# Mixed electron donor autotrophic denitrification processes for groundwater treatment by immobilized biological filters

Jun-feng Su, Dong-hui Liang, Ting-lin Huang, Ting-ting Lian and Wen-dong Wang

## ABSTRACT

An immobilized biological filter (IBF) using Fe(II) and Mn(II) as mixed electron donors was evaluated for nitrate removal in groundwater. Results of the single factor experiments of strain SZ28 under the conditions of electron donor:electron acceptor ratio (1:2, 1.45:1, 3:1), Fe(II):Mn(II) ratio (1:9, 3:7, 5:5) demonstrated that the highest nitrate removal ratio was 100%, 49.6% (Mn(II)) and 100% (Fe(II)) under the conditions of electron donor:electron acceptor ratio of 3:1, Fe(II):Mn(II) ratio of 5:5. Mn(II) and Fe(II) as electron donor was tested for the effects on denitrification in the IBF reactor. Optimal conditions were obtained at an electron donor:electron acceptor ratio of 2:1, hydraulic retention time of 12 h and Fe(II):Mn(II) ratio of 5:5 with the highest removal ratio of nitrate-N (100%), Mn(II) (50.25%) and Fe(II) (99.2%). Results suggest that the optimal condition obtained from the IBF was feasible.

**Key words** | autotrophic denitrification (AD), electron donor, immobilized biological filter (IBF), nitrate

**Jun-feng Su** (corresponding author)
**Dong-hui Liang**
**Ting-lin Huang**
**Ting-ting Lian**
**Wen-dong Wang**
School of Environmental and Municipal
   Engineering,
Xi'an University of Architecture and Technology,
Xi'an 710055,
China
E-mail: sjf1977518@sina.com

**Jun-feng Su**
**Ting-lin Huang**
Key Laboratory of Northwest Water Resources,
   Environment and Ecology,
MOE,
Xi'an 710055,
China

## INTRODUCTION

Excessive use of nitrogenous fertilizers in the agricultural industry and inappropriate disposal of domestic and industrial wastewaters may lead to contamination of surface and ground waters (Liu *et al.* 2009; Sahinkaya & Dursun 2012). Nitrate is a widespread contaminant in drinking water and ingested nitrate under conditions resulting in endogenous nitrosation is suspected to be carcinogenic (Espejo-Herrera *et al.* 2015). It is a step-wise process through which $NO_3^-$ is converted to nitrogen gas via intermediate nitrite ($NO_2^-$), nitrogen monoxide (NO), and gaseous nitrous oxide ($N_2O$) (Ghafari *et al.* 2010).

A biological denitrification process, which comprises heterotrophic denitrification and autotrophic denitrification (AD), is considered an efficient technology to treat nitrate-contaminated water (Su *et al.* 2015). If plenty of organic carbon is available, heterotrophic denitrification is a very effective method. However, if groundwater lacks organic carbon, this could limit the application of heterotrophic denitrifying unless external provide organic carbon source (Smith *et al.* 2005; Sunger & Bose 2009).

Alternatively, an AD process can be used to remove nitrate from drinking water sources. Organic supplementation is not required in the AD process, which leads to low biomass production, decreased risk of bacterial contamination and reduced operational cost (Sahinkaya & Dursun 2012). Recently, AD processes, including sulfur-based denitrification and hydrogen-based AD, were widely studied in the remediation of groundwater and drinking water (Zhang *et al.* 2009). Sulfide-oxidizing AD is performed by oxidizing chemolithotrophic sulfur bacteria, which are capable of oxidizing reduced sulfur compounds (S, SO, $S_2O_3^{2-}$, $SO_3^{2-}$) while reducing oxidized nitrogen compounds

$(NO_3^-, NO_2^-)$ (Moraes & Foresti 2012). AD could be conducted by some bacteria through using hydrogen as the electron donor, inorganic carbon as the carbon source and nitrate/nitrite as the electron acceptor in the absence of oxygen (Mao *et al.* 2012). But the disadvantages such as sulfur-based denitrification, denitrification rate being controlled by the proportion of sulfur/limestone, which makes this technology complex and difficult to operate (Moon *et al.* 2006; Sierra-Alvarez *et al.* 2007). Complex hydrogen delivery systems, the loss of the $H_2$ to improve process cost and create explosive hydrogen atmosphere AD system (Lee & Rittmann 2002).

Because microbes with a denitrification ability utilize a variety of electron donors, such as organic carbon, sulfur, hydrogen, etc., many processes have been developed for each kind of donor (Park & Yoo 2009). But comparative studies on single electron donor, mixed electron donor supplements in groundwater treatment are scarce. Previously, Su *et al.* evaluated the denitrification activity of the mixed electron donor autotrophic denitrifying bacterium *Acinetobacter* sp. SZ28.

In this study, the biological filters filled with quartz-sand employing simultaneous removal of nitrate, Fe(II) and Mn(II) are proposed. The nitrate removal efficiency under different hydraulic retention times (HRTs) (8 h, 10 h and 12 h), electron donor:electron acceptor ratios (1:2, 1.75:1 and 3:1) and Fe(II):Mn(II) ratios (1:9, 3:7, 5:5) were investigated. Meanwhile, find out the comparatively suitable environment on the removal of nitrate, Fe(II) and Mn(II), operate the immobilized biological filter (IBF) reactor under the most suitable conditions.

