Short Communication

Removal of highly elevated nitrate from drinking water by pH-heterogenized heterotrophic denitrification facilitated with ferrous sulfide-based autotrophic denitrification

Bin Huang *, Guangyu Chi, Xin Chen, Yi Shi

Key Laboratory of Pollution Ecology and Environmental Engineering, Institute of Applied Ecology, Chinese Academy of Science, Shenyang 110016, People’s Republic of China
State Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, Chinese Academy of Science, Shenyang 110164, People’s Republic of China

A R T I C L E   I N F O
Article history:
Received 6 June 2011
Received in revised form 7 August 2011
Accepted 10 August 2011
Available online 3 September 2011

Keywords:
Acidic heterotrophic denitrification
Autotrophic denitrification
Ferrous sulfide
Carbon limitation
pH adjustment

A B S T R A C T
The performance of acetic acid-supported pH-heterogenized heterotrophic denitrification (HD) facilitated with ferrous sulfide-based autotrophic denitrification (AD) was investigated in upflow activated carbon-packed column reactors for reliable removal of highly elevated nitrate (42 mg NO$_3$-N l$^{-1}$) in drinking water. The use of acetic acid as substrate provided sufficient internal carbon dioxide to completely eliminate the need of external pH adjustment for HD, but simultaneously created vertically heterogenized pH varying from 4.8 to 7.8 in the HD reactor. After 5-week acclimation, the HD reactor developed a moderate nitrate removal capacity with about one third of nitrate removal occurring in the acidic zone (pH 4.8–6.2). To increase the treatment reliability, acetic acid-supported HD was operated under 10% carbon limitation to remove >85% of nitrate, and ferrous sulfide-based AD was supplementally operated to remove residual nitrate and formed nitrite without excess of soluble organic carbon, nitrite or sulfate in the final effluent.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Excessive nitrogen fertilization in agriculture and inappropriate disposal of sanitary and industrial wastes create a wide spread of nitrate pollution in ground and surface waters (Soares, 2000). The uptake of excessive nitrate from nitrate-polluted drinking water promotes nitrite-induced methemoglobinemia and nitrosamines-induced malformation (Bouchard et al., 1992). Nitrate contamination in groundwater in arid and semi-arid regions is generally serious (Zhang et al., 1996), and especially needs proper remediation since people there largely rely on groundwater as drinking water source.

Among nitrate removal approaches, reverse osmosis and ion exchange generate secondary brine wastes, and chemical reduction (Garron and Epron, 2005) needs to control nitrite and ammonium formation. Nitrate may also be biologically removed, either autotrophically (Kimura et al., 2002; Xia et al., 2009) or heterotrophically (McAdam and Judd, 2007). Efficient hydrogen delivery systems are needed for hydrogen-based autotrophic denitrification (AD) on large scales (Karanasios et al., 2010). Sulfur-based AD is generally suitable for removal of nitrate of relatively low levels (e.g., <15 mg N l$^{-1}$) (Liu et al., 2009) to avoid an excessive increase of sulfate or water salinity while organic carbon-supported heterotrophic denitrification (HD) is widely applied in practice for biological treatment of nitrate-polluted drinking water (Hall, 1997). Heterotrophic denitrification is ideally operated under pH-circumneutral condition for high efficiency and minimized nitrite formation. The need of external pH adjustment may be excluded when a suitable HD substrate is applied to provide sufficient internal carbon dioxide (CO$_2$) to neutralize alkalinity generated during HD. Alcohols (such as methanol) and volatile fatty acids (such as acetic acid) are two major types of HD substrates (Elefsiniotis and Li, 2006; Hall, 1997). The overall stoichiometry of methanol- or acetic acid-supported HD may be simplified as:

$$1.083 \text{CH}_3\text{OH} + \text{NO}_3^- \rightarrow 0.066\text{C}_5\text{H}_7\text{O}_2\text{N} + 0.467\text{N}_2 + \text{OH}^- + 0.753\text{CO}_2$$  

(1)

$$0.813\text{CH}_3\text{COOH} + \text{NO}_3^- \rightarrow 0.066\text{C}_5\text{H}_7\text{O}_2\text{N} + 0.467\text{N}_2 + \text{OH}^- + 1.296\text{CO}_2$$  

(2)

Unlike methanol-supported HD, acetic acid-supported HD generates sufficient internal CO$_2$ to control pH within its initial range (6.5–8.5) when the percentage of the loss of internally generated CO$_2$ is <25%. However, when a common fixed bed system is used for HD of highly elevated nitrate (e.g., >40 mg NO$_3$-N l$^{-1}$) as found in groundwater of northern China (Zhang et al., 1996), the need of acetic acid as carbon source (>149 mg COD l$^{-1}$) will lower pH from <8.3 to <5 when the pretreated water contains <1.5 mM alkalinity.

