JEB
Journal of Environmental Biology

Journal Website : www.jeb.co.in
E-mail : editor@jeb.co.in
ISSN: 0254-8704 (Print)
ISSN: 2394-0379 (Online)
CODEN: JEBIDP

Sulfide-based mixotrophic denitrification for treatment of sulfur, nitrogen and carbon-contaminated wastewater

Li Wei[1], Liang Xiao[2], Liu Lei[1] and Lin Jianguo[1]*

[1]College of Environmental Science and Engineering, Dalian Maritime University, Dalian-116026, China
[2]College of Transportation Equipment and Ocean Engineering, Dalian Maritime University, Dalian-116026, China
*Corresponding Author's Email : weiwei99231@126.com

Publication Info

Paper received:
08 June 2014

Revised received:
30 September 2014

Re-revised received:
01 January 2015

Accepted:
07 February 2015

Abstract

Wastewater contaminated by sulfur, nitrogen and carbon compounds was treated by sulfide-based denitrification process in an expanded granular sludge bed packed with porous sponge. In influent, nitrate and nitrite served as electron acceptors, while sulfide and organic carbon served as electron donors. Both sulfide-based autotrophic denitrification and heterotrophic denitrification were found in the bioreactor. The percentage of heterotrophic denitrification was 36.5%, and the removal rates of sulfide, nitrate, nitrite and organic carbon were 99%, 99%, 95.5% and 80% respectively, which actualized the simultaneous mixotrophic denitrification and desulfurization. The effect factors such as organic carbon category, organic carbon loading rate and nitrite loading rate were also investigated. The 50mgCl^{-1}d^{-1} of glucose and 25mgCl^{-1} d^{-1} acetate sodium were found good for sulfide-based denitrification, while the suitable loading rate of nitrite was 50mgNl^{-1}d^{-1}.

Key words

Denitrification, Desulfurization, Mixotrophic, Nitrite, Organic carbon

Introduction

With rapid development of economy, wastewater emanating from pharmacy, food fermentation, paper pulp and fertilizer industry contain high amount of sulfate, nitrate and organic carbon compounds. Under anaerobic conditions, organic compounds can be removed by reduction of sulfate to sulfide. Sulfide is a major environmental contaminant and its toxicity, corrosiveness and odor causes secondary pollution (Wiessner *et al.*, 2005; Zhang *et al.*, 2013). Meanwhile, nitrate is reduced to nitrite under anaerobic conditions. These nitrogen compounds contribute mainly to eutrophication of water bodies, beside the risks associated with toxicity and bad odors (Baker *et al.*, 1998; Reyes-Avila *et al.*, 2004). In order to remove these contaminants, biological treatments are preferred technologies rather than physical–chemical methods, which are expensive and may generate toxic residuals (Kaksonen *et al.*, 2006; Hossain *et al.*, 2013). The common biotechnologies can offer high removal efficiencies to wastewater containing sulfide or nitrite (Park *et al.*, 2005; Fux *et al.*, 2004). Nevertheless, there are still difficulties on removing sulfide and nitrite simultaneously due to inhibition of microorganisms, representing a tremendous challenge for treatment.

Heterotrophic denitrification process is often used to remove nitrite and nitrate from wastewater, whose stoichiometric reaction between glucose and nitrate is shown by the following reaction given below (Dong *et al.*, 2001).

$$8NO_2^- + C_6H_{12}O_6 \rightarrow 4N_2 + 4H_2O + 4HCO_3^- + 2CO_3^{2-} \quad (1)$$

Heterotrophic denitrification uses several organic compounds as carbon and energy sources. It means that extra organic carbon must be added into wastewater (Zeng *et al.*, 2004). Although this process is a high rate process, it can produce byproducts like nitrous oxide which causes air pollution (Tallec *et al.*, 2006).

Autotrophic denitrification has been found to remove nitrate from wastewater, as nitrate is reduced to nitrogen gas by chemoautotrophic microorganisms under anaerobic conditions

using sulfur as electron donors. Moon *et al.* (2004) showed reduction of nitrate using sulfur as energy source by the following reaction.

$$50NO_3^- + 55S + 20CO_2 + 38H_2O + 4NH_4^+ \rightarrow 25N + 55SO_4^{2-} + 64H^+ + 4C_5H_7O_2N \quad (2)$$

It is often used for removal of nitrate in drinking water or low nutrition wastewater (Hasegawa *et al.*, 2004). Reyes *et al.* (2007) used sulfur/limestone bed to treat underground water. Although the removal rate of nitrate reached 95.8% and no outgrowths were produced, however, much sulfur and sulfate was added in underground water.

Recently, nitrite has been reported to be used in place of nitrate in autotrophic denitrification process (Qaisar *et al.*, 2007). To avoid sulfate secondary pollution, sulfide and nitrate can be degraded to sulfur and nitrogen by autotrophic microorganisms in some processes (Wang *et al.*, 2005). However, the processes mentioned have seldom been used in organic wastewater treatment, because organic carbon is strictly controlled in sulfur-based autotrophic denitrification process.

Autotrophic denitrification and heterotrophic denitrification can be combined together to be called mixotrophic denitrification (Park *et al.*, 2011). Krishnakumar *et al.* (1999) reported that sulfide and nitrate can be degraded by an isolated *Thiobacillus denitrificans* in the presence of organic carbon. Nitrate was reduced to nitrite and accumulated during sulfide oxidized to sulfur and sulfate in almost equal proportions. Sergey *et al.* (2006) reported that anaerobic ammonium oxidation and autotrophic denitrification were combined together for treating baker's yeast effluent, in which nitrate and sulfide were converted to nitrogen and sulfate rather than sulfur.