## METHODS

### Inoculated strain for bioaugmentation

An autotrophic denitrifying bacterium SZ28 was isolated from a sediment. Bacteria were grown in 1,000 mL bottles containing 900 mL medium. The following microbial culture media (FMM) were used ($g·L^{-1}$): 0.5 of $NaHCO_3$; 0.1 of $NaNO_3$; 0.1 of $KH_2PO_4$; 0.05 of $MgSO_4·7H_2O$; 0.05 of $CaCl_2$ and 2 mL of trace elements solution. The components of trace elements in the solution were as follows: $0.5 g·L^{-1}$

$MgSO_4·7H_2O$, $1.0 g·L^{-1}$ EDTA, $0.2 g·L^{-1}$ $ZnSO_4$, $0.1 g·L^{-1}$ $MnCl_2·4H_2O$, $0.5 g·L^{-1}$ $FeSO_4·7H_2O$, $0.5 g·L^{-1}$ $CuSO_4·5H_2O$ and $0.2 g·L^{-1}$ $CoCl_2·6H_2O$.

The final pH of FMM was adjusted to 7.0 and then put into a portable pressure steam sterilizer at 121 °C for 30 min. Finally, the FMM was removed from the pressure cooker, and autoclaved Fe(II) and Mn(II) sources were added from a concentrated anoxic sterile stock solution to achieve a final concentration and incubated at 30 °C.

### Column experiment start-up and operation

The whole experiment ran two parallel reactors at the same time, one acting as control reactor, with immobilized bacteria as the experimental group. Two parallel reactors were operated with total and working volumes of 5 L and 3.5 L. The diameter and the total height of the reactor were 0.08 m and 1.0 m. The reactor was filled with silica sand filtering medium to 0.7 m of the column height. Details of the quartz sand were the same as in Su *et al.* (2016a). The IBF of the AD process was operated at 30 °C. Three sampling ports allowed sampling at the bottom of the columns.

The synthetic water used in this biological filter reactor comprised the following reagents per L: 0.5 g of $NaHCO_3$; 0.1 g of $KH_2PO_4$; 0.05 g of $MgSO_4·7H_2O$ and 0.05 g of $CaCl_2$. Firstly, 10 L synthetic water was added into the reactor. Then influent water was put into the reactor through a pump which set the desired flow velocity. Inorganic electron donor Fe(II) and Mn(II) diluted into the required concentration by deionized water, and added into reactor by high pressure steam pump. The effluent port was set in the right side of the reactor, when the reactor operation measured the nitrate-N, Mn(II), Fe(II) and nitrite-N concentration each day.

### Analysis methods

The concentrations of nitrite, nitrate, Mn(II) and Fe(II) ions were measured using *N*-(1-naphthalene)-diaminoethane spectrophotometry, and ultraviolet, potassium periodate and phenanthroline spectrophotometric methods (DR5000, HACH, USA), respectively. pH values were measured with a pH meter (MM110, HACH, USA).

## Influence of different electron donors on nitrate removal by the bacterium SZ28

The bacterium SZ28 was tested in contaminated groundwater after sterilization by adding extra Fe(II), Mn(II). Serum bottles with 280 mL volume were filled with 250 ml medium, and the bottles were incubated at 30 °C with 10% of inoculum (v/v).

The bottles were performed for a period of 192 h, the experiment was done in an anaerobic environment, and 5 mL samples were taken from the bottles periodically to analyse for nitrate, nitrite, Fe(II), Mn(II) and pH.

### Effect of different Fe(II):Mn(II) ratio on nitrate removal

Testing of nitrate removal rate under the different Fe(II):Mn(II) ratio (1:9, 3:7, 5:5). This study conducted to three cycles and each cycle measure six days to ensure experiment enter into the stable period, The nitrate concentration of experiment keep into 16.5 mg L$^{-1}$ and electron donor:electron acceptor ratio was 2:1, meanwhile in the process of the whole test set up the HRT at 10 h. The concentration of nitrate-N, Mn(II), Fe(II), nitrite-N and pH should be tested on a regular basis.

### Effect of different electron donor:electron acceptor ratio on nitrate removal

Estimate the nitrate removal capacity of the IBF in proportions of electron donor/electron acceptor. The experiment was performed by using Mn(II) and Fe(II) as electron donors (the Mn(II):Fe(II) ratio as 5:5), and using nitrate as electron acceptor, which concentration are 16.5 mg·L$^{-1}$. The concentration of Mn(II), Fe(II) would be changed though electron donor:electron acceptor ratio (1:2, 1.75:1 and 3:1), and the HRT were set at 10 h.

### Effect of HRT on nitrate removal

In this part, detection of nitrate removal efficiency under different HRT (8 h, 10 h and 12 h). In the process of the whole experiment operation, other factors were set to a specific value, the ratio of Fe(II):Mn(II) ratio was set to 5:5, the electron donor:electron acceptor ratio was set to 3:1. Operational conditions for each cycle were maintained until steady state in the Fe(II), Mn(II) and nitrate removal.

### Evaluation of the optimum condition

The three single factor experiment was employed to optimize the operating variables of Fe(II):Mn(II) ratio (1:9, 3:7 and 5:5), HRT (8 h, 10 h and 12 h) and electron donor:electron acceptor ratio (1:2, 1.75:1, 3:1). The model for predicting the optimum conditions, could be expressed according to the single factor experiment. After the preliminary study, the optimum condition of factors used to the experiment.

## RESULTS AND DISCUSSION

### Influence of electron donor:electron acceptor ratio on nitrate removal by the bacterium SZ28

The experiment was performed by using different ratios of electron donor:electron acceptor. As shown in Figure 1, the rates of nitrate removal were noticeably different depending on the electron donor:electron acceptor ratio (1:2, 1.45:1, 3:1). Different ratios of Fe(II):Mn(II) (1:9, 3:7, 5:5) were also used to evaluate their suitability in supporting nitrate removal. As can be seen from the experimental data, the fastest removal rate of nitrate (0.253 mg-N·L$^{-1}$·h$^{-1}$) was observed with an electron donor:electron acceptor ratio of 3:1, the ratio of Fe(II):Mn(II) was 5:5. Nitrate removal rates of 0.064 mg-N·L$^{-1}$·h$^{-1}$ and 0.193 mg-N·L$^{-1}$·h$^{-1}$ were observed when the ratios of electron donor:electron acceptor were 1:2 and 1.54:1, and the ratio of Fe(II):Mn(II) was 5:5. And the whole experiment less 0.5 mg·L$^{-1}$ nitrite was accumulated in effluent. The experimental data showed that the nitrate removal ratio increased when the ratio of electron donor:electron acceptor increased, regardless of the ratio of Fe(II):Mn(II). The growth of bacteria and the nitrate removal required a higher concentration of electron donor. Biofilter with sulfur (BS) was superior on nitrate (89.74% vs 80.72%) and total nitrogen (TN) removal (83.18% vs 70.42%) while biofilter with pyrite (BP) (Kong *et al.* 2016).

