Simultaneous removal of nitrogen and phosphorus from wastewater by means of FeS-based autotrophic denitrification

Ruihua Li, Jianmin Niu, Xinmin Zhan and Bo Liu

ABSTRACT

The efficacy of iron(II) sulfide (FeS)-based autotrophic denitrification in simultaneous nitrogen and phosphorus removal from wastewater was studied with batch experiments. It was efficient at a wide pH range of 5–9, and temperature range of 10–40 °C. The concentrations of NH4⁺-N, Mg²⁺, and \( \text{HCO}_3^-/\text{CO}_3^{2-} \) in the wastewater should be kept over 7.8, 0.24 and 30 mg L\(^{-1}\) for efficient nitrate (NO₃⁻-N) reduction, respectively. The NO₃⁻-N removal rate increased from 0 to 82 mg L\(^{-1}\) d\(^{-1}\) and then leveled off when the NO₃⁻-N concentration increased from 0 to 415 mg L\(^{-1}\) and then to 700 mg L\(^{-1}\), respectively. The NO₃⁻-N removal rate quickly increased, leveled off, and then sharply decreased when the PO₄³⁻-P concentration increased from 0 to 0.1 mg L\(^{-1}\), then to 114.0 mg L\(^{-1}\), and further to 683.8 mg L\(^{-1}\), respectively. The PO₄³⁻-P removal was over 98% when the PO₄³⁻-P concentration ranged 0–683.3 mg L\(^{-1}\). During treatment of the secondary effluent of a local municipal wastewater treatment plant containing NO₃⁻-N of 14.9 mg L\(^{-1}\) and total phosphorus (TP) of 3.9 mg L\(^{-1}\), NO₃⁻-N was reduced to 1.1 mg L\(^{-1}\) and TP was completely removed.

Key words | autotrophic denitrification, FeS, nitrogen removal, phosphorus removal, wastewater treatment

INTRODUCTION

Eutrophication of water bodies is a major global environmental issue. Its main cause is over-discharge of nitrogen (N) and phosphorus (P) to receiving water bodies due to improper treatment of wastewater. Recently, more and more stringent legislation has required low N and P emission limits for wastewater treatment.

Conventional biological N removal (BNR) via heterotrophic denitrification and phosphorus removal via enhanced biological P removal (EBPR) have been proven to be feasible and cost-effective for N (Gómez et al. 2000) and P (Simon & Jennifer 2008) removal from wastewater, respectively. However, when there is not sufficient readily biodegradable organic matter present in wastewater, efficient heterotrophic denitrification and efficient P removal is hard to achieve.

BNR can also be achieved through autotrophic denitrification, in which hydrogen or reduced sulfur compounds are electron donors (Biswas & Bose 2005). In comparison with heterotrophic denitrification, autotrophic denitrification has advantages like no need for addition of external organic carbon sources and low sludge production (Zhang & Lampe 1999).

In sulfur-based autotrophic denitrification, denitrifying bacteria like *Thiobacillus denitrificans*, which are ubiquitous in anoxic marine ecosystems, reduce nitrate (NO₃⁻) to nitrogen gas (N₂). Denitrification with reduced sulfur compounds like iron(II) sulfide (FeS) and elemental S can proceed in accordance with the stoichiometric reactions as follow (Jørgensen et al. 2009), respectively:

\[
5\text{FeS} + 8\text{NO}_3^- + 4\text{H}_2\text{O} \rightarrow 4\text{N}_2 + 5\text{SO}_4^{2-} + 5\text{Fe}^{2+} + 8\text{OH}^- \quad (1)
\]

\[
5\text{S} + 6\text{NO}_3^- + 2\text{H}_2\text{O} \rightarrow 3\text{N}_2 + 5\text{SO}_4^{2-} + 4\text{H}^+ \quad (2)
\]

Elemental S is the most common reduced sulfur source studied and applied in wastewater treatment (Koenig & Liu 1996).
FeS-based autotrophic denitrification has been found to be responsible for NO\textsubscript{3}\textsuperscript{-} reduction in freshwater systems (Garcia-Gil & Golterman 1993; Haaijer et al. 2007) and marine sediments (Simon & Jennifer 2008), and has higher nitrogen removal efficiency than elemental S-based autotrophic denitrification. To date, few researchers have paid attention to FeS-based autotrophic denitrification for nitrogen removal from wastewater.

