Comparing heterotrophic and hydrogen-based autotrophic denitrification reactors for effluent water quality and post-treatment
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ABSTRACT
This work compares a pilot-scale H2-based membrane biofilm reactor (MBfR) and a pilot-scale packed-bed heterotrophic reactor (PBHR) for denitrification of nitrate-contaminated groundwater. The comparison includes the effluent water quality of the denitrification reactors (NO3⁻, NO2⁻, dissolved oxygen, SO4²⁻, (biodegradable) dissolved organic carbon, heterotrophic plate counts (HPC), turbidity, NH4⁺, and pH), and the impact of post-treatment on water quality. At the same nitrate carrier-surface loading, effluent water quality was generally better directly from the MBfR than from the PBHR. However, post treatment including an ozone-contact tank and a post-filter brought the finished-water quality for both systems to roughly the same level, which met all drinking water standards except for HPC.

Key words | autotrophic denitrification, heterotrophic denitrification, post treatment, water quality

INTRODUCTION
Nitrate (NO3⁻) is a pervasive drinking water contaminant that comes from a wide range of human activities: the use of agricultural fertilizers, the discharge of industrial and municipal effluents, and emissions from combustion engines (Soares 2000). The primary health concern regarding nitrate is methemoglobinemia, so-called ‘blue-baby syndrome’ (Kapoor & Viraraghavan 1997). The current maximum contaminant levels (MCL) for nitrate of the United States, Europe, and World Health Organization are 10, 11, and 11 mg N/L, respectively (Soares 2000). Closely related to nitrate is nitrite (NO2⁻), which is an even more serious cause of methemoglobinemia; its MCL is 1 mg N/L (Soares 2000).

Nitrate in drinking water sources can be biologically removed, and this has been practiced in Europe for around four decades (Richard et al. 1980; Rittmann & Huck 1989; Rogalla et al. 1990). Elsewhere, however, biological denitrification for human consumption is not common. For example, the US experience with biological denitrification for drinking water supplies has been limited to pilot-scale reactors, except for a commercial-scale BioDen™ denitrification reactor that was successfully installed and operated in the Town of Coyle, Oklahoma in 1998 (Silverstein & Mann 1998; Rittmann et al. 2011).

Bacteria carry out nitrate reduction only when they are able to oxidize a bioavailable electron donor. Hydrogen gas (H2) and two simple organic chemicals (ethanol (CH3CH2OH) and acetic acid (CH3COOH)) are three electron donors that have been used in full-scale denitrification...
plants (Gros et al. 1988; Rittmann & Huck 1989; Rogalla et al. 1990; Rittmann et al. 2011). Depending on the donor, these processes are called H2-based autotrophic denitrification (using H2 as the donor) and heterotrophic denitrification (using organic chemicals as the electron donor).

The only full-scale H2-based autotrophic denitrification plant in Europe used a packed-bed reactor (Gros et al. 1988) that delivered the H2 through sparging. Off-gassing resulted in significant H2 loss, since H2 gas has a very low solubility (1.6 mg/L at 1 atm and 20 °C), and, in addition, it could have created a combustible atmosphere. These problems can be overcome with a membrane biofilm reactor (MBfR) (Lee & Rittmann 2002), in which H2 gas is delivered by diffusion through the walls of gas-transfer fibers. A biofilm develops on the outside of the bubbleless (i.e., no pores) gas-transfer fibers and as the bacteria oxidize H2 to reduce nitrate, the concentration gradient pulls more H2 across the fiber wall. This allows self-regulation of the H2-delivery rate and eliminates the problems encountered in a H2-sparging system. The MBfR has been intensively tested at the bench-, pilot-, and field-scale in the past decade (e.g., Downing & Nerenberg 2007; Rittmann 2007; Tang et al. 2010; Xia et al. 2010).

At least five full-scale heterotrophic denitrification plants currently operate in Europe including a fluidized-bed reactor and at least four packed-bed heterotrophic reactors (PBHR) (Gayle et al. 1989; Matějů et al. 1992; Bonnelye 2006; Rittmann et al. 2011). Biomass-carriers used in these plants include plastics, ceramics, sand and clay. Four of these plants use ethanol as the electron donor and one uses ethanol and acetic acid.

