

Hydrogenotrophic denitrification of drinking water using a hollow fibre membrane bioreactor

Sarina J. Ergas and Andreas F. Reuss

ABSTRACT

The objective of this research was to investigate the performance of a hollow fibre membrane bioreactor (HFMB) for hydrogenotrophic denitrification of contaminated drinking water. In the HFMB, H₂ flows through the lumen of the hydrophobic hollow fibres and diffuses to an attached H₂ oxidizing biofilm. Nitrate in the contaminated water serves as an electron acceptor. A hydrogenotrophic denitrifying culture was enriched from a wastewater seed. Batch culture experiments were conducted to compare heterotrophic (methanol as electron donor) and hydrogenotrophic denitrification rates and to investigate the conditions required for the HFMB studies. The batch cultures demonstrated mixotrophy, with denitrification rates of 30 g NO₃⁻-N m⁻³ d⁻¹ for heterotrophic and 18 g NO₃⁻-N m⁻³ d⁻¹ for hydrogenotrophic conditions. A laboratory-scale HFMB was constructed that utilized 2,400 polypropylene hollow fibres with an inner diameter of 200 µm, an outer diameter of 250 µm and a 0.05 µm pore size. After a 70-day start-up period, the NO₃⁻ loading rate was gradually increased over a three-month period. The NO₃⁻ utilization rate reached a maximum of 770 g NO₃⁻-N m⁻³ d⁻¹ at an influent NO₃⁻ concentration of 145 mg NO₃⁻-N l⁻¹ and a hydraulic residence time of 4.1 hours. Influent NO₃⁻ concentrations of up to 200 mg NO₃⁻-N l⁻¹ were almost completely denitrified. Tests with contaminated water from the Cape Cod aquifer resulted in an increase in product water turbidity and dissolved organic carbon (DOC) concentrations.

Key words | biological denitrification, bioreactor, drinking water, hydrogen, membranes, nitrate

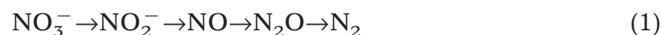
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INTRODUCTION

Worldwide, the problem of NO₃⁻ contamination of drinking water supplies is severe; a significant fraction of groundwater currently used as municipal water supplies exceeds the US Environmental Protection Agency and World Health Organization standards (10 mg NO₃⁻-N l⁻¹). Sources of NO₃⁻ contamination include the use of synthetic fertilizers, industrial and food processing operations and animal and human waste disposal. Methaemoglobinemia, or blue baby syndrome, is a toxic response to NO₃⁻ exposure. Treatment technologies for NO₃⁻ removal from drinking water include ion exchange, reverse osmosis and biological denitrification (Kapoor & Viraraghavan 1997). Problems associated with ion exchange and reverse osmosis include high capital and energy costs and the generation of large volumes of waste brine.

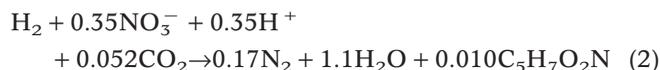
Biological denitrification is carried out by facultative bacteria that can use NO₃⁻ as a terminal electron acceptor for respiration under anoxic conditions. Reduction of NO₃⁻ to nitrogen gas proceeds in a four-step process:



Each step in the process is catalysed by a separate enzyme system. As a group, denitrifying bacteria are genetically diverse and metabolically versatile (Liessens *et al.* 1993; Smith *et al.* 1994). Biological denitrification systems have recently been developed for the treatment of drinking water (Gayle *et al.* 1989; Matéju *et al.* 1992; Kapoor & Viraraghavan 1997). Most of these systems rely on heterotrophic bacteria, which require an organic carbon source,

such as methanol, ethanol or acetate, for energy metabolism and cell synthesis. Advantages of these systems include the high specificity of denitrifying organisms for NO_3^- , low cost and high denitrification rates. Problems with these systems include carryover of added organic carbon and microbial biomass to the product water.

A number of common genera of soil bacteria are able to use H_2 as an electron donor and inorganic carbon for cell synthesis. Some of these species are able to respire on NO_3^- in the absence of molecular oxygen. Using the method of McCarty (1975) the following equation was developed as an estimate of the stoichiometry of hydrogenotrophic (H_2 oxidizing) denitrification:



Based on this equation, the cell yield is approximately 0.24 g cells/g NO_3^- -N, which is considerably lower than the 0.6 to 0.9 g cells/g NO_3^- -N typically reported for heterotrophic denitrification. A number of studies (Gros & Treutler 1986; Kurt *et al.* 1987; Dries *et al.* 1988; Smith & Duff 1988; Liessens *et al.* 1992) have shown that hydrogenotrophic bacteria can denitrify contaminated drinking water to acceptable levels. Gros *et al.* (1988) demonstrated the technical and economic feasibility of such a system at full scale. Advantages of hydrogenotrophic denitrification over heterotrophic denitrification include: (1) lower cell yield; (2) elimination of carryover of added organic electron donor to the product water; (3) the relatively low solubility of H_2 , which makes it easy to remove from the product water by air stripping; and (4) the low cost of H_2 . Disadvantages of hydrogenotrophic denitrification include lower denitrification rates and the difficulty in dissolving sufficient quantities of H_2 into the water due to its low solubility. This paper presents the results of an investigation of a novel system that overcomes these limitations using a hollow fibre membrane bioreactor (HFMB).