In view of the above, the objective of the present study was to evaluate simultaneous biological removal of nitrate and sulfide. Few processes have been reported to remove sulfide, nitrate, nitrite and organic carbon simultaneously under well defined denitrifying conditions.

Materials and Methods

Anoxic composite EGSB bioreactor : Anoxic composite expanded granular sludge bed (EGSB) reactor of 3.52 l in column shape was used (Fig. 1). Constant temperature of 30±1°C inside the reactor was maintained by temperature sensor connected with temperature controller (MWZK-02, China) and heating threads bonded around the reactor. One peristaltic pump was used to feed artificial wastewater from the bottom of the reactor into the system and the other peristaltic pump was used to recirculate sludge and water to achieve uniform mixing. On the top of the reactor, a three-phase separator was used to separate biogas, sludge and effluent. Biogas was collected by water sealing tank. In order to increase biomass inside the reactor, sponge cubes (8mm×8mm×8mm) were applied as attached-growth media, which were washed with distilled water for three times before use. The cubes and the active sludge were held up in the reacting area by three porous baffles in order not to block the recirculating pipe. The oxidation-reduction potential (ORP) detector and pH detector were inserted into the reactor to test ORP and pH. The extent of abiotic sulfide oxidation was assessed by feeding the same wastewater into the anoxic reactor with no prior inoculation.

The composite EGSB reactor was inoculated with 1.5l of sludge collected from a secondary sediment tank, used to treat municipal wastewater, giving a biomass concentration of 15.19 g l^{-1}. During start up, the reactor was fed semi-continuously for 150 days. When the removal rates of contaminants reached 90%, the reactor was started to operate under continuous feeding conditions. Ratio of influent sulfide to nitrate was adjusted 1.6-2.5. Thus the influent concentrations of sulfide, nitrate, nitrite, organic carbon (TOC) and inorganic carbon (IC) were 200mgSl^{-1},

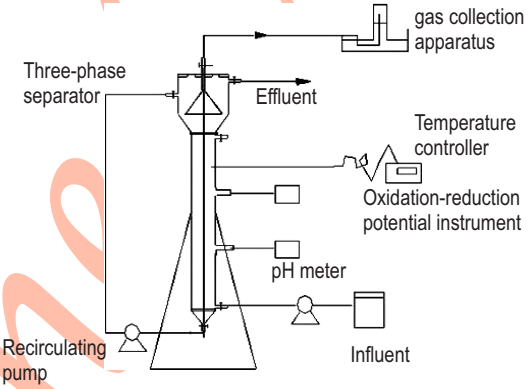

Fig.1 : Diagrammatic representation of anoxic composite EGSB bioreactor

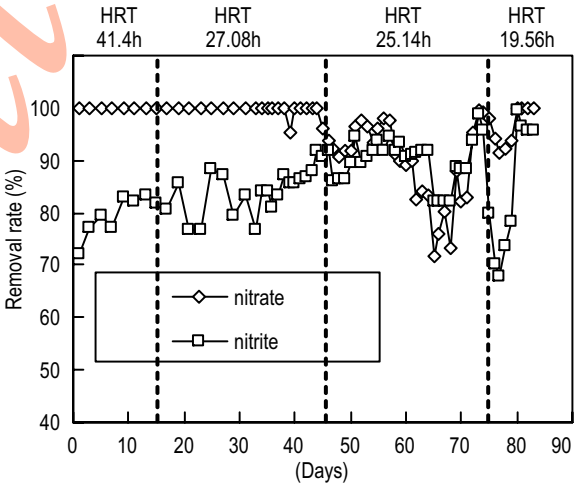

Fig. 2 : Removal rate of nitrite and nitrate

52.5mgNl^{-1}, 20mgNl^{-1}, 20mgCl^{-1} and 113.7mgCl^{-1}, respectively. The hydraulic retention time (HRT) was decreased gradually to increase the loading rates of influent substrates. The effluent was analyzed once in 24 hr.

Batch experiments: Two series of batch experiments were carried out in serum flasks sealed with butyl rubber stoppers. In both series, 10 ml of denitrifying microorganisms from the composite EGSB reactor, operating semi-continuously, for 150 days were added to each flask for a final mixed liquor volatile suspended solid (MLVSS) of 1.5 gd^{-1}. The flasks were kept in shaking bed whose temperature was maintained at 30±0.1°C. The wastewater was let in and out every 24hr in 50 ml and 100ml of flask. Organic carbon source for No. P1-P6 flask was glucose, while for No. C1-C6 flask sodium acetate was carbon. These two groups were used for studying the effects of organic carbon source loading rate and category. The concentration of sulfide, nitrate and inorganic carbon were same in these 12 flasks, which were as follows: 380.86 mgSl^{-1}, 100 mgNl^{-1} and 287mgCl^{-1}, respectively. TOC in these two groups increased gradually, which were 0mgCl^{-1} d^{-1}, 12.5mgCl^{-1}d^{-1}, 25mgCl^{-1}d^{-1}, 37.5mgCl^{-1}d^{-1}, 50mgCl^{-1}d^{-1} and 100mgCl^{-1}d^{-1}. The effect of nitrite loading rate was studied in No.Y1-Y8 flasks. The concentration of influent sulfide and inorganic carbon was maintained at 200mgSl^{-1} and 287mgCl^{-1} in eight flasks, while nitrite loading rate increased gradually from 12.5 mgNl^{-1}d^{-1}, 25 mgNl^{-1}d^{-1}, 50 mgNl^{-1}d^{-1}, 75 mgNl^{-1}d^{-1}, 100 mgNl^{-1} d^{-1}, 150 mgNl^{-1}d^{-1}, 200 mgNl^{-1}d^{-1} to 250 mgNl^{-1}d^{-1}, respectively. Each group mentioned above had two parallel groups to insure the repeatability of data. Meanwhile each group had one blank sample to evaluate the amount of sulfide oxidized by dissolved oxygen in influent.