The MBfR captures the advantages of using H2 as the electron donor: simultaneous reduction of almost all oxidized contaminants, minimized excess biomass production, low cost per electron-equivalent delivered for contaminant reduction, and on-site generation of H2 by electrolysis if desired. It also has two advantages associated with membrane delivery of H2: virtually self-regulating H2-delivery and nearly 100% H2-utilization efficiency (Rittmann 2007; Tang et al. 2010). The identified problems of the MBfR process are membrane fouling and leaking, both of which can be overcome by improvements to membrane fabrication and operating strategy (Rittmann 2007), which are being undertaken by its commercialization company, APTwater, Inc. (Long Beach, California). Because heterotrophic denitrification of groundwater was adapted from tertiary treatment of wastewater, it is a well-studied and widely available approach. The disadvantages of this process are carryover of soluble organic compounds and biomass to the effluent, both of which can be overcome to a degree by using online instrumentation (Soares 2000).

We tested two pilot-scale reactors treating the same nitrate-contaminated groundwater: an MBfR and a HPBR. The loading limits were documented separately by Tang et al. (2010) for the MBfR and Tang et al. (2011a) for the PBHR. Table 1 compares the surface and volumetric loading limits for the two denitrification reactors. In both cases, the loading limit was set by the nitrite MCL of 1 mg N/L. While the PBHR had a higher biomass-carrier surface loading, the MBfR had a higher volumetric loading. The loadings were similar, however, for both systems.

The loading limits depend on the operating conditions. The two most important operating conditions that affect

| Table 1 | Maximum NO3⁻ loadings⁴ to achieve effluent NO3⁻ and NO2⁻ concentrations below the MCLs⁵ |
| --- | --- | --- | --- | --- | --- |
| Biomass-carrier surface loading⁶ (g N/m²/d) | Volumetric loading⁶ (kg N/m³/d) | Influent NO3⁻ (mg N/L) | Effluent NO3⁻ (mg N/L) | Influent NO2⁻ (mg N/L) | Effluent NO2⁻ (mg N/L) |
| MBfR | 5.4 | 6.9 | 12.0 | <1.0 | <0.1 | 1.0 |
| PBHR | 6.3 | 4.0 | 12.0 | <1.0 | <0.1 | 1.0 |

⁴The maximum loadings were interpolated from the flow-rate-increase tests described in Figure 3 of Tang et al. (2010, 2011a). These values correspond to the influent nitrate biomass-carrier surface loading for which the effluent nitrite concentration was the MCL.

⁵Nitrate MCL = 10 mg-N/L, nitrite MCL = 1 mg-N/L.

⁶Nitrate biomass-carrier surface loading = QC/CA, where Q is the influent flow rate [L/d], C is the influent nitrate concentration [g-N/L], and A is the biofilm-carrier surface [m²].

⁷Nitrate volumetric loading = QC/V, where V is the reactor volume [m³].
the loading of the MBfR are the fiber type and the H₂ pressure in the fibers. Here, the H₂ pressure was kept constant at 2.7 atm, which was the maximum bubbleless hydrogen pressure for the 70-micron-thick polyester fibers used in this experiment. In the PBHR, the two most important operating conditions that affect the loading are the type of electron donor and its dose; ethanol was supplied here according to stoichiometry.

The objective of this work was to compare effluent water quality directly from the two denitrification reactors and after post-treatment with ozone and filtration with granular activated carbon (GAC) and sand. To the best of our knowledge, this is the first set of results for this post-treatment configuration for denitrification effluents.

MATERIALS AND METHODS

Configuration and operation

Figure 1 is a line diagram of the reactor system. Groundwater containing nitrate at ≈12 mg N/L was pumped from a well at the Cholla Water Treatment Plant of the City of Glendale, Arizona, and stored in a raw water storage tank (8 m³). Downstream of the raw water tank, the piping split to provide influent to the MBfR and PBHR. The MBfR (0.028 m³) was a two-stage reactor that had polyester fibers for H₂ delivery and biofilm attachment. The PBHR (0.028 m³) was a two-stage reactor filled with plastic media for biofilm attachment and supplemented with ethanol as the organic electron donor. Effluent from one denitrification reactor at a time was routed to a single post-treatment train, which included an ozone-contact tank (0.01 m³) followed by a post-filter (0.01 m³).

The groundwater characteristics and denitrification reactor configurations and operations were described in detail in Tang et al. (2010, 2011a). Here, we provide the detailed configurations of the post-treatment and operating conditions that correspond to comparison of water quality in the effluents of the two denitrification reactors, the ozone-contact tank, and the post-filter.

The MBfR was operated at a flow rate of 3.8 L/min, corresponding to a nitrate biomass-carrier surface loading of 2.0 g N/m² d. The PBHR was operated at a flow rate of 1.9 L/min, corresponding to the same biomass-carrier surface loading of 2.0 g N/m² d. This loading was selected because it was the highest value for which both denitrification reactors achieved nearly complete denitrification (Tang et al. 2010, 2011a).