A conceptual diagram of the HFMB is shown in Figure 1. Hydrogen gas flows through the lumen of the hydrophobic hollow fibres and diffuses through the gas-filled pores of the membrane to the attached biofilm. Nitrate contaminated water surrounds the biofilm on the shell

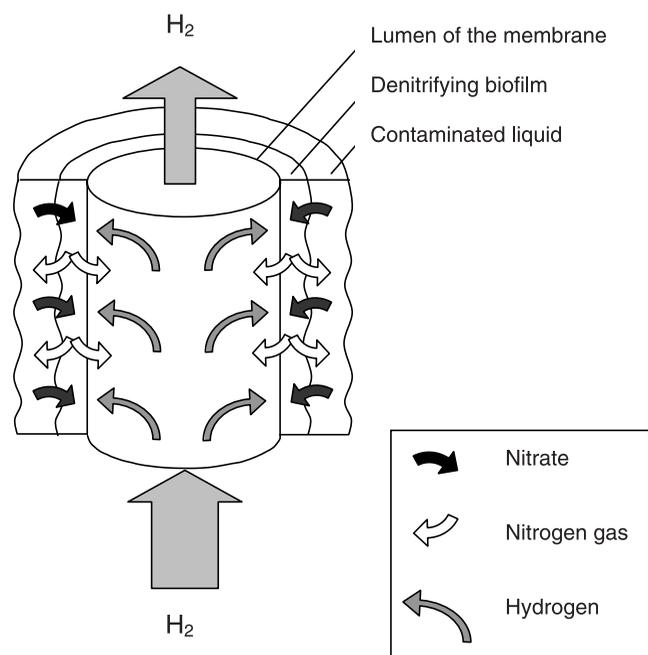


Figure 1 | Conceptual model of the phases of the hollow fibre membrane bioreactor.

side of the reactor. Within the biofilm, H_2 and NO_3^- are utilized by the hydrogenotrophic population creating a driving force for mass transfer. A number of researchers have presented mathematical models of mass transfer and biodegradation of substrates by biofilms growing on the surfaces of gas-transfer membranes (Livingston 1993; Ergas *et al.* 1999; Essila *et al.* 2000). Advantages of the HFMB over systems that employ traditional gas sparging include higher gas transfer rates, higher biomass densities and bubbleless operation, which prevents the waste of excess H_2 and the accumulation of explosive gases in a confined space (Gantzer 1995; Brindle & Stephenson 1996; Stephenson *et al.* 2000). Combining hydrogenotrophic denitrification with HFMB technology has the potential for the production of high quality drinking water in a small reactor volume.

Hollow fibre membranes have been used in a number of gas transfer applications including bubble free aeration of bioreactors (Coté *et al.* 1988, 1989; Ahmed & Semmens 1992a, b) and treatment of volatile organic compounds (VOCs) in the gas phase (Ergas & McGrath 1997; Ergas *et al.* 1999). Gantzer (1995) used a hollow fibre membrane

gas transfer system to deliver H_2 to hydrogenotrophic denitrifying populations in a two-stage system. Hydrogen transfer occurred in the first stage and denitrification was carried out in a fixed bed bioreactor. Greater than 99% removal efficiency was achieved with an influent NO_3^- concentration of $15 \text{ mg } NO_3^- \text{-N l}^{-1}$. Lee & Rittmann (2000) carried out hydrogenotrophic denitrification in a single stage HFMB. Greater than 92% removal efficiency was achieved with an influent NO_3^- concentration of $12.5 \text{ mg } NO_3^- \text{-N l}^{-1}$. Nitrate fluxes of up to $2.2 \text{ g } NO_3^- \text{-N m}^{-2} \text{ d}^{-1}$ were achieved. Both studies used sealed end hollow fibre membranes and reported high H_2 utilization efficiencies.

The objective of this research was to study the performance of a HFMB for hydrogenotrophic denitrification of drinking water with varied NO_3^- loading rates and influent concentrations. A flow-through membrane system was used for H_2 transfer. Batch culture experiments were initially conducted to compare heterotrophic and hydrogenotrophic denitrification rates and to investigate the conditions required for the HFMB studies. The effect of HFMB treatment on product water quality was investigated using water from a NO_3^- contaminated aquifer.

MATERIALS AND METHODS

Batch culture studies

Mixed liquor suspended solids (MLSS) were collected from an anoxic rotating biological contactor (RBC) at the Berkshire Mall wastewater treatment plant (WWTP) in Lanesboro, Massachusetts (methanol supplied as an external electron donor). Synthetic groundwater (80 ml) was added to each of eight 160 ml glass bottles. Bottles were sparged with a N_2/CO_2 gas mixture (70% N_2 , 30% CO_2), inoculated with MLSS and sealed with septum caps.

Two of the bottles initially received 30 μl of methanol injected directly into the headspace. Additional methanol was added when GC analysis showed that methanol concentrations had decreased below the detection limit of 0.5 ppm_v. After approximately 20 days, bottles receiving methanol were switched to H_2 as an electron donor. Four

bottles received H_2 as the sole electron donor, with daily exchange of headspace H_2 , throughout the experiments.

Nitrate concentrations were determined in all bottles on approximately a daily basis. Fresh synthetic groundwater was added to the bottles, when required, by allowing the contents of the bottles to settle, removing 40 ml of the contents with a syringe and adding 40 ml of fresh synthetic groundwater.

Bioreactor studies

A schematic of the laboratory scale HFMB system is shown in Figure 2. The bioreactor housing was a 7.5 cm diameter by 29 cm height glass cylinder with stainless steel endplates. The overall volume of the system was 1.2 l. A Masterflex C/L pump (Vernon Hills, Illinois) was used to supply CO_2 sparged synthetic groundwater to the reactor. A Parker (Jacksonville, Alabama) adjustable pressure-regulating valve was used at the liquid outlet to maintain the liquid pressure in the bioreactor slightly higher than the inlet H_2 pressure. The reactor contents were continuously recirculated using a Fluid Metering Inc. (Syosset, New York) pump.