Substrates : Artificial wastewater containing sodium sulfide as electron donor, potassium nitrate and sodium nitrite as electron acceptors, glucose and sodium acetate as organic carbon sources, sodium bicarbonate as inorganic carbon source and potassium dihydrogen phosphate as phosphorus source for bacteria growth was used as feed in reactor. Wastewater was diluted by tap water to supply other microelements nutrition for microorganisms and pH was adjusted to 7.0 using 1moll^{-1} HCl.

Analytical methods : To measure nitrate, nitrite and sulfate, liquid samples were filtrated through 0.45 mm filter and injected into an ion chromatography (DIONEX ICS 3000, USA) equipped with an inhibitory type conductivity detector and an Ionpac column (AG4A AS4A-SC, 4mm). The flow rate of carrier liquid was 1.0ml in one minute. Sulfide and ammonium were measured by spectrophotometer (UV-2550, Japan). Nitrogen gas was analyzed by gas chromatography (Agilent 4890D, USA), equipped with thermal conductivity detector and molecular screen column (5Å). The temperatures of column, injector and detector were maintained at 60°C, 100°C and 100°C, respectively. TOC and IC content were estimated by TOC analyzing instrument (TOC-V_{CPH}, Japan). MLVSS were analyzed according to the

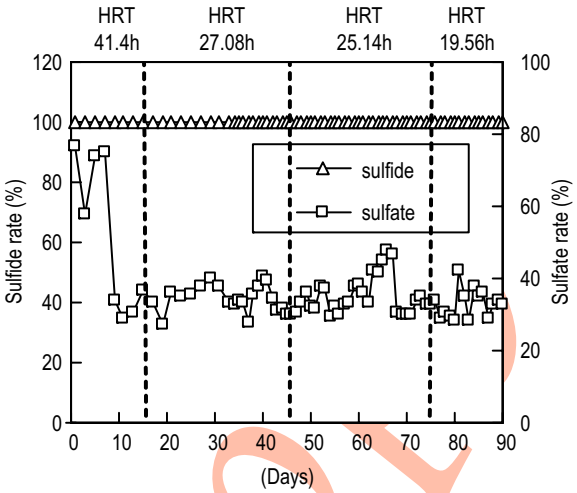

Fig. 3 : Removal rate of sulfide and generated sulfate percent of removal sulfide

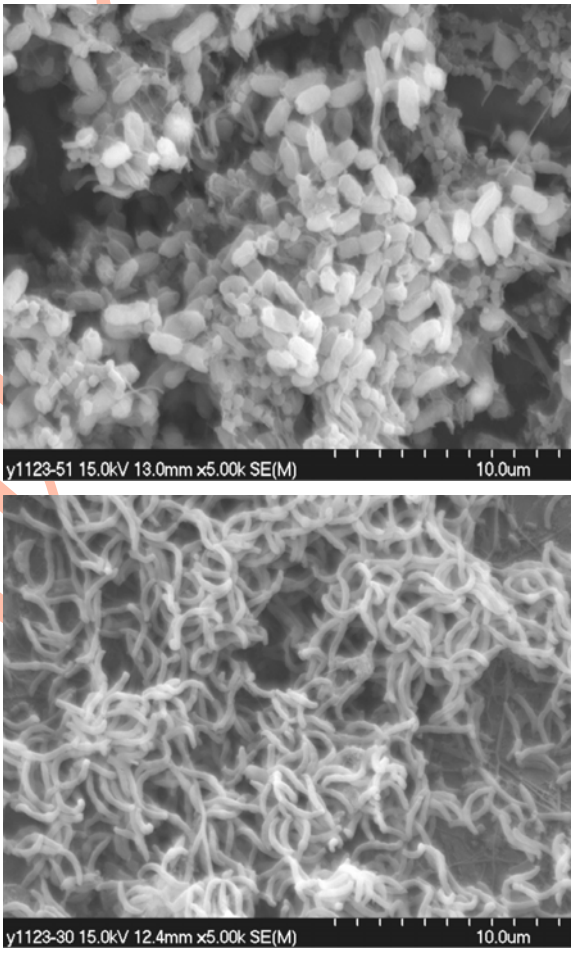

Fig. 4 : Microorganisms attached on sponge cube

standard methods of APHA (2005). ORP and pH were measured by pH meter (pHs-3c, China).