* Corresponding author. Tel.: +86 24 88087717; fax: +86 24 83970540.
E-mail address: huangbin@iae.ac.cn (B. Huang).
In this situation, only if HD can proceed at acceptable rates at pH <5 to increase pH above 6 but does increase operational cost, then, acetic acid-supported HD may be applied to completely eliminate external pH adjustment during treatment of nitrate-heavily polluted drinking water. However, studies on application of HD at initial pH <5 in a fixed bed system for water treatment have not been reported.

Reliable treatment of nitrate-polluted drinking water by HD also needs proper dosing of organic carbon. Slight overdosing of organic carbon has been suggested to avoid incomplete nitrate removal (Hall, 1997), but easily causes organic carbon breakthrough in the effluent (McAdam and Judd, 2007). To solve this problem, carbon-limited HD may be applied to remove the majority of nitrate, and residual nitrate and/or formed nitrite is supplementally removed by chlorine oxidation (McAdam and Judd, 2007) or elemental sulfur (S0)-based AD (Liu et al., 2009). Solid sulfide-based AD may also be applied to facilitate carbon-limited HD. Pyrite (FeS2)-based AD occurs at low rates in groundwater environment (Juncher Jørgensen et al., 2009). Ferrous sulfide (FeS) is expected to be more efficient than FeS2 (Davison, 1991) to support AD but still will not release much free sulfide to cause incomplete denitrification or ammonia formation (Brunet and Garcia-Gil, 1996). Meanwhile, FeS-based AD generates less acidity and sulfate compared to S0-based AD, according to the following equations:

\[
0.556\text{FeS} + \text{NO}_3^- \rightarrow 0.278\text{Fe}_2\text{O}_3 + 0.556\text{SO}_4^{2-} + 0.111\text{H}^+
\]

(3)

\[
0.588\text{FeS} + \text{NO}_3^- \rightarrow 0.196\text{Fe}_2\text{O}_3 + 0.588\text{SO}_4^{2-} + 0.176\text{H}^+
\]

(4)

\[
0.8335 + \text{NO}_3^- \rightarrow 0.8335\text{SO}_4^{2-} + 0.667\text{H}^+
\]

(5)

In this study, acetic acid-supported pH-heterogenized HD and FeS-based AD were cooperatively applied in fixed bed reactors for reliable treatment of nitrate-heavily polluted drinking water. The main purposes of this study are: (1) to investigate the feasibility of acetic acid-supported pH-heterogenized HD of highly elevated nitrate without the need of external pH adjustment; (2) to investigate the performance of FeS-based AD for removal of nitrate of low levels; (3) to investigate if carbon-limited cooperative operation of acetic acid-supported pH-heterogenized HD and FeS-based AD can reliably treat nitrate-heavily polluted drinking water without excess of nitrite, soluble organic carbon and/or sulfate in the effluent.

2. Methods

2.1. Experimental setup

Experiments were performed in an HD–AD treatment system consisted of an HD reactor and an AD reactor. Each reactor was made of cylindrical plexiglass with an inner diameter of 100 mm and a height of 100 cm. The main part of the HD reactor was packed with granular activated carbon (GAC) (2.0–2.8 mm), and at the 0–5, 18–22, 38–42, 58–62, 78–82 and 90–95 cm depths packed with coarse quarts (4.0–4.8 mm) for improving water flow during operation. Sampling ports were located at the 20, 40, 60 and 80 cm heights of the HD reactor. The AD reactor was packed with GAC (2.0–2.8 mm) and amorphous FeS (1.2–2.8 mm) (Guoyao Group, Shanghai, China) at a volume ratio of 3:1 at the 5–75 cm depths, with GAC (2.0–2.8 mm) at the 75–90 cm depths, and with coarse quarts (4.0–4.8 mm) at the 0–5 and 90–95 cm depths. The effective liquid volume of each reactor was 2.5 l.