The results for the removal of Fe(II) and Mn(II) were also shown in Figure 1. The removal ratio of Fe(II) was
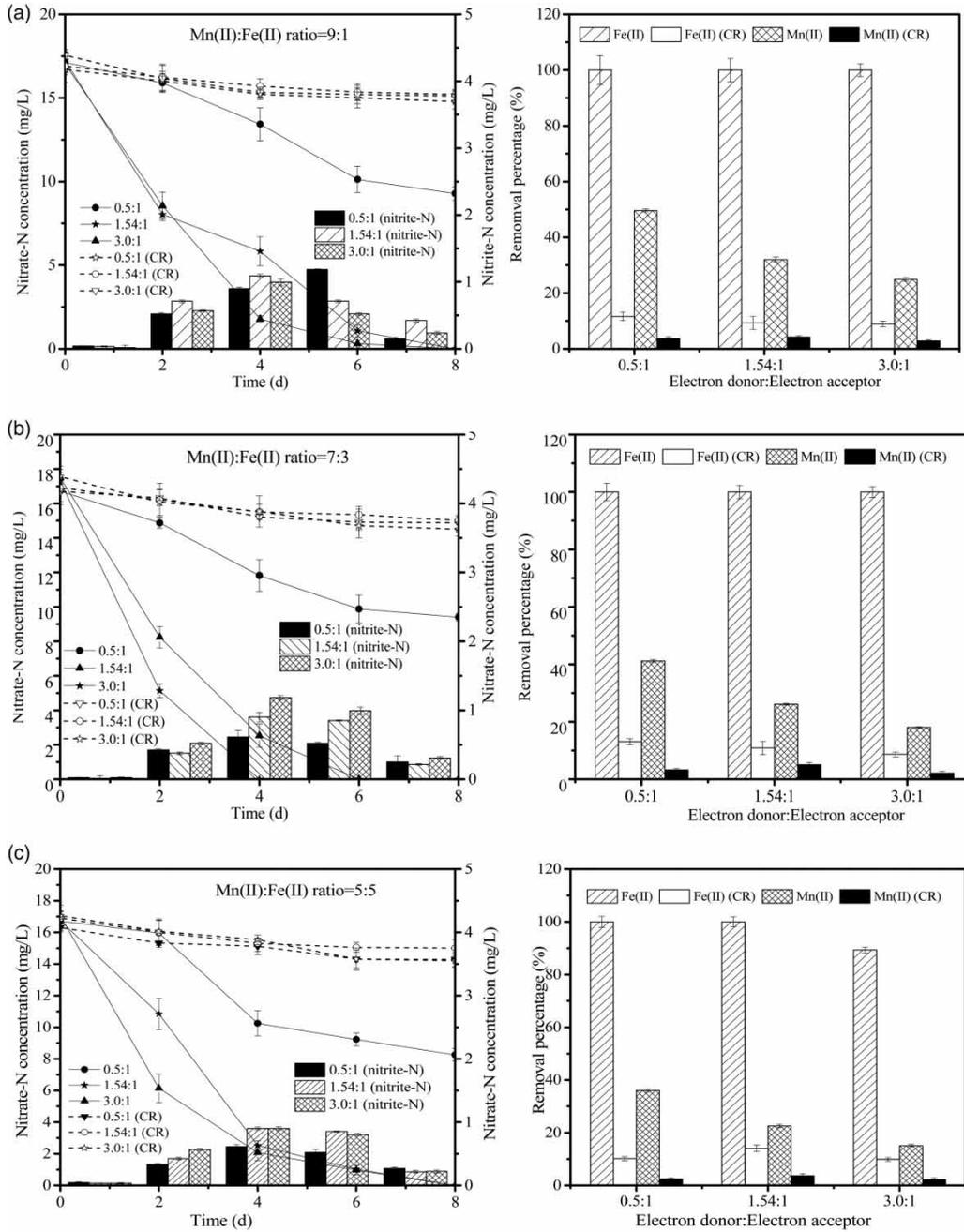
**Figure 1** │ Effect of different ratios of Fe(II):Mn(II) on the denitrification. (a) Fe(II):Mn(II) ratio = 1:9, (b) Fe(II):Mn(II) ratio = 3:7, (c) Fe(II):Mn(II) ratio = 5:5.

invariable when the ratio of electron donor:electron acceptor was increased from 1:2 (49.5%) to 3:1 (100%). However, the Mn(II) decreased was slower compared with removal of Fe(II). The maximum removal ratio of Mn(II) (49.6%) was observed with the electron donor:electron acceptor ratio was 1:2, the ratio of Fe(II):Mn(II) was 1:9.

When the electron donor:electron acceptor ratio was 1.45:1 and 3:1, the Mn(II) removal rate would be lower slightly. In the groundwater, which exhibited extremely low concentration of nutrient (low total organic carbon (TOC) and TN), only 40% Mn(II) was oxidized (Liang *et al.* 2015).