Results and Discussion

The removal rates of nitrate and nitrite are illustrated in Fig. 2. At 41.4hr and 27.08hr HRT, the nitrate removal rate was close to 100%, while nitrite removal rate reached 90.65%. With increasing influent loading rate, the nitrate removal rate decreased and had large fluctuation at HRT 25.14hr, while nitrite loading rate had little variation. On 64th day, the removal rate of nitrate and nitrite decreased with much oxygen leakage into reactor, which destructed the anoxic environment and inhibited denitrification. When HRT decreased to 19.56hr on 73rd day, degradation of nitrate and nitrite sharply degenerated while nitrite removal rate decreased to 68%. Finally, on 83rd day, the removal rates of nitrate and nitrite recovered to 99.98% and 99.55%, respectively.

In anoxic environment, the microbial characteristics has important effect on bioreactor operation (Xu *et al.*, 2014a). Initially, the substrates loading rates were at low level and had little impact on microorganisms. Later, when the substrate loading rates climbed to a higher level, microorganisms in the bioreactor were influenced and their degradation ability decreased, which caused decline of removal rates of nitrate and nitrite. With adaptation of microorganisms on new loading rates, the removal rates of nitrate and nitrite recovered. Comparing the impact from the destruction of anoxic environment, increasing influent loading rates had larger impact on nitrate removal and had less impact on nitrite removal. Adaptabilities of nitrate and nitrite to surging loading rates decreased with increasing influent loading rates. This phenomenon was similar to the results of Xu e*t al.* (2014b).

Degradation of sulfide and generation of sulfate in bioreactor is shown in Fig. 3. The removal rate of sulfide remained high at 99% for different HRTs. Under abiotic conditions, 20% of sulfide disappeared and 0.1% of organic compounds were oxidized. Only 160mgSl^{-1}(200mgSl^{-1}x 0.8) of sulfide was used for denitrification by microorganisms, considering abiotic oxidation. As sulfide was easily oxidized to sulfate, the abiotic formation of sulfate was about 40mgSl^{-1} (200mgSl^{-1}x0.2). Biooxidation of sulfide to sulfur and sulfate in the presence of nitrate and nitrite was concluded to occur through reactions shown below:

$$5S_2^- + 2NO_3^- + 12H^+ \rightarrow 5S + N_2 + 6H_2O \quad (3)$$

$$5S + 6NO_3^- 2H_2O \rightarrow 5SO_4^{2-} + 3N_2 + 4H^+ \quad (4)$$

$$5S_2^- + 8NO_3^- 8H^+ \rightarrow 5SO_4^{2-} + 4N_2 + 4H_2O \quad (5)$$

$$3S_2^- + 2NO_2^- + 8H^+ \rightarrow 3S + N_2 + 4H_2O \quad (6)$$

$$3S + 6NO_2^- \rightarrow 3SO_4^{2-} + 3N_2 \quad (7)$$

$$3S_2^- + 8NO_2^- + 8H^+ \rightarrow 3SO_4 2^- + 4N_2 + 4H_2O \quad (8)$$

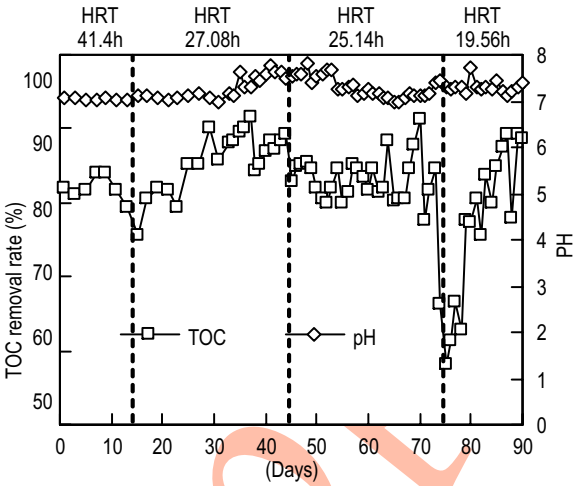

Fig. 5 : Variation of pH and removal rate of organic carbon

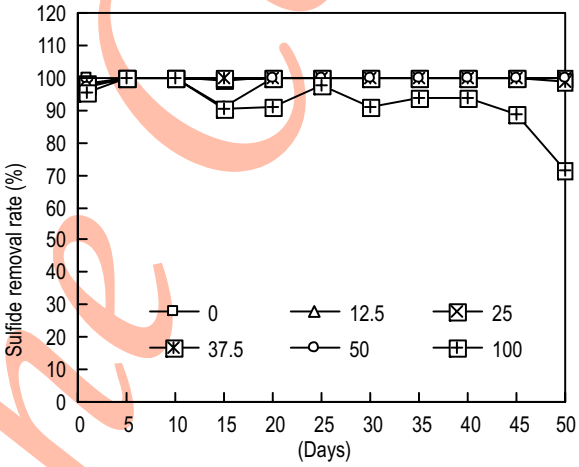

Fig. 6 : Removal rate of sulfide using glucose as carbon source

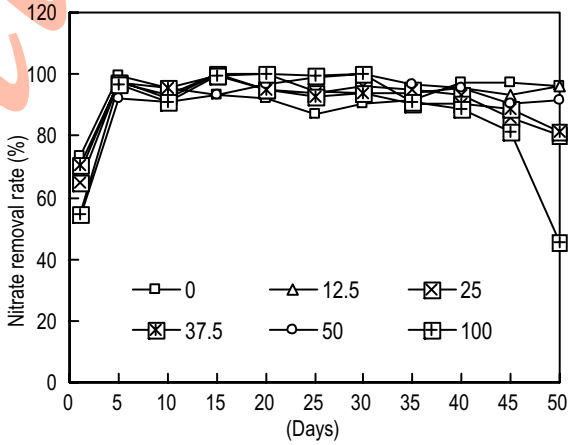

Fig. 7 : Removal rate of nitrate using glucose as carbon source

The stoichiometry of reaction 3 and reaction 6 indicated that 160 mgl^{-1} of sulfide could be converted to sulfur by 35 $mgNl^{-1}$ 0 (160 mgl^{-1}x14/64) of nitrite and nitrate. 140 $mgNl^{-1}$ of nitrite and nitrate was needed for 160 $mgSl^{-1}$ (160 mgl^{-1}x28/32) of sulfide conversion to sulfate via reaction 5 and reaction 8. Observed concentration of nitrogen compounds (NO_3^-, NO_2^-) at various loading rates was about 50 $mgNl^{-1}$, which was closer to theoretical amount for oxidization of sulfide to sulfur than that for oxidization of sulfide to sulfate. In view of abiotic formation of sulfate, the ratio of sulfate produced to removal sulfide was about 40%. Judging by the ratio of sulfate produced to sulfide removed, it was noted that oxidization of sulfide to elemental sulfur predominated the reactor. This novel respiratory process could be explained in terms of microbial diversity present in the reactor, as shown in Fig. 4. Predominant bacteria were found to be *Bacillus* and *Coccus*. Groups of microorganisms were considered to be main reason for simultaneous removal of organic carbon compounds, sulfide, nitrite and nitrate in anoxic bioreactor (Lee and Wong, 2014a).

Fig. 5 illustrates the removal rate of organic carbon and variation of pH in the reactor. The removal rate of organic carbon stabilized at 80%, while fluctuating at 73rd-76th day, was attributed to high influent loading rates strike. In view of influent organic carbon (20$mgCl^{-1}$), the corresponding amount of nitrogen compounds was 24.8$mgNl^{-1}$ (20mgl^{-1}x0.8x14/9) according to reaction 1. The pH of effluent remained 7.4 for hydrogen ion consumption during sulfur formation, according to reaction 3 and reaction 6.

According to the above data, autotrophic denitrification occurred together with heterotrophic denitrification in this EGSB system. The level of heterotrophic denitrification could be indicated by removal rate of organic carbon (Lee and Wong, 2014b). As the total removed nitrogen (NO_3^-, NO_2^-) was about 68 $mgNl^{-1}$, the level of heterotrophic denitrification was only 36.5% (24.8 $mgNl^{-1}$/68 $mgNl^{-1}$). This meant that sulfide-based autotrophic denirification dominated the whole process. And further investigation was suggested to be on influent organic carbon to enhance the level of heterotrophic denitrification.

Degradation of sulfide and nitrate using glucose as carbon source is shown in Fig. 6 and 7. With glucose loading rate increasing stepwise from 0 $mgCl^{-1}d^{-1}$ to 50 $mgCl^{-1}d^{-1}$, the removal rates of sulfide and nitrate remained at high level. However, at 100 $mgCl^{-1}d^{-1}$, the removal rates of substrates decreased to fewer than 70%.

At low TOC load, the process of sulfide-based autotrophic denitrification was not inhibited, while activity of heterotrophic denitrification enhanced with increasing TOC. Some nitrate was converted into nitrogen gas using organic carbon as electron donors (Erkan *et al.*, 2013). However, at high TOC loading rate, the amount of sulfide and nitrate for autotrophic denitrification decreased, as this process was inhibited. As heterotrophic denitrification had less impact on this reactor than autotrophic denitrification, the removal rates of sulfide and nitrate declined

