	54–61	60
B	61–71	100
C	71–85	140
D	85–96	280

concentrations were observed, particularly in runs B–D, indicating that nitrate was only partially reduced at higher oxygen supply rates. However, nitrite was rapidly removed when the nitrate concentration approached zero.

Pesticide removal with suspended culture

The ability of the suspended mixed culture to remove pesticides was tested using four common pesticides that have frequently been found in groundwater: aldicarb, atrazine, alachlor, and malathion. These pesticides belong to different chemical classes. Aldicarb is a carbamate, atrazine a triazine, alachlor an acetanilide, and malathion an organophosphorus. Their persistence in the environment varies greatly depending on conditions. Atrazine is generally considered the most persistent with a half-life of 60 to >100 days (Extoxnet 2007). Schwab *et al.* (2006) estimated the half-lives to 66–106 days in a well mixed aquifer and 206–710 days in a static one. Aldicarb has a half-life typically ranging from 1 day to a few months (Extoxnet 2007), although half-lives up to 350–4,600 days have been estimated for aquifer sediments (Kazumi & Capone 1995). For alachlor, Schwab *et al.* (2006) observed half-lives ranging from 7 to 21 days in columns containing well-mixed soil and aquifer materials. Knapp *et al.* (2003) investigated alachlor degradation in water and observed half-lives ranging from 16 to 122 days. Malathion is the least persistent of the four pesticides with a water half-life of less than 1 week (Extoxnet 2007).

The mixed methanotrophic culture was able to some extent remove aldicarb, alachlor, and malathion, but not atrazine (Figure 4). The pesticide reduction is likely due to microbial activity since the non-inoculated controls did not show reduction. The reduction of malathion observed also in the controls was due to microbial contamination of these vials as evidenced by an increase in the optical density

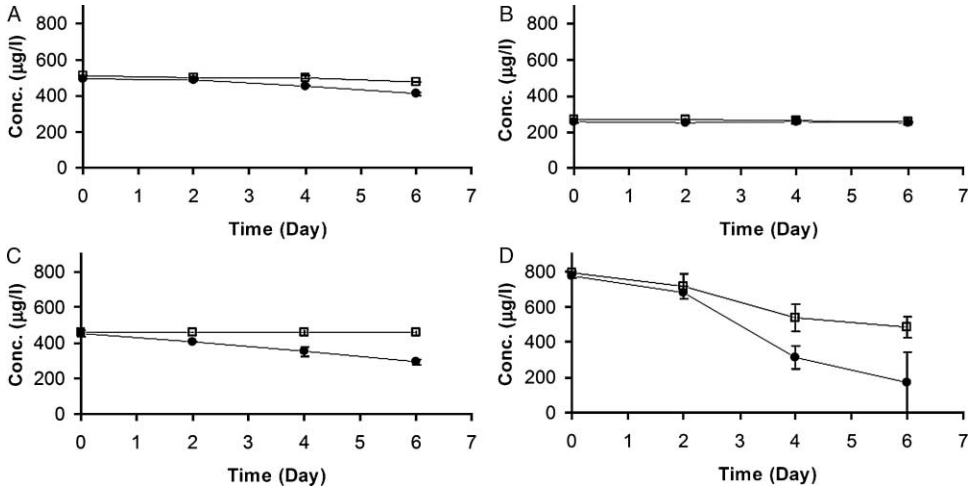

Figure 4 | Removal of the pesticides aldicarb (A), atrazine (B), alachlor (C), and malathion (D) by the mixed methanotrophic culture. Non-inoculated controls (open squares) and inoculated vials (filled circles). Values are averages of two replicas. Error bars show the variation between the two replicas. Some bacterial growth was observed in the control for D, which explains the drop in concentration.

(data not shown). The removal of alachlor commenced immediately and proceeded at a steady rate of 27 $\mu g\,l^{-1}\,d^{-1}$. The removal of aldicarb and malathion commenced after day 2. The removal rates were 20 $\mu g\,l^{-1}\,d^{-1}$ for aldicarb, and 128 $\mu g\,l^{-1}\,d^{-1}$ for malathion. The occurrence of potential metabolites was not investigated in this study. The biomass in the vials continuously increased during the 6 day experiment. The pesticide removal rate on a per biomass basis was calculated using the average biomass concentration in the vials during the period of degradation for each pesticide. The removal rates were: aldicarb 65; alachlor 88; and malathion 372 $\mu g\,gVSS^{-1}\,d^{-1}$.