## Effect of electron donor:electron acceptor ratio

The initial nitrate concentration was 16.5 mg·L$^{-1}$, HRT was set to 10 h and the ratio of Fe(II):Mn(II) set to 5:5. In Figure 2(a) was showed the effect of different electron



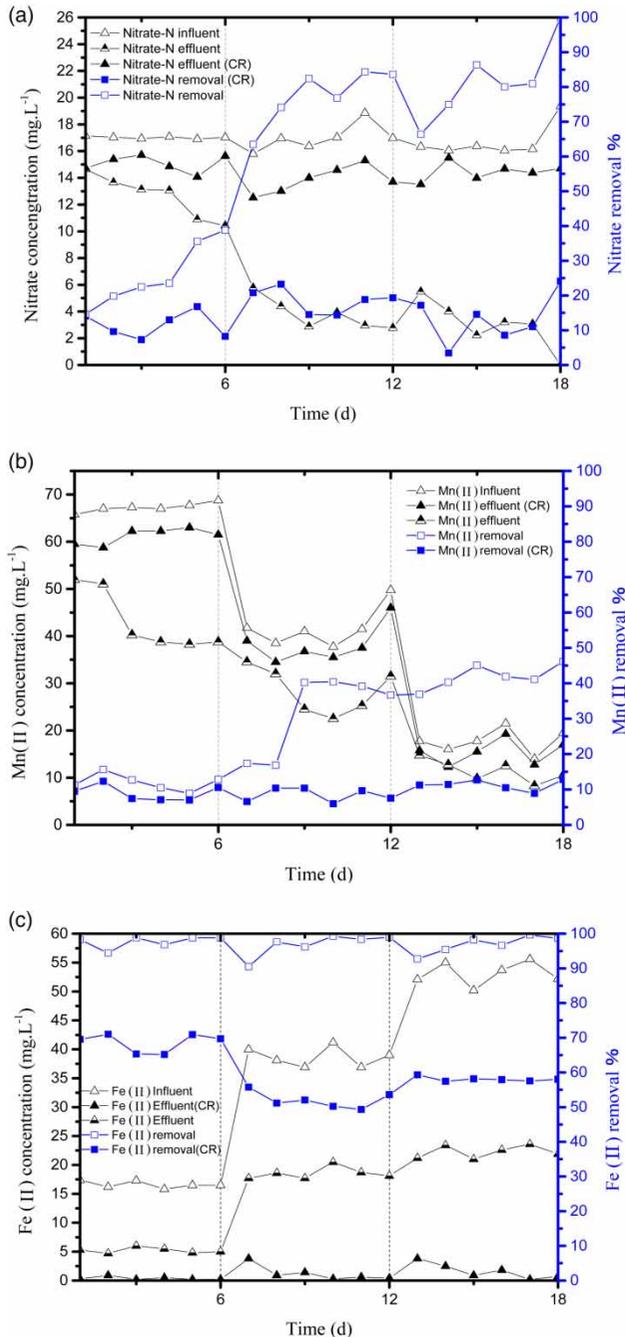donor:electron acceptor ratio on the AD using Fe(II) and Mn(II) as electron donors. In this experiment the nitrate removal efficiency increased when the electron donor:electron acceptor ratio was increased from 1:2 to 2:1. When the electron donor:electron acceptor ratio was 2:1, the maximum nitrate removal ratio was observed approximately 100% (1.935 mg-N·L$^{-1}$·h$^{-1}$). Nitrate removal ratio of 83.64% (1.421 mg-N·L$^{-1}$·h$^{-1}$) and 38.81% (0.661 mg-N·L$^{-1}$·h$^{-1}$) were observed when 1.25:1 and 1:2 were used as the ratio of electron donor:electron acceptor, respectively.

The denitrification efficiency significantly changed when using different ratio of electron donor:electron acceptor. Compared with nitrate removal at ratio of 1:2 and 2:1, can conclude that the nitrate removal ratio required higher concentration of electron donor. In another study, an up-flow bioreactor inoculated with strain B6 could achieve nitrate reduction coupled with Fe(II) concentration (Zhang *et al.* 2016).

Compared with removal of nitrate, the Mn(II) removal had different changes. Figure 2(b) shows that with the increase of electron donor, Mn(II) removal ratio is decrease. The maximum removal ratio of 46.15% (0.9 mg-N·L$^{-1}$·h$^{-1}$) was achieved using the electron donor:electron acceptor ratio of 1:2. However, the Fe(II) removal ratio of over 90.00% could be obtained at the whole experiment. This might be due to with the increase of electron donor, the concentration of Fe(II) increase which is easy used by bacteria, the needed electronic most provide by Fe(II). So bacteria showed good Fe(II) removal capability under all condition, but the removal ratio of Mn(II) instead of down which hard to use by bacteria. The Fe(II) oxidation were attributed to the biological role and the chemical role of oxygen (Bai *et al.* 2016). It has been documented that nitrate dependent Fe(II) oxidation is a spontaneous process because the redox potential of ferrous/ferric couples is about +200 mV, which is lower than nitrate/nitrogen gas (+710 mV) at neutral pH (Hedrich *et al.* 2011).

The results for the control reactor are shown in Figure 2. The results show that a nitrate removal ratio about 20% was obtained at whole experiment, the maximum removal ratio of 22.65% (0.376 mg·L$^{-1}$·h$^{-1}$). And the removal ratio of Mn(II) about 10% was obtained at whole experiment, the maximum removal ratio of 10.75% (0.96 mg·L$^{-1}$·h$^{-1}$) was obtained at seventh day. Compared with experimental group revealed that the IBF accelerated simultaneous removal of Fe(II), Mn(II) and nitrate.

**Figure 2** │ Effect of electron donor:electron acceptor ratio on denitrification processes. Nitrate-N = 16.5 mg/L, HRT = 10, Fe(II):Mn(II) = 5:5.