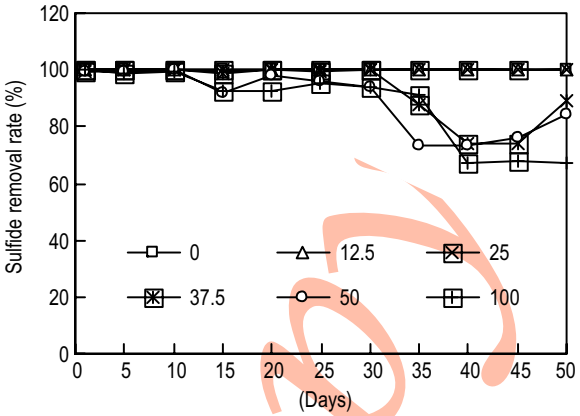

Fig. 8 : Removal rate of sulfide using acetate sodium as carbon source

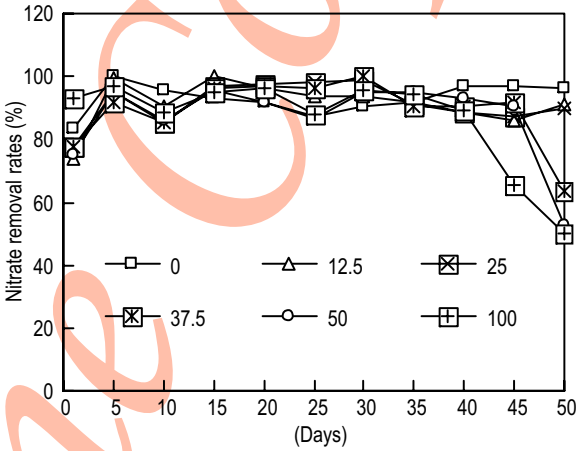

Fig. 9 : Removal rate of nitrate using acetate sodium as carbon source

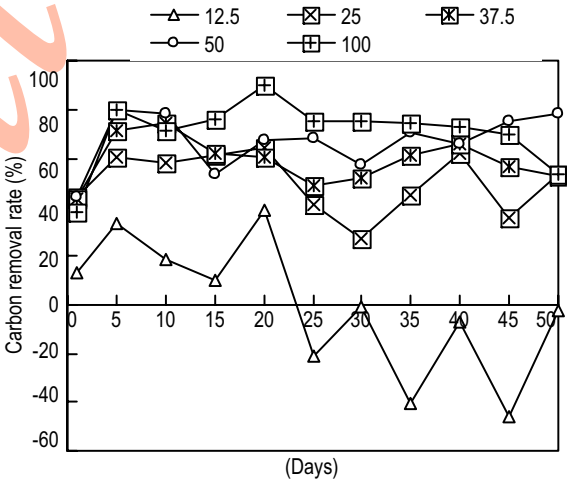

Fig. 10 : Removal rate of TOC using glucose as carbon source

gradually, despite heterotrophic denitrification was good for nitrate removal.

Degradation of sulfide and nitrate using acetate sodium as carbon source is illustrated in Fig. 8 and Fig. 9. The removal rates of sulfide and nitrate reached 95% and 90%, respectively, regarding acetate loading rate at 0 $mgCl^{-1}d^{-1}$-25 $mgCl^{-1}d^{-1}$. With acetate loading rate increasing stepwise from 37.5$mgCl^{-1}d^{-1}$ to 100 $mgCl^{-1}d^{-1}$, degradation of nitrate and sulfide declined for inhibition of high organic carbon on autotrophic denitrification.

The effect of glucose and acetate on TOC removal was compared and illustrated in Fig. 10 and 11. When glucose loading rate was low (12.5$mgCl^{-1}d^{-1}$) TOC was cumulated in flask for low activity of heterotrophic denitrification. At loading rate of 25 $mgCl^{-1}d^{-1}$-100 $mgCl^{-1}d^{-1}$, TOC removal rate increased with increasing activity of heterotrophic denitrification. Taking degradation of sulfide, nitrate and organic carbon all around, mixotrophic denitrification showed best efficiencies at glucose loading rate of 50$mgCl^{-1}d^{-1}$, where removal rates of sulfide, nitrate and organic carbon were capable of reaching 99.99%, 98.87% and 79.76%.

The removal rate of acetate increased with increasing influent loading rate. Nevertheless, the acetate cumulation was less than glucose at low organic loading rate. The removal rates of sulfide, nitrate and organic carbon were 99.98%, 97.94% and 56.16% at TOC loading rate of 25$mgCl^{-1}d^{-1}$. The removal rates of sulfide and nitrate began to decrease when acetate loading rate increased to 25$mgCl^{-1}d^{-1}$. Thus 25$mgCl^{-1}d^{-1}$ was most suitable for denitrification, despite TOC removal rate was not at highest level.

The sulfide removal rate is an important parameter to reflect autotrophic denitrification activity (Guo *et al.*, 2013). Thus, according to different removal rates of substrates at different glucose and acetate loadings, it could be concluded that glucose was suitable for mixotrophic denitrification and had less inhibition on autotrophic denitrification than acetate.

Degradation of sulfide using nitrite as electron acceptors is shown in Fig. 12 and 13. Degradation of sulfide was low at nitrite loading of 12.5$mgNl^{-1}d^{-1}$. From 25$mgNl^{-1}d^{-1}$ to 75$mgNl^{-1}d^{-1}$, the sulfide removal rate reached 99%, while nitrite removal rate increased gradually to 95%. With 100$mgNl^{-1}d^{-1}$ to 250$mgNl^{-1}d^{-1}$, the degradation of nitrite declined. It could be analyzed as follows: at low nitrite loading, sulfide was surplus as compared to nitrite and caused inhibition on microorganisms (Moraes *et al.*, 2012). At medium level of nitrite, sulfide became limited and nitrite degradation enhanced. With nitrite loading rate increasing to high level, microorganisms were inhibited by high nitrite and degradation of nitrite declined.

The ratio of generated sulfate to removed sulfide is illustrated in Fig. 14. According to reaction 6, sulfide was surplus and low sulfate was generated at nitrite loading rate of 12.5 $mgNl^{-1}d^{-1}$. With nitrite loading rate increasing from 25$mgNl^{-1}d^{-1}$ to