Nitrate-polluted drinking water was synthetically prepared and used as the feed for the HD and AD reactors during acclimation and operation (see Sections 2.2 and 2.3). The HD feeding tank was purged with nitrogen gas and maintained anoxic with a floating cover to limit dissolved oxygen below 1.0 mg l\(^{-1}\). The AD feeding tank was maintained anoxic by the presence of nitrogen gas in the headspace. The feed was delivered through a peristaltic pump (BT01-DG2, Xieda Inc., Tianjin, China). The experiments were conducted at 22–25 °C.

2.2. Inoculation and acclimation

The inoculation and acclimation of the HD and AD reactors were simultaneously conducted. The effluent of an HD system in the laboratory was pumped into the upflow HD reactor for inoculation. Then, the HD reactor was fed with a nitrate-containing solution. The solution was prepared from tap water with initial pH at 7.5–7.6. The pH of the solution became 4.8 after additions of 70 mg NO\(_3^--\)N\(^{-1}\) and 156 mg COD\(^{-1}\) in form of acetic acid. The HD effluent was used as feed for the AD reactor. The upflow HD–AD treatment system was operated at hydraulic retention time (HRT) 6 h for 1 week, 4 h for 2 weeks and 3 h for 2 weeks for respective acclimation of heterotrophic and autotrophic denitrifiers in the HD and AD reactors. At the end of 5-week acclimation, profiles of pH, nitrate and acetate in the HD reactor, and nitrate change in the water after treatment by the AD reactor were measured for preliminary evaluation of HD in the HD reactor and AD in the AD reactor.

2.3. Operation

At first, the HD and AD reactors were operated in a separated mode for determining suitable nitrate loading rates for the HD and AD reactors. The feed for the HD reactor contained 4 mg NO\(_3^--\)N\(^{-1}\) and 156 mg COD\(^{-1}\) in form of acetic acid. The feed pH was 4.8 after acetic acid addition. The HD reactor was operated at HRT 3.0, 2.5, 2.0 or 1.5 h to determine the suitable HRT for removal of >95% of nitrate. The feed for the AD reactor contained 14 mg NO\(_3^--\)N\(^{-1}\). The feed pH was adjusted to 7.8 with 1 M NaHCO\(_3\) to simulate the AD feed pH observed during acclimation. The AD reactor was operated at HRT 3.0, 2.5, 2.0 or 1.5 h to determine the suitable HRT for removal of 4 mg NO\(_3^--\)N\(^{-1}\).

Then, based on the suitable nitrate loading rates determined during separate operation, the HD and AD reactors was cooperatively operated under organic carbon limitation to increase the reliability of biological removal of highly elevated nitrate. The HD feed contained 42 mg NO\(_3^--\)N\(^{-1}\) and 141 mg COD\(^{-1}\) (10% shortage) in form of acetic acid. The feed pH was 4.9 after acetic acid addition. The majority of nitrate in the feed was removed by the HD reactor. The HD effluent containing residual nitrate and formed nitrite was then treated by the AD reactor. The cooperative operation of the HD and AD reactors was conducted at HRT 2.5 h (suitable for both HD and AD reactors) for 4 weeks.

The performance of the HD–AD treatment system on nitrate removal was evaluated by chemical analysis of the effluents of the HD and AD reactors. Besides the measurements of pH, nitrate and nitrite, relevant changes of acetate and sulfate were also measured to respectively reveal the utilization of acetate in the AD reactor and of FeS in the AD reactor. Soluble organic carbon (SOC) and soluble ferrous iron in the effluents were also measured to reveal the treatment quality. Under each specific operational condition, the system was run for at least 4 days in order that the system reached a quasi-steady state. Then, measurements were conducted under the quasi-state condition, and values were reported as averages of three repeated measurements.