## Effect of Fe(II):Mn(II) ratio

The initial nitrate concentration was 16.5 mg/L, with the HRT of reactor set to 12 h. As shown in Figure 3(a). The nitrate removal ratio was related to different Fe(II):Mn(II) ratios (1:9, 3:7, 5:5). Experimental results indicated that the IBF could achieve nitrate removal coupled with Fe(II) concentration. When the ratio of Fe(II):Mn(II) increased from 1:9 to 5:5, the nitrate removal ratio increased from 64.6% to 97.5%, and the maximum nitrate removal ratio was observed at Fe(II):Mn(II) ratio of 5:5. The nitrate removal ratios were 97.5% ($1.567 \ mg·L^{-1}·h^{-1}$) (Fe(II): Mn(II) ratio of 5:5) close to nitrate removal ratio 95.28% ($1.309 \ mg·L^{-1}·h^{-1}$) when the Fe(II):Mn(II) ratio of 3:7. At the same time, the control reactor showed low Mn(II) and nitrate removal ratios (about 20% and 10%, respectively). But compared with removal of nitrate and Mn(II), the Fe(II) removal ratio was slightly higher (about 50%).

Fe(II) and Mn(II) as electron donors are very important in the AD process, the vulnerable electron donor Fe(II) was enough to bacteria using, but the biological removal of manganese requires more stringent conditions than the biological removal of iron (Katsoyiannis & Zouboulis 2004).

Moreover, Figure 3(b) shows the effect of different Fe(II):Mn(II) ratios on the Mn(II) removal process of the IBF. The Mn(II) removal efficiency increased when the Fe(II):Mn(II) ratio was increased from 1:9 (53.6%) to 5:5 (57.3%). But when the Fe(II):Mn(II) ratio was 3:7 there was almost no effect on the Mn(II) removal efficiency, the Fe(II):Mn(II) ratio of 1:9 (53.6%), the Fe(II):Mn(II) ratio of 3:7 (53.5%). But the Fe(II) removal ratio of over 90.0% obtained at the whole experiment.

It might be that when the Fe(II):Mn(II) ratio of 1:9, little electronic provided by Fe(II) is not enough to satisfy the denitrification, so bacteria can only use Mn(II) as electron donor to meet the needs of the denitrification. And when Fe(II):Mn(II) ratio of 5:5, along with the nitrate removal rate rise, the required amount of electron donor rose, after consumption of Fe(II) which easy to be used, bacteria in reactor can only use Mn(II) as electronic donor provide electronic to remove nitrate. Mn(II) is at an almost complete ionic state at a low pH, indicating its potential as an electron acceptor for denitrification (Su et al. 2016b).
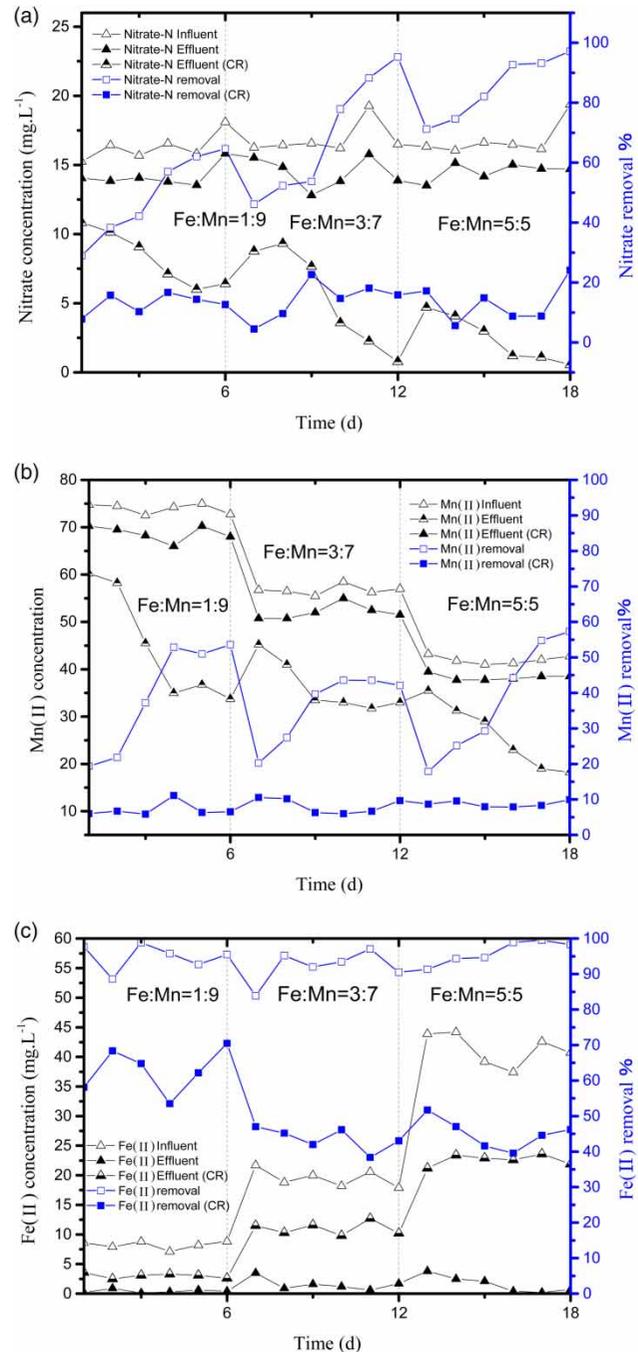


**Figure 3** | Effect of Fe(II):Mn(II) ratio on denitrification processes. Nitrate-N = 16.5 mg/L, HRT = 12, electron donor:electron acceptor = 1.25:1.