Membrane bundles (polypropylene hollow fibre membranes potted in polysulfone fittings) used in this research were manufactured by Spectrum Microgon (Laguna Hills, California). These flow-through bundles were developed for oxygen transfer to cell cultures. The hollow fibre membranes had an inner diameter of 200 μm , an outer diameter of 250 μm and an active fibre length of 19.5 cm. Porosity (pore density) was nominally 50%, with a 0.05 μm pore size. There were 2,400 fibres in each bundle and the total outer surface area was 0.37 m^2 . High purity H_2 was supplied to the membrane bundle from a pressurized tank (Merriam Graves, Charlestown, New Hampshire). An Aalborg Instruments (Orangeburg, New York) mass flowmeter was used to monitor the H_2 flow rate, which was manually controlled using two needle valves. Excess H_2 gas flowed through the outlet to a laboratory hood.

Contaminated groundwater

Synthetic contaminated groundwater consisted of well water amended with sufficient KNO_3 to provide the

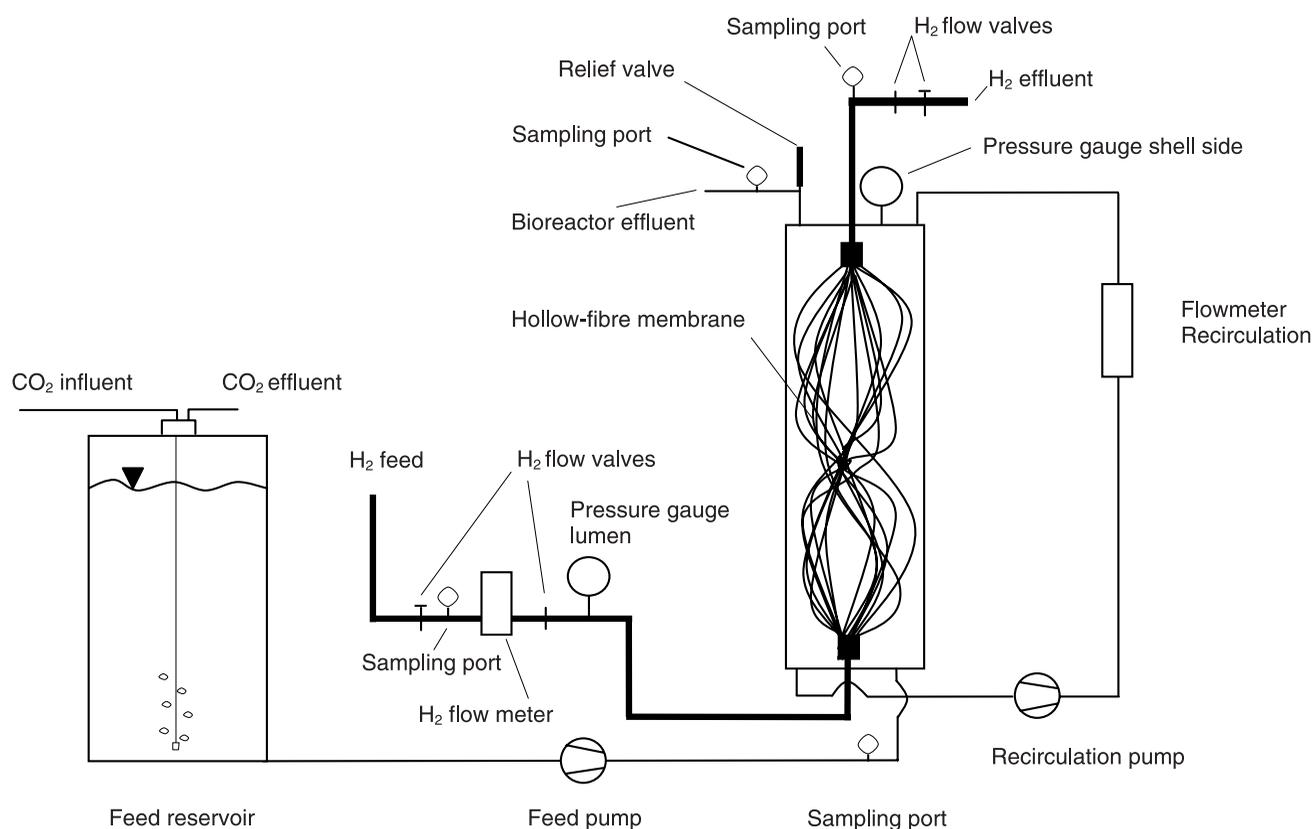


Figure 2 | Laboratory-scale bioreactor schematic.

desired NO_3^- concentration and phosphate buffer ($980 \text{ mg l}^{-1} \text{ KH}_2\text{PO}_4$ and $1,190 \text{ mg l}^{-1} \text{ K}_2\text{HPO}_4$). For most of the experiments, well water from a private residence in Leverett, Massachusetts, was used. Measured NO_3^- concentrations in the Leverett well water were less than $0.5 \text{ mg NO}_3^- \text{-N l}^{-1}$ and measured total organic carbon (TOC) was 0.28 mg l^{-1} . On days 84–96 of the experiment, water from a NO_3^- contaminated aquifer on Cape Cod, Massachusetts, was used as feed water for the HFMB. Water was pumped from a USGS monitoring well located near Ashumet Pond in Falmouth, Massachusetts. Disposal of treated wastewater into infiltration sand beds at Otis Air Force base since 1936 has resulted in a plume of contaminated groundwater characterized by elevated concentrations of dissolved solids, boron, chloride, sodium, phosphorus, ammonium, NO_3^- , detergents and VOCs. LeBlanc (1993) presented a detailed discussion of the site.