FeS-based autotrophic denitrification produces alkali and Fe\textsuperscript{2+} (Reaction (1)). Fe\textsuperscript{2+} is oxidized and precipitated in the aqueous solutions, as described in Reactions (3) and (4):

\[
5\text{Fe}^{2+} + \text{NO}_3^- + 12\text{H}_2\text{O} \rightarrow 5\text{Fe(OH)}_3 + 0.5\text{N}_2 + 9\text{H}^+ \quad (3)
\]

\[
\text{Fe}^{2+} + 2\text{H}_2\text{O} \rightarrow \text{Fe(OH)}_2 \downarrow + 2\text{H}^+ \quad (4)
\]

Polymerization of Fe\textsuperscript{(OH)}\textsubscript{3} and Fe\textsuperscript{(OH)}\textsubscript{2} occurs, which is similar to the polymerization process after the addition of iron coagulants into wastewater. As a consequence, P can be removed from wastewater via chemical precipitation and adsorption by means of the ‘iron’ coagulant:

\[
3\text{Fe}^{2+} + 2\text{PO}_4^{3-} \rightarrow \text{Fe}_3(\text{PO}_4)_2^- \quad (5)
\]

\[
\text{pPO}_4^{3-} + \text{Fe}_n(\text{OH})_m \rightarrow (\text{PO}_4)_{ip} \times \text{Fe}_n(\text{OH})_m \text{adsorption} \quad (6)
\]

Fe\textsuperscript{2+}, Fe\textsuperscript{(OH)}\textsubscript{2} and Fe\textsuperscript{(OH)}\textsubscript{3} are efficient in P removal via chemical precipitation and adsorption (Wei et al. 2008). Therefore, in addition to N removal from wastewater, FeS-based autotrophic denitrification can simultaneously achieve efficient phosphorus removal.

The objectives of this study were to investigate factors affecting FeS-based autotrophic denitrification and to examine its performance in nitrogen and phosphorus removals from synthetic wastewater and secondary effluent taken from a local municipal wastewater treatment plant.

**Enrichment and isolation of sulfur autotrophic denitrifiers**

Five millilitres of anaerobic sludge taken from a laboratory-scale continuous upflow anaerobic sludge bed reactor was inoculated into a 150-mL conical flask filled with 100 mL culture medium. The medium was made from distilled water containing 5 g L\textsuperscript{-1} Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3}-5H\textsubscript{2}O, 2 g L\textsuperscript{-1} K\textsubscript{2}HPO\textsubscript{4}, 2 g L\textsuperscript{-1} KNO\textsubscript{3}, 1 g L\textsuperscript{-1} NaHCO\textsubscript{3}, 0.5 g L\textsuperscript{-1} NH\textsubscript{4}Cl, 0.5 g L\textsuperscript{-1} MgCl\textsubscript{2}-6H\textsubscript{2}O and 0.01 g L\textsuperscript{-1} FeSO\textsubscript{4}-7H\textsubscript{2}O; this composition was adopted from Baalsrud’s research (Baalsrud & Baalsrud 1954). The conical flask was flushed with CO\textsubscript{2} to exclude air, sealed with a rubber stopper and then placed in a thermostatic water bath shaker for incubation at 28°C for 4 d. Successful enrichment of sulfur autotrophic denitrifiers was determined with development of turbidity in addition to nitrogen removal. After enrichment five times with the procedure mentioned above, enriched samples were streaked on the culture medium with 2% agar and anaerobically incubated at 28°C in the anaerobic box. Isolated colonies were re-inoculated in the anaerobic culturing medium and, if these cultures grew well with the observation of nitrogen removal, the plating and inoculating procedure was repeated again and again. All of the isolated colonies on plates possessed the form of tiny white colonies of Gram-negative rods (Kuenen & Tuovinen 1988) when observed with a microscope. They were preserved at 4°C.

**Wastewater**

Two types of wastewater were treated in this study. One was synthetic wastewater with the composition of 0.4 g L\textsuperscript{-1} K\textsubscript{2}HPO\textsubscript{4}, 0.4 g L\textsuperscript{-1} KNO\textsubscript{3}, 0.2 g L\textsuperscript{-1} NaHCO\textsubscript{3}, 0.1 g L\textsuperscript{-1} NH\textsubscript{4}Cl, 0.1 g L\textsuperscript{-1} MgCl\textsubscript{2}-6H\textsubscript{2}O and 0.002 g L\textsuperscript{-1} FeSO\textsubscript{4}-7H\textsubscript{2}O, if not specially indicated. The concentrations of NO\textsubscript{3}\textsuperscript{-}N and PO\textsubscript{4}\textsuperscript{3-}-P in the synthetic wastewater were 55 and 78.6 mg L\textsuperscript{-1}, respectively. A higher P concentration in the wastewater would help to examine the capacity of this technology for P removal.