Ozone was generated on site and dosed at ~2 mg/L for oxygenation and disinfection. The ozone-contact tank was a 10-cm diameter, 120-cm height column and operated at 1.9 L/min, corresponding to an empty bed contact time (EBCT) of 5 min. The ozone-contact tank effluent was routed to a 7.5-cm diameter post-filter, which included 180 cm of exhausted granular activated carbon (Filtrasorb F820; effective size of approximately 1.1 mm) over 30.5 cm of coarse sand (effective size of approximately 0.7 mm). The exhausted GAC was from a filter in the Cholla Water Treatment Plant (Glendale, Arizona). The filter had been operated for about 12 months, and the GAC was exhausted when the total organic carbon in the filter effluent reached 2.8 mg C/L; thus the role of adsorption (but not biodegradation) was negligible. The post-filter was operated at a flow rate of 0.55 L/min, corresponding to an EBCT of 17 min and a hydraulic loading of 7.5 m³/m²-h. Water from the ozone-contact that did not go through the post filter was wasted. The objective of post-filtration was to remove biodegradable organic matter and to decrease the turbidity and heterotrophic plate counts (HPC) prior to final disinfection, although final disinfection was not studied.

Sampling and analysis for water quality

The methods followed the standard procedures listed in Tang et al. (2010, 2011a). For each reactor, the influent, stage-1 effluent, stage-2 effluent, ozone-contact tank effluent,
and post-filter effluent were assayed daily for pH, temperature, total dissolved solids (TDS), hardness, alkalinity, and turbidity. The samples were also assayed three times per week for NO$_3^-$, NO$_2^-$, NH$_4^+$ and PO$_4^{3-}$, and once per week for dissolved oxygen (DO), SO$_4^{2-}$, HPC, dissolved organic carbon (DOC) and biodegradable organic carbon (BDOC). To understand the composition of DOC and BDOC, the samples were assayed 13 times over the course of the study using HPLC (high-performance liquid chromatography) for ethanol and short-chain organic acids, including formate, acetate, butyrate, iso-butyrate, valerate, iso-valerate, caproate and lactate.

RESULTS AND DISCUSSION

NO$_3^-$ and NO$_2^-$

Figure 2 contains the NO$_3^-$ and NO$_2^-$ concentrations in the effluents from the denitrification reactors, ozone tank and post-filter. The general trend for the NO$_3^-$ concentration was that it was lowest in the denitrification reactors, and then it increased in the ozone contact tank and further in the post-filter. The increase in NO$_3^-$ during post-treatment can be attributed to the release of NH$_4^+$ from biomass oxidation in the ozone contact tank, its oxidation to NO$_3^-$ during ozonation, and additional biological oxidation in the post-filter. Compared to the autotrophic train, the heterotrophic train had higher NO$_3^-$ concentrations in the ozone-contact tank and post-filter, a result of the higher biomass yield of heterotrophic denitrification (Rittmann & McCarty 2001), which led to higher turbidity in the PBHR effluent (shown below). For NO$_2^-$ concentrations, all values were below 0.2 mg N/L (the method detection limit is 0.001 mg NO$_2^-$-N/L).

DO and sulfate

DO was reduced from 4.5 ± 0.8 to below the method detection limit (0.1 mg/L) in both denitrification reactors (18 samples), and it then increased to ~6 mg/L in the ozone-tank effluent. Since the dose of ozone was <2 mg/L, most of the DO increase was attributed to oxygen intrusion into the storage tank located between the denitrification reactors and the ozone-contact tank. The sulfate concentration was 108 ± 2 mg/L in the influent and 107 ± 2 mg/L (41 samples) in the effluent of both denitrification systems, indicating negligible sulfate reduction, a result of successfully limiting the electron-donors (H$_2$ and ethanol) to just enough for complete DO and nitrate reduction (Tang et al. 2010, 2011a).

DOC

Figure 3(a) shows that the DOC concentration in the influent was approximately 0.2 mg-C/L, increased to approximately 0.4 mg-C/L in the MBfR and to around 1.0 mg-C/L in the PBHR, and after the post-filter decreased to 0.3–0.4 mg-C for both reactors. The DOC increase in the MBfR was from soluble microbial products (SMP) generated by the autotrophic denitrifiers (Rittmann & McCarty 2001). When the MBfR effluent was assayed for short-chain
organic acids, only iso-valerate was detected and only in one sample and at a low concentration (<0.2 mg-C/L). The DOC was substantially higher in the PBHR compared to the MBfR. However, measurements of short-chain organic acids and ethanol indicated that the DOC in the PBHR effluent did not include either organic acids or ethanol. Thus, the DOC in the PBHR effluent was probably SMP from the heterotrophic denitrifiers. This trend is consistent with the understanding that heterotrophs produce more SMP than do autotrophs (de Silva & Rittmann 2000; Merkey et al. 2009).