## Effect of HRT

The nitrate removal process was sensitive to HRT, initial HRT was set to 8 h, 10 h and 12 h. The initial nitrate was controlled to be 16.5 mg/L. After 18 days of operation,

The nitrate removal efficiency enhanced significantly along with the HRT was increased, the optimum HRT of IBF for nitrate removal was 12 h, the nitrate removal ratio of 73.25% ($1.479 mg \cdot L^{-1} \cdot h^{-1}$) with the HRT was 8 h, 95.28% ($1.571 mg \cdot L^{-1} \cdot h^{-1}$) with the HRT was 10 h, 100% ($1.379 mg \cdot L^{-1} \cdot h^{-1}$) with the HRT was 12 h. The part of experiment points out that long HRT can promote the nitrate removal as indicated in Figure 4(a). Oxidation of iron was dependent on the reduction of nitrate in the absence of other electron donors or acceptors (Li *et al.* 2013). This might be due to the HRT increasing residence times in the reactor, this allowed the bacteria had enough time to adapt the new environment and to removal nitrate contaminant successfully. In other study, a maximum nitrate removal efficiency was 79.7% at long HRT (17 h) (Zhang *et al.* 2016).

The results for the removal of Mn(II) are shown in Figure 4(b). When the HRT increased from 8 h to 12 h, the Mn(II) removal ratio rise at the same time, from 36.46% ($4.125 mg \cdot L^{-1} \cdot h^{-1}$) to 44.88% ($3.375 mg \cdot L^{-1} \cdot h^{-1}$). But the Mn(II) removal ratio was not substantial discrepancy when HRT between 10 h (43.59%) and 12 h (44.88%). The results suggest that HRT did not play an important role in the process of Mn(II) removal. Similarly when the HRT increased from 8 h to 12 h, the Fe(II) removal ratio did not vary, a removal efficiency about 90% at all stage. The result indicated that the bacteria (SZ28) can use Fe(II) and Mn(II) as electron donors, simultaneously. Compared with Figure 4(a) and 4(b). This indicated that higher Mn(II) removal ratio was not extremely necessary for a higher denitrification efficiency.

## The reactor performance under the optimum conditions

As shown in the three single factor experiment, the optimum conditions was suggested with Fe(II):Mn(II) ratio of 5:5, HRT of 12 h and electron donor:electron acceptor ratio of 3:1, these conditions were used to carry out the following experiment. As shown in Figure 5, the removal ratio of nitrate-N and Mn(II) did not remain stable at the beginning due to the reactor being in the start-up stage, with the nitrate and Mn(II) removal ratio of 78.49% ($1.128 mg \cdot L^{-1} \cdot h^{-1}$) and 27.66% ($1.083 mg \cdot L^{-1} \cdot h^{-1}$), respectively, after the three days the reactor keep stable, it may be that bacterium need some
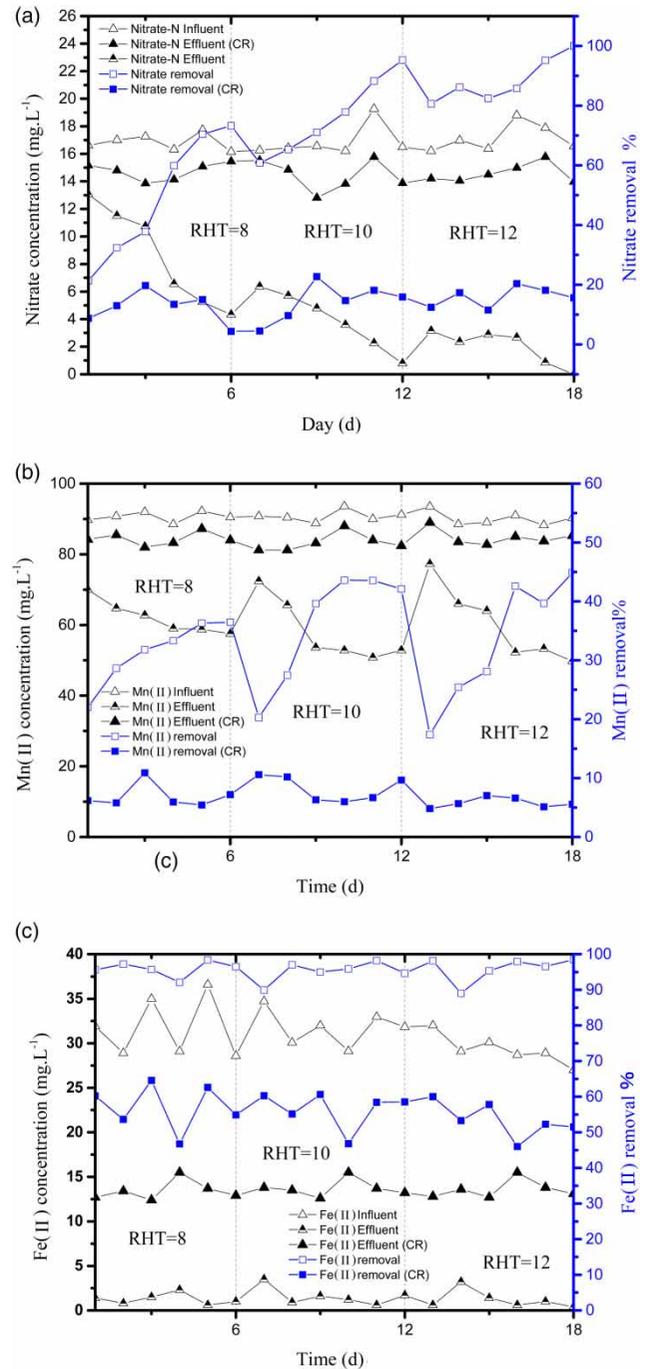


**Figure 4** | Effect of HRT on denitrification processes. Nitrate-N = 16.5 mg/L, Fe(II):Mn(II) = 3:7, electron donor:electron acceptor = 2:1.