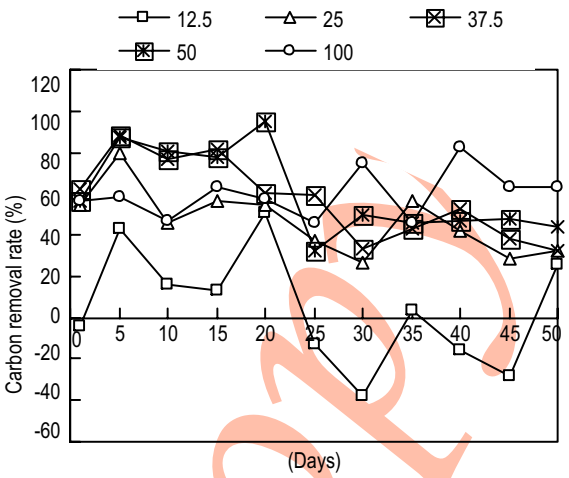

Fig. 11 : Removal rate of TOC using acetate as carbon source

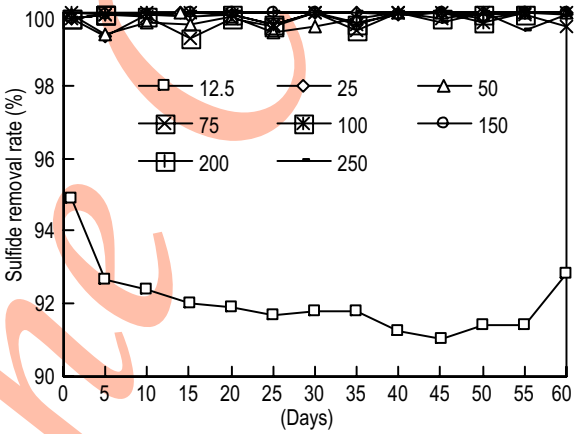

Fig. 12 : Removal rates of sulfide for different nitrite loading rates

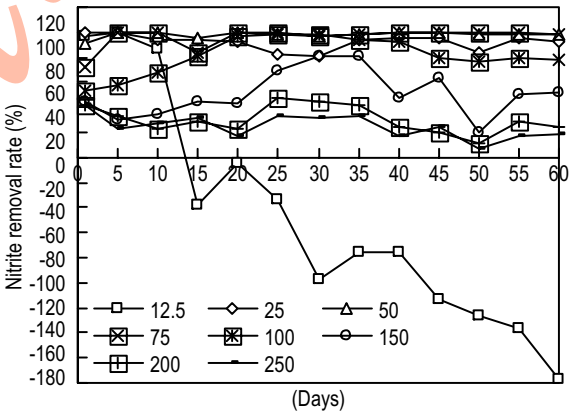

Fig. 13 : : Removal rates of nitrite for different nitrite loading rates

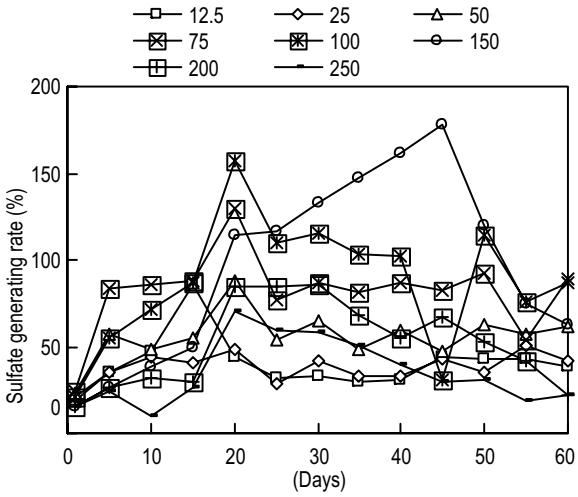

Fig. 14 : Ratio of generated sulfate to removed sulfide for different nitrite loading rates

150mgNl^{-1}d^{-1}, nitrite became surplus and much sulfate cumulated in view of reaction 8. Inhibition on microorganisms due to high nitrite concentration, generation of sulfate decreased for 200mgNl^{-1}d^{-1}-250mgNl^{-1}d^{-1}. The ratio of generated sulfate to removed sulfide was high at 75mgNl^{-1}d^{-1}, which was not suitable for removal of sulfur element, despite sulfide and nitrite removal rates remained high. Hence, nitrite loading rate of 50mgNl^{-1}d^{-1} was good for sulfide and nitrite removal.

In the present study, anoxic composite EGSB reactor simultaneous removed sulfide, nitrate, nitrite and organic carbon by mixotrophic denitrification and desulfurization. Acetate loading rate higher than 37.5mgCl^{-1}d^{-1} was not suitable for mixotrophic denitrification and desulfurization, while glucose loading rate higher than 100mgCl^{-1}d^{-1} was not suitable. Additionally, nitrite loading rate of 50mgNl^{-1}d^{-1} was suitable for mixotrophic denitrification and desulfurization. Enhancement of sulfur production by increasing nitrite and organic carbon loading rate is under investigation.

Acknowledgments

The authors wish to thank the National Natural Science Foundation of China (51208070), China Postdoctoral Science Foundation (2013M530150)), Foundation of Liaoning Education Department (L2014201) and Fundamental Research Funds for the Central Universities of China (3132014102, 3132015083). This work was supported in part by a grant from these funds.

References

APHA: Standard Methods for the Examination of Water and Wastewater. 21st Ed. APHA, AWWA, WPCF, Washington D. C., USA (2005).

Baker, L.A.: Design considerations and application for wetland treatment of high-nitrate waters. *Water Sci. Technol.*, **38**, 389-95 (1998).

Lee, D.U., S. Lee, Y.D. Choi and J.H. Bae: Effects of external carbon source and empty bed contact time on simultaneous heterotrophic and sulfur-utilizing autotrophic denitrification. *Process Biochem.*, **36**, 1215-1224 (2001).

Erkan, S., K. Adem, C. Beste and T. Yasemin: Simultaneous bioreduction of nitrate and chromate using sulfur-based mixotrophic denitrification process. *J. Hazard. Mater.*, **262**, 234-239 (2013).