The other wastewater was the secondary effluent in Suojingcun wastewater treatment plant where the conventional activated sludge process is applied. The concentrations of NO\textsubscript{3}\textsuperscript{-}N and total phosphorus (TP) in the secondary effluent were 14.9 and 3.9 mg L\textsuperscript{-1}, respectively.

**Batch experiments**

The effects of temperature, initial pH, initial concentrations of NO\textsubscript{3}\textsuperscript{-}N and PO\textsubscript{4}\textsuperscript{3-}-P, carbon sources, and concentrations...
of NH$_4^+$-N and Mg$^{2+}$ on the NO$_3^-$-N removal rate were investigated with batch experiments. Before the batch experiment commenced, the preserved isolated colonies were activated twice with the culturing medium under the anaerobic condition at 28°C, and then the suspended solution was homogenized and used as the inoculum. Batch experiments were conducted in plastic centrifuge tubes with a working volume of 50 mL. Thirty grams of FeS and 2.5 mL of inoculum were added into the tubes, and then synthetic or real wastewater was added to reach the liquid volume of 50 mL. The centrifuge tubes were flushed with CO$_2$ to exclude air, airtightened and placed in the thermostatic water bath shaker at 28°C for 2 d if not especially indicated. The pre-experiment showed that 80–90% of nitrate was removed within 2 d. The initial pH value of wastewater was adjusted to 7.0 by adding NaOH or HCl, except in the batch experiment examining effects of the initial pH on the NO$_3^-$-N removal rate, in which the initial pH varied from 5 to 9. The basic experiment conditions were temperature of 28°C, initial pH of 7 and with the synthetic wastewater. When effects of a certain factor on the autotrophic denitrification efficiency were tested, only this factor was changed.

Sampling and analysis

As batch experiments ended, the mixed liquor in the centrifuge tubes was filtered with 0.45 μm filter paper and then the water samples were analyzed for NO$_3^-$-N, TP, and pH. NO$_3^-$-N and TP were determined according to the standard methods (MEPPRC 2002). pH was measured with a portable pH meter (PHB-4, Leici, Shanghai). The concentrations of heavy metal ions were determined with inductively coupled plasma spectrometry (Optima 5300DV, Perkin Elmer, USA).

RESULTS AND DISCUSSION

Effects of temperature and initial pH

The effects of initial pH of wastewater on the NO$_3^-$-N removal rate are shown in Figure 1. As initial pH values rose from 5 to 7, the NO$_3^-$-N removal rate decreased from 17.5 to 14.0 mg L$^{-1}$ d$^{-1}$ and at the same time final pH values increased from 6.5 to 7.9. As initial pH values were further increased from 7 to 9, the NO$_3^-$-N removal rate increased from 14.0 to 16.2 mg L$^{-1}$ d$^{-1}$ at initial pH of 8.0 and then slightly decreased to 15.8 mg L$^{-1}$ d$^{-1}$ at initial pH of 9.0, and the final pH values decreased from 7.9 to 6.9. In the tested initial pH range of 5–9, the NO$_3^-$-N removal rates were over 14.0 mg L$^{-1}$ d$^{-1}$ and were higher than those measured in the elemental S-based autotrophic denitrification system (Cardoso et al. 2006), 11.2 mg L$^{-1}$ d$^{-1}$.

Apart from Reactions (1) and (3), the following reactions also proceed in the FeS system:

FeS $\rightarrow$ Fe$^{2+}$ + S$^{2-}$ \hspace{1cm} (7)

S$^{2-}$ + H$_2$O $\rightarrow$ HS$^{-}$ + OH$^{-}$ \hspace{1cm} (8)

Reactions (1) and (8) generate OH$^{-}$, which can neutralize H$^+$; Reactions (3) and (4) produce H$^+$, which can neutralize OH$^{-}$. Hence, FeS autotrophic denitrification possesses a high buffering capacity, causing the final pH values of the water phase to be around 7. Consequently, in consideration with the fact that the optimal pH range for Thiobacillus denitrificans is 6.2–7.0 (Kuenen & Tuovinen 1981; Donovan & Ann 2000), efficient denitrification in the FeS system was achieved in the initial pH range of 5–9. In elemental sulfur-based systems (Zhang & Lampe 1999), the addition of limestone is required to keep pH stable in the reactors; however, this practice is not needed in the FeS system that has an efficient buffering capacity.