The dominant nitrogen species in the region where the samples were taken is NO_3^- . The Cape Cod water was sparged with CO_2 and amended with KNO_3 to bring the NO_3^- concentration to the desired level. From days 92–96, phosphate buffer was also added to the Cape Cod water.

Analytical methods

For the batch culture studies, headspace methanol concentrations were determined using a Varian 3500 gas chromatograph equipped with a flame ionization detector and a 30 m Restek (Bellefonte, Pennsylvania) RTX-624 megabore capillary column (isothermally at 60°C with N_2 carrier at 20 ml min^{-1}). Samples were withdrawn from the bottles through septa using Hamilton (Reno, Nevada) gas-tight syringes equipped with SampleLock™ valves.

Headspace concentrations were converted to liquid phase concentrations using Henry's law.

Hydrogen concentration was measured using a Trace Analytical (Menlo Park, California) Reduction Gas Analyser equipped with a 3 m long Supelco (Bellefonte, Pennsylvania) Carbosieve S-II column. For analysis of liquid phase samples, 1 ml samples were withdrawn from the reactor using gas-tight syringes. The liquid samples were injected into 250 ml bottles equipped with septum caps and the headspace H_2 concentration was determined. Headspace concentrations were converted to liquid phase concentrations using Henry's law.

Nitrate concentration was determined using an Orion (Beverly, Massachusetts) 720A ISE meter equipped with a 9707 ion plus NO_3^- electrode (Standard Methods 1995; 4500 NO_3^- D). Nitrite concentration was determined spectrophotometrically using Hach (Loveland, Colorado) AcuVac nitrite reagent ampoules and a Perkin-Elmer (Norwalk, Connecticut) Lambda 3A spectrophotometer (Standard Methods 1995; 4500 NO_3^- B). Turbidity was determined using a Monitek (Hayward, California) nephelometer (Standard Methods 1995; 2130 B). Alkalinity was determined using the titration method (Standard Methods 1995; 2320 B). TOC and dissolved organic carbon (DOC) concentrations were determined using a Shimadzu (Columbia, Maryland) 4000 TOC analyser (Standard Methods 1995; 5310 C).

RESULTS AND DISCUSSION

Batch culture experiments

Batch cultures initially receiving methanol as an electron donor began to denitrify with no lag period, as shown in Figure 3. Denitrification rates of $30 \text{ g } NO_3^- \text{-N m}^{-3} \text{ d}^{-1}$ and final NO_3^- concentrations of less than $0.5 \text{ mg } NO_3^- \text{-N l}^{-1}$ were achieved. After switching the cultures to H_2 as an electron donor, denitrification rates slowed to $18 \text{ g } NO_3^- \text{-N m}^{-3} \text{ d}^{-1}$. No lag period was observed, indicating that the organisms were facultative chemolithotrophs (Madigan *et al.* 1997).

In batch cultures initially receiving H_2 as an electron donor, an initial period of high rate denitrification (up to

$30 \text{ g } NO_3^- \text{-N m}^{-3} \text{ d}^{-1}$) was observed (data not shown). A slowing of the denitrification rate was observed after 12–15 days. The initial high rate of denitrification may have been due to the metabolism of organic matter transferred along with the MLSS in addition to the H_2 supplied. Simultaneous oxidation of H_2 and organic compounds (mixotrophy) has been previously observed in denitrifying organisms (Kluyver & van Niel 1956). High denitrification rates have been observed under these conditions (Revsbech *et al.* 1989). After approximately 40 days, the cultures that initially received methanol and the cultures on a constant H_2 feed were denitrifying at approximately the same rate (average of $18 \text{ g } NO_3^- \text{-N m}^{-3} \text{ d}^{-1}$).

Bioreactor acclimation

During a 70-day start-up period, the HFMB was operated as a batch system by recirculating the contents of the reactor to avoid washout and promote attachment of the biomass to the membranes. Initially, the recirculation velocity was set at 0.72 cm s^{-1} ; however, the velocity was reduced to 0.19 cm s^{-1} on day 37 to further promote biomass attachment. Mathematical modelling and experimental studies with the same reactor configuration (Ergas *et al.* 1999) showed that recirculation velocity had no significant impact on bioreactor performance once a biofilm was established on the membranes. The need for high recirculation velocities to control biofilm thickness during later stages of the study is discussed below.

Lumen side (H_2) pressure was maintained at 28 kPa and shell side (liquid) pressure was maintained at 56 kPa. A pressure differential of approximately 30 kPa between the shell and lumen side was required to maintain the proper conditions for system operation. A higher pressure differential resulted in water entry into the membranes while a lower pressure differential was found to result in bubble formation.

During the start-up period, hydrogenotrophic cultures from the batch experiments were repeatedly transferred to the HFMB. Fresh synthetic groundwater was added to the system whenever the NO_3^- concentration decreased below $10 \text{ mg } NO_3^- \text{-N l}^{-1}$. Nitrite concentrations of up to $1.0 \text{ mg } NO_3^- \text{-N l}^{-1}$ were observed at the beginning of the start-up period but decreased to below the detection limit