**BDOC**

The influent contained no BDOC, as seen in Figure 3(b). BDOC increased in the MBfR to around 0.2 mg C/L due to SMP production, and its concentration did not change downstream. The BDOC increased to 0.7 mg C/L in the PBHR effluent and decreased to about 0.2 mg C/L through post-treatment. Though BDOC, like DOC, is not regulated, it is associated with biomass growth in distribution systems (Rittmann & Snoeyink 1984; Rittmann & Huck 1989), and the acceptable concentration of BDOC or DOC depends on the concentrations of chlorine residual in the distribution systems (Woolschlager et al. 2002). While a BDOC concentration of 0.16 mg/L may promote excessive growth of heterotrophs with no chlorine residual, 0.32 mg/L may be tolerable if a chlorine residual of 2 mg/L is maintained throughout the system (Woolschlager et al. 2002). 2 mg Cl2/L is half of the Maximum Residual Disinfectant Level in the distribution system regulated by US EPA and four times the minimum chlorine residual that utilities must maintain at all points along the distribution network (US EPA 2009). Thus the BDOC concentrations in the post-treatment effluents of the post-filter reported here should be acceptable when a chlorine residual of 2 mg/L is maintained. An increase in BDOC and DOC likely increases the formation potential for disinfection products, which are regulated (Escobar & Randall 2001).

**HPC**

Demonstrated in Figure 3(c), the HPC levels in the influent water were approximately 5.0 × 10⁴ CFU/mL, and they increased to around 2.0 × 10⁵ CFU/mL in the MBfR and 9.0 × 10⁵ CFU/mL in the PBHR. Although no external organic substance was added to the MBfR, the HPC increase in the MBfR effluent was possible because heterotrophic bacteria grew by oxidizing SMP released by the autotrophic bacteria. Heterotrophic bacteria can oxidize BDOC while reducing nitrate in the MBfR; thus, their presence is
The HPC increase in the PBHR was higher than that in the MBfR, an expected result, since all of the denitrifiers in the PBHR were heterotrophic. The HPC concentrations decreased in the ozone contact tank and post-filter. The HPC concentrations in the effluents of the post-filter were approximately $1.0 \times 10^4$ CFU/mL for the MBfR system and $6.0 \times 10^4$ CFU/mL for the PBHR system. Thus, HPC declined across the autotrophic train and the values were only slightly larger for the heterotrophic train. Since the US EPA requires HPC in drinking water lower than 500 CFU/mL (US EPA 2009), further disinfection would be required for both trains.

**Turbidity**

Turbidity (Figure 3(d)) was about 0.2 NTU in the influent and effluent of the MBfR, and it was about 0.7 NTU in the effluent from the PBHR. Turbidity increased more in the PBHR mainly due to higher biomass production for heterotrophic bacteria compared to autotrophic bacteria (Rittmann & McCarty 2001). Through ozonation and filtration, the turbidity was reduced to <0.1 NTU in the autotrophic system effluent and <0.2 NTU in the heterotrophic system effluent, meeting the US EPA’s requirement for filtered water turbidity: ≤1 NTU at all times for systems that use conventional or direct filtration, and <0.3 NTU in at least 95% of the samples in any month (US EPA 2009).

**Ammonium**

Ammonium, generated from biomass decay, was monitored throughout the study and was detected only in the effluents from the denitrification reactors and only when electron donors were suddenly cut off due to an empty H₂ tank or when the ethanol-supply tubing became clogged. The maximum concentrations detected were 0.3 mg N/L in the MBfR and 1.0 mg N/L in the PBHR, but ammonium was completely removed in the post-filter by nitrification. The ammonium spikes appeared within two days after the electron donors were cut off, and they disappeared within two days after the electron-donor supply was re-instated. (Samples were taken every two days for ammonium analysis.) The ammonium spikes were coincident with the nitrate and nitrite spikes.

**pH**

The effect of denitrification on pH in the two reactors and the pH-control measures were summarized in Tang et al. (201b). In brief, pH increased more in the MBfR than in the PBHR. Acid (e.g., HCl) addition is the preferred pH-control method for the PBHR, and CO₂ addition is the preferred method for the MBfR.

**CONCLUSIONS**

1. The effluent concentrations of DOC, BDOC, HPC and turbidity were higher in the PBHR effluent than in the MBfR effluent. However, post-treatment that included an ozone-contact tank and a post-filter brought them to the same level; the finished water met drinking water standards except for HPC, which would require further disinfection.

2. Ammonium was only detected in the denitrification reactor effluents during a sudden cut-off of electron donors, resulting in biomass decay, but ammonium was completely removed by the post-filter.

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