2.4. Analytical methods

Water samples were filtered (<0.45 mm), stored at 4 °C and analyzed within 4 days after sampling. One set of the AD effluent samples were acidified to pH <3 before filtration for Fe\(^{3+}\) analysis. The water samples were analyzed for determination of
NO₃⁻, NO₂⁻, and SO₄²⁻ by an ion chromatograph (Dionex-ICS-900, Dionex Inc., Sunnyvale, CA, USA), and for determination of SOC by a TOC analyzer (Multi N/C 3100, Analytikjena, Germany). The detection limits of NO₃⁻, NO₂⁻, SO₄²⁻ and SOC were determined as 0.1, 0.1, 0.2 and 0.2 mg l⁻¹, respectively. The AD effluent samples were analyzed for colorimetical determination of Fe²⁺ using Ferrozine with a detection limit of 0.05 mg l⁻¹. The water pH was measured using a 9165BN pH electrode connected to an Orion-868 pH Analyzer (Thermo Orion Research Inc., Beverly, MA, USA).

3. Results and discussion

3.1. Acclimation of the HD–AD treatment system

The HD–AD treatment system was fed with 70 mg NO₃-N l⁻¹ and 156 mg COD l⁻¹ (40% shortage) in form of acetic acid for simultaneous acclimation of heterotrophic and autotrophic denitrifiers. At the end of 5-week acclimation, NO₃-N and NO₂-N in the effluents from HD and AD reactors were 28.3, 4.1 and 12.9, 2.5 mg l⁻¹ respectively, equivalent to a capacity to effectively remove more than 37 and 14 mg NO₃-N l⁻¹ at HRT 3 h, respectively. The use of acetic acid as the sole carbon source resulted in vertically heterogenized pH varying from 4.8 to 7.8 and heterogenized nitrate removal in the HD reactor (Fig. 1). About 35% of nitrate removal occurred in the acidic zone (0–40 cm) with pH varying from 4.8 to 6.2, and 46% in the pH-circumneutral zone (40–60 cm). The low removal of nitrate at the 60–80 cm depths should be due to the shortage of acetate as organic carbon (Fig. 1).

The HD effluent pH of 7.8 (Fig. 1) indicated that the internally generated CO₂ from acetic acid-supported HD was sufficient to maintain the HD effluent pH below 8.0 without the need of external pH adjustment. The pH of the AD effluent was little changed due to the low generation of acidity from FeS-based AD (<0.2 mM) and the elevated bicarbonate alkalinity (>3.5 mM) after treatment by the HD reactor.

3.2. Nitrate removal capacities of the HD and AD reactors

With 156 mg COD l⁻¹ in form of acetic acid, more than 95% of nitrate (42 mg N l⁻¹) was removed at HRT from 3.0 to 2.0 h, but residual nitrate, formed nitrite and acetate breakthrough in the effluent became considerable at HRT 1.5 h (Fig. 2a). Overall, the HD reactor after acclimation could effectively remove 0.48 g NO₃-N l⁻¹ d⁻¹ at HRT 2.5 h. This nitrate removal capacity is moderate among fixed-bed systems (AbuGhararah, 1996).

The operation of the AD reactor with feed of 14 mg NO₃-N l⁻¹ showed that >95% of nitrate was removed at HRT 2.5 h (equivalent to 0.13 g NO₃-N l⁻¹ d⁻¹) (Fig. 2b). The release of sulfate in the AD effluent at various HRTs confirmed the occurrence of FeS-based AD (Fig. 2b). Amorphous FeS-based AD observed in this study was more efficient than FeS₂-based AD in natural environments (Juncher Jørgensen et al., 2009), but less efficient than soluble sulfide-based AD (Chen et al., 2009). Nevertheless, the operation of FeS-based AD at pH 7.8 had little risk of over-releasing free sulfide to cause incomplete denitrification or ammonia formation (Brunet and Garcia-Gil, 1996), and would be ideal to reliably remove nitrate and nitrite of small amounts remaining in the effluent from the HD reactor operated under carbon limitation.

3.3. Nitrate removal by the HD–AD treatment system under carbon limitation

The cooperative operation of the HD and AD reactors was conducted at HRT 2.5 h with feed of 42 mg NO₃-N l⁻¹ and 156 mg COD l⁻¹ in form of acetic acid at HRT 3 h at the end of 5-week acclimation.