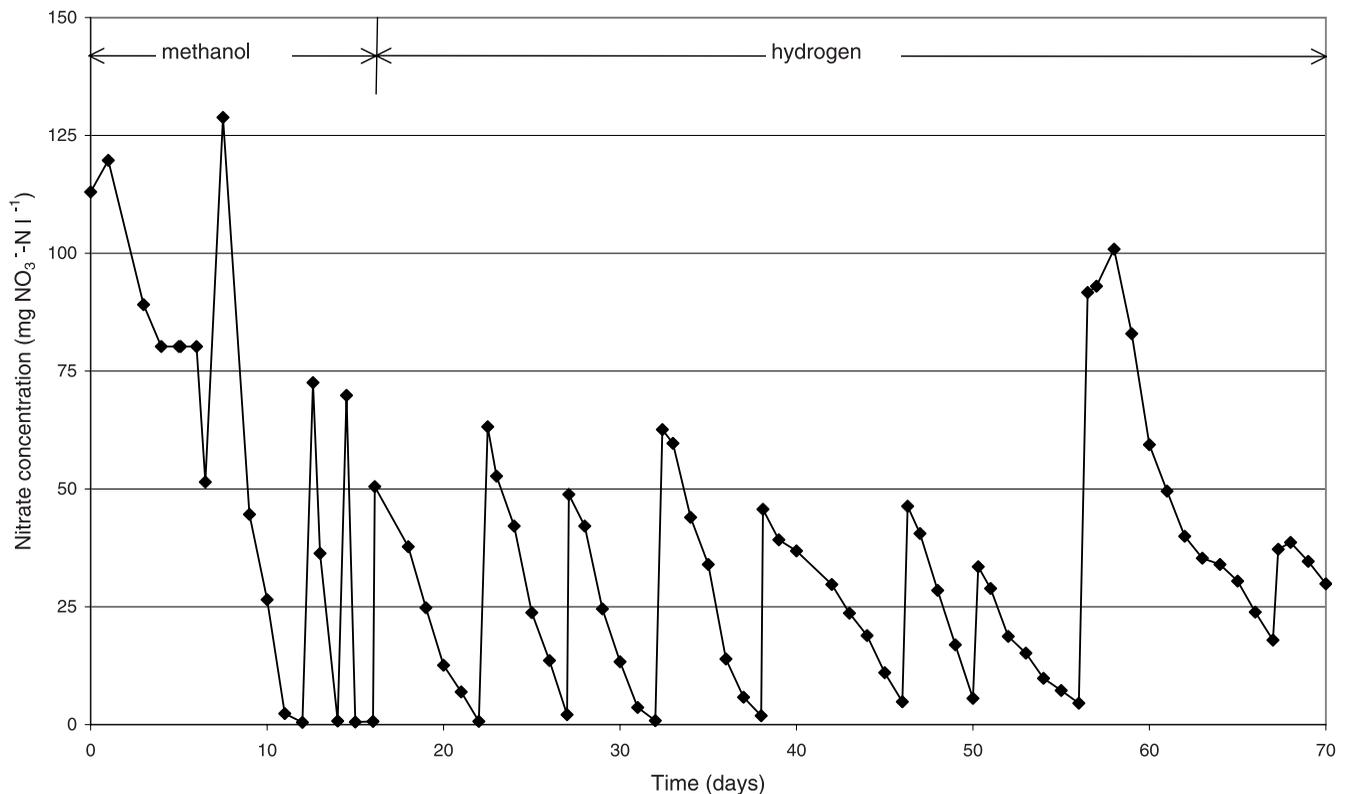


Figure 3 | Typical nitrate concentration (as N) vs. time results for the batch culture studies. The nitrate concentration was increased, when required, by replacing half the volume with fresh synthetic groundwater solution. On day 18, the electron donor was switched from methanol to hydrogen.

of $1.5 \mu\text{g NO}_3^- \text{-N l}^{-1}$ after the denitrification rate stabilized (data not shown). Liessens *et al.* (1993) found that NO_2^- temporarily accumulated in hydrogenotrophic denitrifying cultures that lacked an organic carbon source. Once the biofilm density was high enough to produce significant amounts of internal organic carbon, NO_2^- accumulation was no longer observed. After approximately 50 days, a thin layer of biofilm was visible covering the surface of the membranes. The biofilm layer was thicker near the H_2 inlet and thinner near the outlet. At the end of the start-up period, the denitrification rate averaged $20 \text{ g NO}_3^- \text{-N m}^{-3} \text{ d}^{-1}$.

Bioreactor performance

After continuous stable denitrification was established, the NO_3^- loading rate was gradually increased by increas-

ing the influent NO_3^- concentration in the feed water and decreasing the hydraulic residence time. Results are shown in Figures 4 and 5. During the first 23 days after the start-up period ended, the influent NO_3^- concentration was maintained at between 65 and $72 \text{ mg NO}_3^- \text{-N l}^{-1}$. The system hydraulic residence time was decreased each time the effluent concentration decreased below $10 \text{ mg NO}_3^- \text{-N l}^{-1}$. The effluent concentration averaged $13 \text{ mg NO}_3^- \text{-N l}^{-1}$ and the average NO_3^- utilization rate was $59 \text{ g NO}_3^- \text{-N m}^{-3} \text{ d}^{-1}$ during this period. Effluent NO_2^- concentrations were consistently below detection limits.

On day 84, KNO_3 amended Cape Cod aquifer water was introduced to the reactor. On day 92, phosphate buffer was added to the Cape Cod water due to an observed decrease in pH to 5.7 in the bioreactor due to CO_2 sparging of the unbuffered feedwater. After day 92, the NO_3^- loading rate was increased repeatedly by both