Nitrate and pesticide removal with the M-MBfR

The M-MBfR was fed with NMS medium containing about 20 mg NO_3-N l^{-1}, which is slightly less than twice the WHO standard concentration of 11.3 mg NO_3-N l^{-1}. As shown in Figure 5, a hydraulic retention time (HRT) of approximately one hour was required to completely remove the incoming nitrate. At an HRT of 0.5 hrs, the effluent TIN concentration was less than 10 mgN l^{-1}; however, 2.8 mg NO_2-N l^{-1} was observed. The effluent DOC concentration was normally around 8 mg l^{-1}, though it was lower at shorter HRT. The methane and oxygen gas pressures were consistently kept at 12 kPa and 20 kPa, respectively, except on one occasion when the oxygen pressure dropped to 13 kPa, as indicated in Figure 5. When the oxygen pressure dropped, the effluent DOC concentration significantly increased to about 20–25 mg l^{-1}. This indicates that the oxygen pressure is an important parameter to control the DOC concentration in the effluent. The dissolved methane and oxygen concentrations in the system were consistently zero as measured by the headspace method described in Materials and Methods. Biofilm formation was observed in the membrane modules. The total amount of biomass growing in the four modules was approximately 180 mg which appeared to be fairly evenly distributed between the four modules. However, significant microbial growth was also observed in the recirculation tubing, which makes it difficult to accurately estimate nitrate removal of a per biomass basis in the M-MBfR.

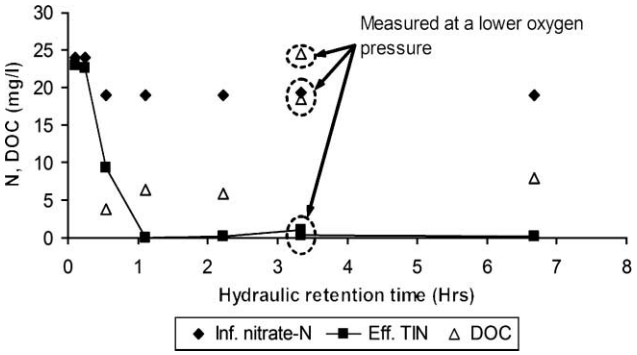

Figure 5 | Influent nitrate-N concentration, and effluent total inorganic nitrogen (TIN) and dissolved organic carbon (DOC) concentrations in the membrane biofilm reactor. The methane pressure was normally around 12 kPa, and the oxygen pressure around 20 kPa. The circled data points were measured at an oxygen pressure of 13 kPa.

Table 2 | Pesticide removal rates in the M-MBfR

Pesticide	Removal rate ($\mu g\,l^{-1}\,d^{-1}$)	($\mu g\,gVS^{-1}\,d^{-1}$)
Aldicarb	421	59
Atrazine	0	0
Alachlor	782	109
Malathion	1,790	249

The ability of the M-MBfR system to remove aldicarb, atrazine, alachlor, and malathion was examined. Just as the batch experiments with suspended culture, the M-MBfR was able to remove aldicarb, alachlor, and malathion, but not atrazine. Control experiments were carried out with a non-inoculated membrane module to determine the extent to which the pesticides adsorbed onto the membrane and tubing materials in the system. Aldicarb was the only pesticide that did not adsorb. Thus, it can be concluded that the aldicarb reduction in the M-MBfR was due to microbial activities only. For alachlor and malathion, the microbial removal is assumed to equal the difference in removal between the biofilm-containing and non-inoculated MBfR systems. For atrazine, the removal in the control was slightly higher than in the biofilm-containing system indicating that the biofilm did not degrade atrazine. During the pesticide removal experiments no growth was observed in the recirculation tubing. As shown in Table 2, the removal rates on a per biomass basis were similar to the removal rates measured with suspended culture. The nitrate removal rate during this experiment reached $24.4\,mg\,N\,gVS^{-1}\,d^{-1}$, which also is similar to the nitrate removal rates measured with suspended culture.

CONCLUSION

Nitrate and pesticide removal was tested both with suspended culture and with the M-MBfR setup. The experiments with suspended culture showed that denitrification occurred at a rate of up to $36.8\,mg\,N\,gVSS^{-1}\,d^{-1}$. Of the tested pesticides, aldicarb, alachlor, and malathion were removed by the culture whereas atrazine was not. The M-MBfR system could completely remove an influent nitrate concentration of about $20\,mg NO_3\text{-}N\,l^{-1}$ in approximately one hour. The same pesticides were removed by the biofilm as were removed in suspended culture. The nitrate and pesticide removal rates measured in the M-MBfR and with suspended culture were similar, indicating that in terms of performance the suspended and membrane-attached cultures were equivalent.

A drawback of the M-MBfR in this study was that the effluent contained about $8\,mg\,l^{-1}$ of DOC. However, this concentration could potentially be controlled by manipulating the oxygen supply. It was observed that when the oxygen supply pressure dropped, the effluent DOC significantly increased. Moreover, the membrane modules had a very high fiber density, which meant only a small volume was available for microbes to grow. This occasionally led to blocking of the modules due to excessive biofilm growth.

In summary, this study showed that removal of nitrate and certain pesticides from water is possible using a microbial culture living on methane as the sole source of carbon, and that the M-MBfR is a promising treatment configuration for this purpose.

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