time to adapt to the new condition. And in the sixth day, the removal ratio of nitrate and Mn(II) was 93.49% ($1.5 mg \cdot L^{-1} \cdot h^{-1}$) and 42.87% ($1.867 mg \cdot L^{-1} \cdot h^{-1}$), these were higher than other article. The strain reduced 30% of
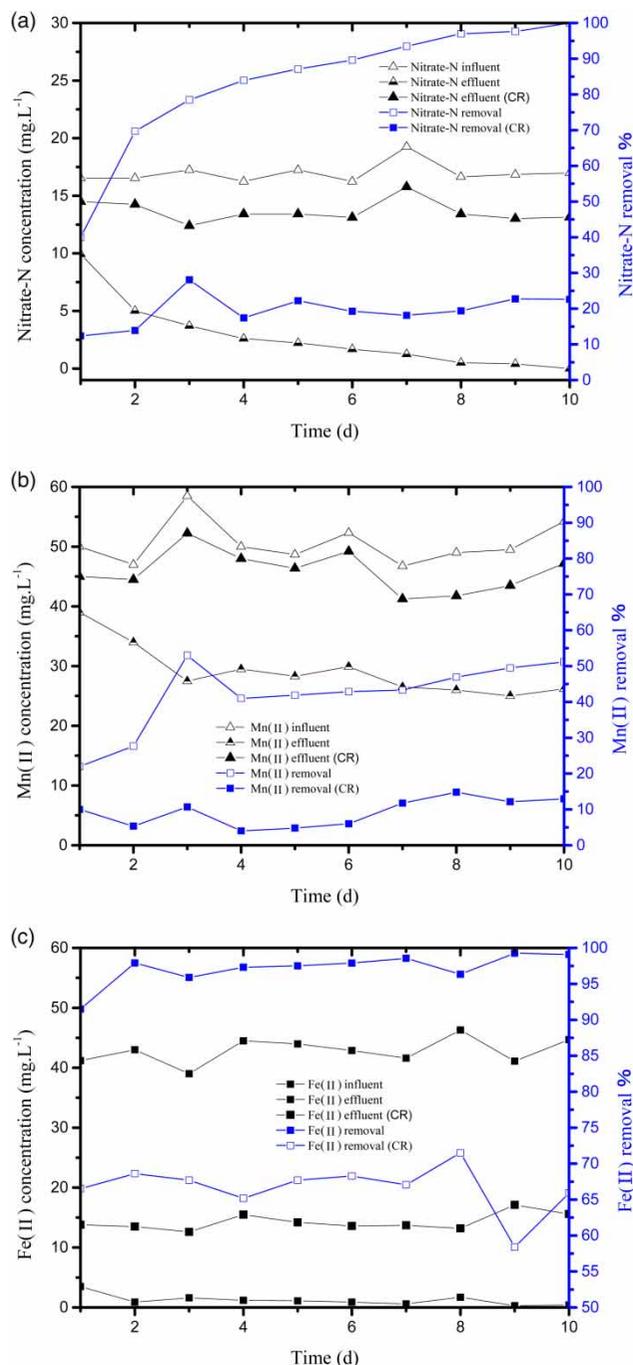
**Figure 5** │ Nitrate-N, Mn(II) as well as Fe(II) variation in an immobilized biological filter when the Fe(II):Mn(II) ratio, HRT and electron donor:electron acceptor ratio were set at the conjectural optimum values.

nitrate and oxidized 85% of Fe(II) over 72 h with an initial Fe(II) concentration of 3.4 mM and nitrate concentration of 9.5 mM (Li *et al.* 2014).

Furthermore, Fe(II) removal ratios of over 90% ($3.14 \ mg \cdot L^{-1} \cdot h^{-1}$) were obtained at the whole experiment. This may be because the Fe(II) was easily used by bacterium, and the Fe(II) oxidation was attributed to the biological role and the chemical role of oxygen.

According to the above, nitrate-N, Mn(II) and Fe(II) removal efficiency was 100% ($1.415 \ mg \cdot L^{-1} \cdot h^{-1}$), 50.25% ($1.708 \ mg \cdot L^{-1} \cdot h^{-1}$) and 99.2% ($3.40 \ mg \cdot L^{-1} \cdot h^{-1}$) under the optimum conditions, respectively. This indicated that the conjectural optimum conditions obtained from the reactor were feasible.

## CONCLUSION

The single factor experiments under different electron donor: electron acceptor ratio and Fe(II):Mn(II) ratio, the results demonstrated that the highest nitrate removal efficiency was occurred under the conditions of electron donor:electron acceptor ratio of 3:1, Fe(II):Mn(II) ratio of 5:5. Different conditions were set for the IBF reactor (HRT (8 h, 10 h and 12 h), electron donor:electron acceptor ratio (1:2, 1.75:1 and 3:1) and Fe(II):Mn(II) ratio (1:9, 3:7, 5:5), through the single factor test conclude the optimal conditions of IBF reactor, the optimal conditions were obtained at electron donor:electron acceptor ratio of 2:1, HRT of 12 h and Fe(II):Mn(II) ratio of 5:5. nitrate-N, Mn(II) and Fe(II) removal efficiency was 100% ($1.415 \ mg \cdot L^{-1} \cdot h^{-1}$), 50.25% ($1.708 \ mg \cdot L^{-1} \cdot h^{-1}$) and 99.2% ($3.40 \ mg \cdot L^{-1} \cdot h^{-1}$), respectively.

## ACKNOWLEDGEMENTS

## REFERENCES

Bai, Y. H., Yang, T. T., Liang, J. S. & Qu, J. H. 2016 The role of biogenic Fe-Mn oxides formed in situ, for arsenic oxidation

and adsorption in aquatic ecosystems. *Water Research* **98** (1), 119–127.

Espejo-Herrera, N., Cantor, K. P., Malats, N., Silverman, D. T., Tardón, A., García-Closas, R., Serra, C., Kogevinas, M. & Villanueva, C. M. 2015 Nitrate in drinking water and bladder cancer risk in Spain. *Environmental Research* **137**, 299–307.