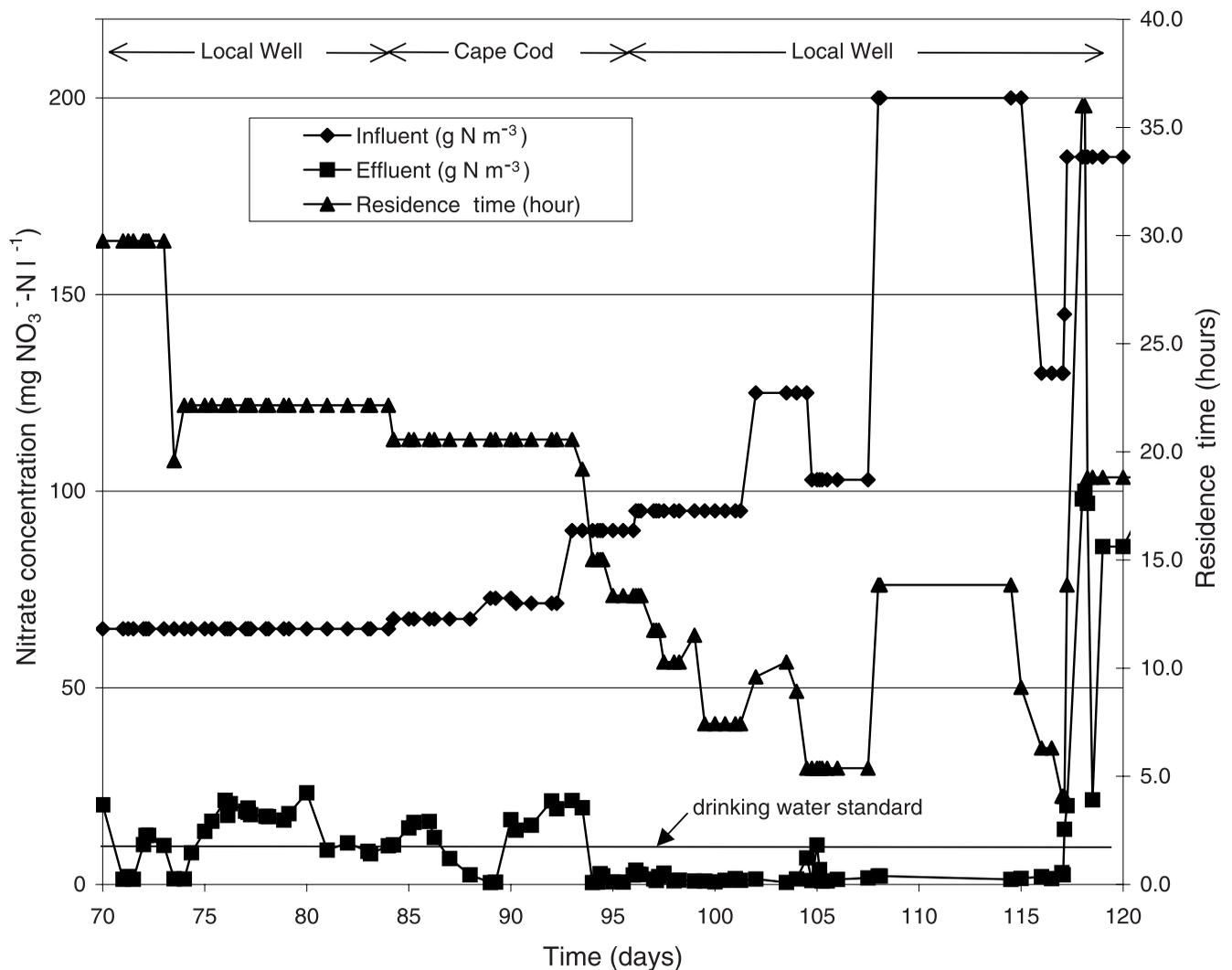


Figure 4 | Influent and effluent nitrate concentration (as N) and liquid residence time for the HFMB after the start-up period.

increasing the NO_3^- concentration and decreasing the hydraulic residence time (Figure 4). The average effluent NO_3^- concentration during this period was $1.9 \text{ mg NO}_3^- \text{ N l}^{-1}$. The utilization rate reached a maximum of $770 \text{ g NO}_3^- \text{ N m}^{-3} \text{ d}^{-1}$ at an influent NO_3^- concentration of $145 \text{ mg NO}_3^- \text{ N l}^{-1}$ and a hydraulic residence time of 4.1 hours. Influent NO_3^- concentrations of up to $200 \text{ mg NO}_3^- \text{ N l}^{-1}$ were almost completely denitrified.

An estimate of the NO_3^- flux to the membranes was determined by dividing the mass removal rate of NO_3^- by the membrane surface area using the following equation:

$$J = \frac{Q_L(C_i - C_e)}{A_m} \quad (3)$$

where Q_L is the feedwater flow rate (l d^{-1}), C_i and C_e are the influent and effluent NO_3^- concentrations ($\text{mg NO}_3^- \text{ N l}^{-1}$), respectively and A_m is the membrane surface area (m^2). The maximum NO_3^- flux to the membranes was $2.5 \text{ g NO}_3^- \text{ N m}^{-2} \text{ d}^{-1}$. This compares favourably with the values obtained by Lee & Rittmann (2000) of $2.2 \text{ g NO}_3^- \text{ N m}^{-2} \text{ d}^{-1}$ and by Gantzer (1995) of $2.0 \text{ g NO}_3^- \text{ N m}^{-2} \text{ d}^{-1}$ in their work with hydrogenotrophic denitrification