Figure 1(b) shows that the NO$_3^-$-N removal rate decreased with increasing of the final pH values. A lower pH value facilitates Reaction (1). It also benefits the solubilization of FeS particles, thus increasing reaction kinetics. The slightly decreased NO$_3^-$-N removal rates when the initial pH value was over 8 could be due to the formation of iron precipitates on the surface of FeS particles, adversely...
affecting the contact between sulfur autotrophic denitrifiers and FeS particles.

The NO$_3^-$-N removal rate rose from 3.5 to 17.0 mg L$^{-1}$ d$^{-1}$ as temperature increased from 5 to 10 $^\circ$C, and with further increase in temperature from 10 to 28 $^\circ$C the NO$_3^-$-N removal rate slightly rose from 17.0 to 21.0 mg L$^{-1}$ d$^{-1}$ (Figure 2). When temperature increased from 28 to 40 $^\circ$C, the NO$_3^-$-N removal rate decreased from 21.0 to 16.9 mg L$^{-1}$ d$^{-1}$. The results indicate that the FeS system can be effective in the temperature range of 10–40 $^\circ$C. Much lower or higher temperature would inhibit the physiological function of microbes and then decrease the NO$_3^-$-N removal rate. Claus & Kutzner (1985) found that the effective temperature ranges of elemental S- and Na$_2$S$_2$O$_3$-based autotrophic denitrification systems were 20–40 $^\circ$C and 20–35 $^\circ$C, respectively. A broader effective temperature range of the FeS system is an advantage when applying this technology to wastewater treatment.

Effects of NH$_4^+$ and Mg$^{2+}$ nutrients

Sulfur autotrophic denitrifiers prefer to use NH$_4^+$ as the nitrogen source rather than NO$_3^-$. Growth of sulfur autotrophic denitrifiers needs trace nutrients like Mg$^{2+}$. Effects of the concentrations of NH$_4^+$ and Mg$^{2+}$ on the NO$_3^-$-N removal rate are shown in Figure 5. The NO$_3^-$-N removal rate sharply rose with increasing the NH$_4^+$-N concentration up to 7.8 mg L$^{-1}$, and then there was little change as the NH$_4^+$-N concentration was further increased. With increasing Mg$^{2+}$ concentration up to 0.24 mg L$^{-1}$, the NO$_3^-$-N removal rate sharply rose and then slightly increased as the Mg$^{2+}$ concentration was increased. Accordingly, in order for optimal denitrification, it is better to have concentrations of NH$_4^+$-N and Mg$^{2+}$ in wastewater of 7.8 and 0.24 mg L$^{-1}$, respectively. However, these two nutrients are not necessary; when the concentrations of NH$_4^+$ and Mg$^{2+}$ were zero, the NO$_3^-$-N removal rates were 11.3 and 12.0 mg L$^{-1}$ d$^{-1}$, respectively.

Effects of carbon sources

Organic carbon sources that can enhance the growth of heterotrophic denitrifying bacteria might inhibit the activity of sulfur autotrophic denitrifiers. The effects of NaHCO$_3$ and glucose, representing inorganic and organic carbon sources, respectively, on the NO$_3^-$-N removal rate were tested in this study (Figure 4).
With increasing the NaHCO$_3$ concentration from 0 to 30 mg L$^{-1}$, the NO$_3^-$-N removal rate increased rapidly, and then increased slowly as the NaHCO$_3$ concentration further increased. The optimal concentration of NaHCO$_3$ should be 30 mg L$^{-1}$, which was nearly the same as that found in the Na$_2$S$_2$O$_3$ autotrophic denitrification system (Niu 2010).

The NO$_3^-$-N removal rate slightly changed when the glucose concentration in the synthetic wastewater varied in the range of 0–1,000 mg L$^{-1}$. It indicates that glucose did not influence FeS autotrophic denitrification. The reason is that, in the present study, only isolated sulfur autotrophic denitrifiers were used in the batch experiment.

**Effects of the NO$_3^-$ concentration**

With respect to FeS-based autotrophic denitrification, NO$_3^-$-N is the electron acceptor for autotrophic denitrifying bacteria. The effects of the initial NO$_3^-$-N concentration on the NO$_3^-$-N removal are shown in Figure 5. With increasing the initial NO$_3^-$-N concentration to 277 mg L$^{-1}$, the NO$_3^-$-N removal was over 99%, but further increase resulted in linear decrease in the NO$_3^-$-N removal efficiency. There was a limit for NO$_3^-$-N removal in the period of the experiment; thus, as the total amount of NO$_3^-$-N in the wastewater exceeded the limit, the NO$_3^-$-N removal efficiency decreased clearly.