![Fig. 1. Vertical variation of pH, nitrate and acetate in the HD reactor operated with feed of 70 mg NO₃-N l⁻¹ and 156 mg COD l⁻¹ in form of acetic acid at HRT 3 h at the end of 5-week acclimation.](image-url)

![Fig. 2. Nitrate, nitrite, acetate and sulfate in the effluents from the HD (a) and AD (b) reactors operated at different HRTs. The HD feed contained 42 mg NO₃-N l⁻¹ and 156 mg COD l⁻¹ in form of acetic acid, and the AD feed contained 14 mg NO₃-N l⁻¹ and 37 mg SO₄²⁻ l⁻¹.](image-url)
of increasing the reliability of biological removal of highly elevated nitrate. The contents of NO$_3^-$-N and NO$_2^-$-N in the HD effluent were 4.1 and 1.5 mg l$^{-1}$, respectively, indicating that >85% of nitrate was removed by the HD reactor. The subsequent removal of nitrate and nitrite remaining in the HD effluent by the AD reactor at HRT 2.5 h was considerably lower than its nitrate removal capacity. As a result, NO$_3^-$-N and NO$_2^-$-N in the effluent from the AD reactor were constantly below 1.0 and 0.1 mg l$^{-1}$, respectively. The recovery of sulfate in the AD effluent was averagely 38% lower than would be expected from complete oxidation of FeS due to the incomplete oxidation of FeS to S$_0$ observed in the AD reactor.

Nitrate-polluted drinking water generally needs proper post processing such as Cl$_2$ oxidation after biological treatment (McAdam and Judd, 2007) to remove residual SOC, formed nitrite and/or kill bacteria to meet compliance. Acetate was found below detection in the HD effluent, primarily due to the operation of carbon-limited HD at the suitable HRT. The low level of SOC in the HD effluent (3.4 mg l$^{-1}$) confirmed the lowered release of microbially derived SOC from carbon-limited HD (McAdam and Judd, 2007). The level of Fe$^{2+}$ in the AD effluent was found below 0.3 mg l$^{-1}$. Overall, the lowered release of SOC, nitrite and Fe$^{2+}$ in the effluent from the HD–AD treatment system would lower the cost of post processing.

### 3.4. Advantages of the HD–AD treatment system for treatment of nitrate-heavily polluted drinking water

Nitrate removal mainly occurs in the first half part of a conventional fixed bed system and the second half part can only be operated under capacity due to low substrate availability. In contrast, in the acetic acid-supported pH-heterogenized HD reactor, about one third of nitrate removal occurred in the initially acidic zone, and about a half occurred in the pH-circumneutral central part, but the overall HD capacity was moderate among fixed bed systems (AbuGhararah, 1996). More importantly, acetic acid-supported HD provided sufficient internal CO$_2$ to eliminate the need of external pH adjustment for biological treatment of nitrate-highly polluted water.

Nitrate removal by carbon-limited HD has been suggested to lower the risk of organic carbon breakthrough in the effluent (McAdam and Judd, 2007). Nitrate and nitrite remaining in the effluent from carbon-limited HD could be removed by a supplemental process such as S$_0$-based AD (Liu et al., 2009) or FeS-based AD as proposed by this study. Compared to S$_0$-based AD, FeS-based AD generates less acidity and sulfide. Also, FeS-based AD has little risk of releasing soluble sulfide at pH above 7.5 even when nitrate is not available. So, the supplemental operation of FeS-based AD should increase the operational reliability of carbon-limited HD without excess of SOC, NO$_3^-$ or SO$_4^{2-}$ in the final effluent.

In addition, FeS per se and the subsequently formed ferric iron oxides after FeS oxidation may absorb toxic metals. Further studies are needed on simultaneous removal of heavy metals and nitrate from drinking water by the HD–AD system.

### 4. Conclusions

Through acclimation at heterogenized pH from being moderately acidic to circumneutral, acetic acid-supported HD was able to biologically remove highly elevated nitrate at a moderate efficiency without the need of external pH adjustment during and after treatment. To increase the treatment reliability and lower the cost of post processing, acetic acid-supported HD was operated under 10% carbon limitation to remove >85% of nitrate, and FeS-based AD was supplementally operated to reliably remove residual nitrate and formed nitrite without excess of SOC, NO$_3^-$ or SO$_4^{2-}$ in the final effluent.

### References


Zhang, W.L., Tian, Z.X., Zhang, Y.H., Zhong, F.H., 2009. A continuous stirred hydrogen-based AD was supplementally operated to reliably remove residual nitrate and formed nitrite without excess of SOC, NO$_3^-$ or SO$_4^{2-}$ in the final effluent.