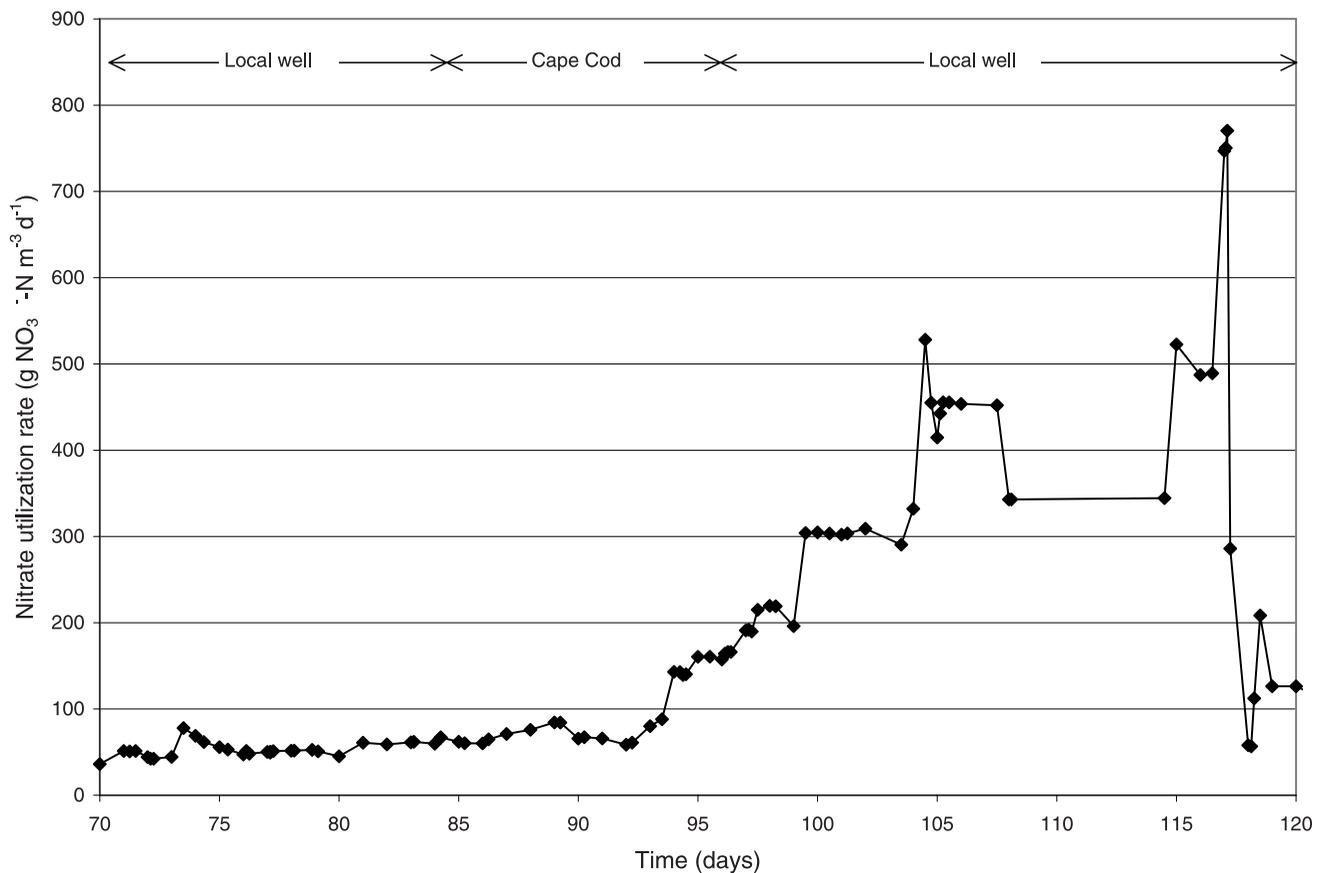


Figure 5 | Nitrate utilization rate vs. time for the HFMB after the start-up period. From day 70 to 118, the nitrate loading rate was gradually increased by increasing the nitrate concentration and decreasing the hydraulic residence time.

in membrane bioreactors. Mansell & Schroeder (1999) reported a NO_3^- flux of $4 \text{ g NO}_3^- \text{-N m}^{-2} \text{ d}^{-1}$ for a membrane bioreactor using methanol as an electron donor; however, carryover of methanol to the product water was a problem in their system. For comparison, reported NO_3^- flux values for RBC systems using organic electron donors range from 1.8 (Mohseni-Bandpi & Elliott 1998) to $7.2 \text{ g NO}_3^- \text{-N m}^{-2} \text{ d}^{-1}$ (Barnes & Bliss 1983).

Changing the feedwater source from the local well water to Cape Cod aquifer water and controlling the bioreactor pH resulted in a significant increase in the denitrification rate and the development of a dense biofilm layer on the hollow fibre membranes. The increased denitrification rate may have been due to the introduction of trace minerals or organic compounds with the Cape Cod water. The TOC of the Cape Cod water was 12 mg l^{-1} and

mixotrophic metabolism has been shown to significantly increase denitrification rates, as discussed above. Reactor performance did not improve immediately nor did it decrease for over three weeks after changing the feedwater source back to the low TOC local well water.

The Cape Cod water may also have introduced a more robust population of denitrifying bacteria into the reactor. Cape Cod water used in this study was pumped from an active zone of denitrification (LeBlanc 1993). In fact, addition of KNO_3 to the Cape Cod site water was required for this study since natural attenuation had reduced the NO_3^- concentrations to below $10 \text{ mg NO}_3^- \text{-N l}^{-1}$. In addition, Smith *et al.* (1994) isolated several strains of hydrogenotrophic denitrifying bacteria from the Cape Cod site.

Throughout the study, the mass flow rate of H_2 was adjusted, as required, so that it was approximately 60%

Table 1 | Water quality parameters measured at the end of the Cape Cod studies

Parameter	Units	Influent	Effluent
Nitrate	mg NO ₃ ⁻ -N l ⁻¹	90	0.6
Turbidity	NTU	< 1	8
Suspended solids	mg l ⁻¹	nd	4.0
Volatile suspended solids	mg l ⁻¹	nd	3.8
Total organic carbon	mg l ⁻¹	12	32
Dissolved organic carbon	mg l ⁻¹	11	31
Particulate organic carbon	mg l ⁻¹	1	1
Alkalinity	mg l ⁻¹ as CaCO ₃	400	650
pH	—	6	7

nd=not determined.

greater than the stoichiometric requirements for denitrification (calculated using Equation 2). Excess H₂ gas flowed through the membranes and was wasted via a laboratory hood. The resulting H₂ utilization efficiency was maintained at approximately 40%. Hydrophobic hollow fibres have been developed with sealed hydrophilic ends that enable 100% utilization efficiency in gas-to-liquid mass transfer applications (Ahmed & Semmens 1992a, b). Gantzer (1995) and Lee & Rittmann (2000) used sealed end hollow fibre membranes in their research on hydrogenotrophic denitrification; however, these membranes were unavailable at the start of this research project.