With increasing the initial NO$_3^-$-N concentration to just less than 415 mg L$^{-1}$, the NO$_3^-$-N removal rate increased linearly, and then leveled off at 82 mg L$^{-1}$ d$^{-1}$ as the NO$_3^-$-N concentration increased to 700 mg L$^{-1}$. This clearly shows that high NO$_3^-$-N concentrations did not inhibit the activity of sulfur autotrophic denitrifiers in the FeS system.

**Effects of PO$_4^{3-}$-P concentration and phosphorus removal**

P is necessary for cell synthesis, and lack of P would inhibit denitrification. Effects of the PO$_4^{3-}$-P concentration on the NO$_3^-$-N removal rate and performance in P removal are shown in Figure 6. With increasing the initial PO$_4^{3-}$-P concentration, the NO$_3^-$-N removal rate changed in three stages: in Stage 1 (the initial PO$_4^{3-}$-P concentration was 0–0.11 mg L$^{-1}$), the NO$_3^-$-N removal rate rose sharply; in Stage 2 (the initial PO$_4^{3-}$-P concentration was 0.11–114.0 mg L$^{-1}$), the NO$_3^-$-N removal rate increased slowly and then leveled off at around 20.5 mg L$^{-1}$ d$^{-1}$; in Stage 3 (the initial PO$_4^{3-}$-P concentration was 114.0–683.8 mg L$^{-1}$), the NO$_3^-$-N removal rate decreased. As PO$_4^{3-}$-P was over 114.0 mg L$^{-1}$, the phosphorus precipitates formed on the surface of FeS particles obstructed the contact of denitrifiers with FeS particles (Elsetinow et al. 2003). This caused the decreased NO$_3^-$-N removal rate. With increasing initial PO$_4^{3-}$-P concentrations from 0 to 683.8 mg L$^{-1}$, the final PO$_4^{3-}$-P concentration only rose from 0 to 10.9 mg L$^{-1}$ and the phosphorus removal reached 98–100%.

According to Reactions (1) and (5), the removed PO$_4^{3-}$-P due to precipitation of Fe$_3$(PO$_4$)$_2$ was no more than 58 mg L$^{-1}$ even if NO$_3^-$-N (55 mg L$^{-1}$) in the wastewater was completely reduced. Then the adsorption of PO$_4^{3-}$ on the surface of FeS particles and iron hydroxide precipitates was an important mechanism for PO$_4^{3-}$-P removal.

---

**Figure 5** | Effects of the initial NO$_3^-$-N concentration on the NO$_3^-$-N removal and the NO$_3^-$-N removal efficiency.

**Figure 6** | Effects of the initial PO$_4^{3-}$-P concentration on the NO$_3^-$-N and the phosphorus removal.
Treatment of the secondary municipal effluent

The secondary municipal effluent was treated with the FeS system in batch flask experiments (Figure 7). The NO$_3$-N concentration linearly decreased with time. After 16 hours since commencement of the experiment, the NO$_3$-N concentration decreased to 1.1 mg L$^{-1}$, and NO$_2$-N was not detectable. After 4 hours, the TP concentration was too low to be detectable.

CONCLUSIONS

In this study synthetic and real wastewater without biodegradable organic matter was treated based on FeS autotrophic denitrification. The results obtained are as follows.

1. Efficient autotrophic denitrification was achieved at pH ranging 5–9 and temperature ranging 10–40 °C.
2. The limit concentrations of NH$_4$+–N, Mg$^{2+}$, and NaHCO$_3$ for efficient denitrification were 7.8, 0.24 and 50 mg L$^{-1}$, respectively. The denitrification rate increased with increasing NO$_3$-N concentration up to 415 mg L$^{-1}$.
3. The denitrification rate was high and did not change when the PO$_4^{3-}$-P concentration varied between 0.1 and 114.0 mg L$^{-1}$. The P removal efficiency was over 98% when the initial PO$_4^{3-}$-P concentration ranged 0–683.5 mg L$^{-1}$.
4. As the secondary municipal effluent was treated, NO$_3$-N was reduced from 14.9 to 1.1 mg L$^{-1}$ and TP was completely removed.

ACKNOWLEDGEMENTS

This study was financially supported by the National Natural Science Foundation of China (50978153) and an FP7 Marie Curie IIF Fellowship (PIIF-GA-2010-272511).

REFERENCES


MEPPRC (Ministry of Environmental Protection of People’s Republic of China) 2002 *Standard Methods for the
Examination of Water and Wastewater, 4th edn, Environmental Science Press, Beijing, China.


First received 31 July 2012; accepted in revised form 18 February 2013