Ghafari, S., Hasan, M. & Aroua, M. K. 2010 A kinetic study of autohydrogenotrophic denitrification at the optimum pH and sodium bicarbonate dose. *Bioresource Technology* **101** (7), 2236–2242.

Hedrich, S., Schlömann, M. & Johnson, D. B. 2011 The iron-oxidizing proteobacteria. *Microbiology* **157** (6), 1551–1564.

Katsoyiannis, I. A. & Zouboulis, A. I. 2004 Biological treatment of Mn(II) and Fe(II) containing groundwater: kinetic considerations and product characterization. *Water Research* **38** (7), 1922–1932.

Kong, Z., Li, L., Feng, C. Q., Dong, S. S. & Chen, N. 2016 Comparative investigation on integrated vertical-flow biofilters applying sulfur-based and pyrite-based autotrophic denitrification for domestic wastewater treatment. *Bioresource Technology* **211**, 125–135.

Lee, K. C. & Rittmann, B. E. 2002 Applying a novel autohydrogenotrophic hollow-fiber membrane biofilm reactor for denitrification of drinking water. *Water Research* **36** (8), 2040–2052.

Li, B. H., Tian, C. Y., Zhang, D. Y. & Pan, X. L. 2013 Anaerobic nitrate-dependent iron (II) oxidation by a novel autotrophic bacterium. Strain PXL1. *Geomicrobiology* **31** (2), 138–144.

Li, B. H., Tian, C. Y., Zhang, D. Y. & Pan, X. L. 2014 Anaerobic nitrate-dependent iron (II) oxidation by a novel autotrophic bacterium, *Citrobacter freundii* strain PXL1. *Geomicrobiology Journal* **31** (2), 138–144.

Liang, J. S., Bai, Y. H., Hu, C. Z. & Qu, J. H. 2015 Cooperative Mn(II) oxidation between two bacterial strains in an aquatic environment. *Water Research* **89**, 252–260.

Liu, H. J., Jiang, W., Wan, D. J. & Qu, J. H. 2009 Study of a combined heterotrophic and sulfur autotrophic denitrification technology for removal of nitrate in water. *Journal of Hazardous Materials* **169**, 23–28.

Mao, Y., Xia, Y. & Zhang, T. 2012 Characterization of *Thauera*-dominated hydrogen-oxidizing autotrophic denitrifying microbial communities by using high-throughput sequencing. *Bioresource Technology* **128**, 703–710.

Moon, H. S., Chang, S. W., Nam, K., Choe, J. & Kim, J. Y. 2006 Effect of reactive media composition and co-contaminants on sulfur-based autotrophic denitrification. *Environmental Pollution* **144** (3), 802–807.

Moraes, B. S. & Foresti, E. 2012 Determination of the intrinsic kinetic parameters of sulfide-oxidizing autotrophic denitrification in differential reactors containing immobilized biomass. *Bioresource Technology* **104**, 250–256.

Park, J. Y. & Yoo, Y. J. 2009 Biological nitrate removal in industrial wastewater treatment: which electron donor we can choose. *Applied Microbiology and Biotechnology* **82** (3), 415–429.

Sahinkaya, E. & Dursun, N. 2012 Sulfur-oxidizing autotrophic and mixotrophic denitrification processes for drinking water treatment: elimination of excess sulfate production and alkalinity requirement. *Chemosphere* **89** (2), 144–149.

Sierra-Alvarez, R., Beristain-Cardoso, R., Salazar, M., Gomez, J., Razo-Flores, E. & Field, J. A. 2007 Chemolithotrophic denitrification with elemental sulfur for groundwater treatment. *Water Research* **41** (6), 1253–1262.

Smith, R. L., Buckwalter, S. P., Repert, D. A. & Miller, D. N. 2005 Small-scale, hydrogenoxidizing-denitrifying bioreactor for treatment of nitrate-contaminated drinking water. *Water Research* **39** (10), 2014–2023.

Su, J. F., Shao, S. C., Huang, T. L., Ma, F., Yang, S. F., Zhou, Z. M. & Zheng, S. C. 2015 Anaerobic nitrate-dependent iron(II) oxidation by a novel autotrophic bacterium, *Pseudomonas* sp. SZF15. *Journal of Environmental Chemical Engineering* **3**, 2187–2193.

Su, J. F., Shi, J. X., Huang, T. L., Ma, F., Lu, J. S. & Yang, S. F. 2016a Effect of nitrate concentration, pH, and hydraulic retention time on autotrophic denitrification efficiency with Fe(II) and Mn(II) as electron donors. *Water Science & Technology* **74** (5), 1185–1192.

Su, J. F., Zheng, S. C., Huang, T. L., Ma, F., Shao, S. C., Yang, S. F. & Zhang, L. N. 2016b Simultaneous removal of Mn(II) and nitrate by the manganese-oxidizing bacterium *Acinetobacter* sp. SZ28 in anaerobic conditions. *Geomicrobiology Journal* **33** (7), 586–591.

Sunger, N. & Bose, P. 2009 Autotrophic denitrification using hydrogen generated from metallic iron corrosion. *Bioresource Technology* **100** (18), 4077–4082.

Zhang, X. X., Li, A., Szewzyk, U. & Ma, F. 2016 Improvement of biological nitrogen removal with nitrate-dependent Fe(II) oxidation bacterium *Aquabacterium parvum*, B6 in an up-flow bioreactor for wastewater treatment. *Bioresource Technology* **219**, 624–631.

Zhang, Y. H., Zhong, F., Xia, S., Wang, X. J. & Li, J. X. 2009 Autohydrogenotrophic denitrification of drinking water using a polyvinyl chloride hollow fiber membrane biofilm reactor. *Journal of Hazardous Materials* **170** (1), 203–209.