On day 118 of the study, the effluent NO₃⁻ concentration increased significantly as shown in Figure 4. At this point, a thick film (0.5–1 mm, estimated) had developed on the outer fibres of the membrane module, most likely consisting of attached biomass and precipitated minerals. Fibres on the outside of the module were cemented together with biofilm; however, many of the inner fibres were not completely covered with biofilm. Decreased HFMB performance has been observed in bioreactors after the development of a thick biofilm due to substrate mass transfer limitations, membrane fibre plugging, decreased

biomass activity, and/or metabolite accumulation (Brindle & Stephenson 1996; Freitas dos Santos *et al.* 1997). Several operational strategies have been used to maintain film thickness at an optimum level including the use of cross-flow membrane configurations (Ahmed & Semmens 1996) and periodic shearing of biomass from the membranes using high liquid velocities combined with scouring with gas bubbles (Pankhania *et al.* 1994; Dolasa & Ergas 2000). An attempt was made to shear some of the biomass from the membranes by increasing the recirculation velocity to 0.72 cm s⁻¹. Due to pumping limitations significant wasting of biomass from the system was not possible. During the period of decreased performance (days 118–142, data not shown), the average NO₃⁻ removal efficiency was 52% (± 9%) and the average NO₃⁻ utilization rate was 134 g NO₃⁻-N m⁻³ d⁻¹ (± 59 g NO₃⁻-N m⁻³ d⁻¹).

Water quality testing

At the end of the Cape Cod water studies, influent and effluent samples were taken from the reactor and analysed for turbidity, SS, VSS, TOC, DOC, alkalinity and pH in

addition to NO_3^- . The results are shown in Table 1. As was discussed previously, NO_3^- was almost completely removed from the influent water. The observed increase in pH and alkalinity was expected due to the denitrification reaction. Turbidity and TOC increased significantly, most likely as a result of sloughing of biomass from the reactor. Volatile components made up almost all of the suspended solids in the effluent. By assuming a molecular formula for the biomass of $\text{C}_5\text{H}_7\text{O}_2\text{N}$, the VSS should have contributed 2.0 mg l^{-1} of particulate organic carbon ($\text{POC} = \text{TOC} - \text{DOC}$) in the effluent, which was close to the observed value. A very large increase in the effluent DOC, from 11 to 31 mg l^{-1} , was observed. Lee & Rittmann (2000) also reported an increase in DOC in their work with a hydrogenotrophic denitrification HFMB. The source of the effluent DOC is most likely to be soluble microbial products, such as proteins and polysaccharides, leaking from the microbial cells. The results of the water quality testing indicate that further treatment (e.g. aerobic biological treatment, GAC adsorption) is necessary to remove biological products from the water prior to distribution.

CONCLUSIONS

A hydrogenotrophic denitrifying culture was enriched from a heterotrophic denitrifying wastewater seed. In batch culture experiments, denitrification rates of $30 \text{ g NO}_3^- \text{-N m}^{-3} \text{ d}^{-1}$ were observed for heterotrophic and $18 \text{ g NO}_3^- \text{-N m}^{-3} \text{ d}^{-1}$ for hydrogenotrophic conditions. The cultures appeared to be mixotrophic, with higher denitrification rates occurring when an organic substrate was present.

A HFMB was operated for hydrogenotrophic denitrification over a four-month period. During the 70-day start-up period, a biofilm developed on the surface of the membranes and system operating parameters were established. Nitrite concentrations of up to $1 \text{ mg NO}_2^- \text{-N l}^{-1}$ were observed at the beginning of the start-up period, possibly due to a lack of sufficient internal organic carbon, but decreased to below $1.5 \text{ } \mu\text{g NO}_2^- \text{-N l}^{-1}$ for the rest of the study. After the start-up period, denitrification rates of

up to $770 \text{ g NO}_3^- \text{-N m}^{-3} \text{ d}^{-1}$ were achieved with an influent NO_3^- concentration of $145 \text{ mg NO}_3^- \text{-N l}^{-1}$ and a hydraulic residence time of 4.1 hours. Influent NO_3^- concentrations of up to $200 \text{ mg NO}_3^- \text{-N l}^{-1}$ were almost completely denitrified. The maximum NO_3^- flux to the biofilm obtained, of $2.2 \text{ g NO}_3^- \text{-N m}^{-2} \text{ d}^{-1}$, compares favourably with values obtained by other authors.

Denitrification rates appeared to increase significantly after the introduction of feedwater from a NO_3^- contaminated aquifer on Cape Cod, possibly due to the introduction of trace minerals, trace organics, or a more robust microbial population. Water quality testing with the Cape Cod aquifer water showed an increase in pH, alkalinity, turbidity, TOC and DOC, making further treatment of the product water necessary prior to distribution.

After approximately four months of operation, denitrification rates decreased significantly due to the build up of a thick layer of biofilm on the surface of the membranes. Periodic shearing of microbial biomass from the surface of the membranes to achieve sustained high denitrification rates is currently under investigation. Hydrogen utilization efficiencies averaged 40%; however, increased H_2 utilization efficiency is possible through the use of sealed-end hollow fibre membranes.

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