Fux, C. and H. Siegrist: Nitrogen removal from sludge digester liquids by nitrification/denitrification or partial nitritation/anammox: Environmental and economical considerations. *Water Sci. Technol.*, **50**, 19-26 (2004).

Guo, H., C. Chen, D. Lee, A. Wang and N. Ren: Sulfur-nitrogen-carbon removal of *Pseudomonas* sp. C27 under sulfide stress. *Enzyme Microb. Tech.*, **53**, 6-12 (2013)

Hasegawa K., K. Shimizu and K. Hanaki: Nitrate removal with low N_2O emission by application of sulfur denitrification in actual agricultural field. *Water Sci. Technol.*, **50**, 145-151 (2004).

Hossain, M.M., K. Nakata, T. Kawaguchi and K. Shimazu: Reduction of nitrate on electrochemically pre-reduced tin-modified palladium electrodes. *J. Electroanal. Chem.*, **707**, 59-65 (2013).

Kaksonen, A.H., J.J. Plumb, W.J. Robertson, M. Riekkola-Vanhanen, P.D. Franzmann and J.A. Puhakka: The performance, kinetics and microbiology of sulfidogenic fluidized-bed treatment of acidic metal- and sulfate-containing wastewater. *Hydrometallurgy*, **83**, 204-213 (2006).

Krishnakumar, B. and V.B. Manilal: Bacterial oxidation of sulphide under denitrifying conditions. *Biotechnol. Lett.*, **21**, 437-440 (1999).

Lee, D. and B. Wong: Denitrifying sulfide removal and nitrososulfide complex: *Azoarcus sp.* NSC3 and *Pseudomonas sp.* CRS1 mix. *Bioresource Technol.*, **166**, 616-619 (2014a)

Lee, D. and B. Wong: Denitrifying sulfide removal by enriched microbial consortium: Kinetic diagram. *Bioresource Technol.*, **164**, 386-393 (2014b)

Moon, H.S., K.H. Ahn, S. Lee, K. Nam and J.Y. Kim: Use of autotrophic sulfur-oxidizers to remove nitrate from bank filtrate in a permeable reactive barrier system. *Environ. Pollut.*, **129**, 499-507 (2004).

Moraes, B.S., T.S.O. Souza and E. Foresti: Effect of sulfide concentration on autotrophic denitrification from nitrate and nitrite in vertical fixed-bed reactors. *Process Biochem.*, **47**, 1395-1401 (2012)

Park, D., D.S. Lee, J.Y. Joung and J.M. Park: Comparison of different bioreactor systems for indirect H_2S removal using iron oxidizing bacteria. *Process Biochem.*, **40**, 1461-1467 (2005).

Park, S., J. Yu, I. Byun, S. Cho, T. Park and T. Lee: Microbial community structure and dynamics in a mixotrophic nitrogen removal process using recycled spent caustic under different loading conditions. *Bioresource Technol.*, **102**, 7265-7271 (2011).

Qaisar, M., Z. Ping, C. Jing, W. Donglei, H. Baolan and L. Jinye: Anoxic sulfide biooxidation using nitrite as electron acceptor. *J. Hazard. Mater.*, **147**, 249-256 (2007).

Reyes, S.A., R.B. Cardoso, M. Salazar, J. Gómez, E.R. Flores and J.A. Field: Chemolithotrophic denitrification with elemental sulfur for groundwater treatment. *Water Res.*, **41**, 1253-1262 (2007).

Reyes-Avila, J.S., E. Razo-Flores and L. Gomez: Simultaneous biological removal of nitrogen, carbon and sulfur by denitrification. *Water Res.*, **38**, 3313-3321 (2004).

Sergey, K., G. Marina, M. Arnold and V. Bram: DEAMOX—New biological nitrogen removal process based on anaerobic ammonia oxidation coupled to sulphide-driven conversion of nitrate into nitrite. *Water Res.*, **40**, 3637-3645 (2006).

Tallec, G., J. Garnier and M. Gousailles: Nitrogen removal in a

wastewater treatment plant through biofilters: Nitrous oxide emissions during nitrification and denitrification. *Bioproc. Biosyst. Eng.*, **29**, 323-333 (2006).

Wang, A.J., D.Z. Du, N.Q. Ren, X. Cheng and C.S. Liu: Tentative study on a new way of simultaneous desulfurization and denitrification. *Chin. J. Chem. Eng.*, **13**, 422-425 (2005).

Wiessner, A., U. Kappelmeyer, P. Kuschk and M. Kästner: Sulphate reduction and the removal of carbon and ammonia in a laboratory-scale constructed wetland. *Water Res.*, **39**, 4643–4650 (2005).

Xu, X.J., C. Chen, A.J. Wang, H. Yu, X. Zhou, H.L. Guo, Y. Yuan, D.J. Lee, J. Zhou and N.Q. Ren: Biroreactor performance and functional gene analysis of microbial community in a limited-oxygen fed bioreactor for co-reduction of sulfate and nitrate with high organic input. *J. Hazard. Mater.*, **279**, 250-257 (2014a).

Xu, X.J., C. Chen, A.J. Wang, H.L. Guo, Y. Yuan, D.J. Lee, J. Zhou and N.Q. Ren: Kinetics of nitrate and sulfate removal using a mixed microbial culture with or without limited-oxygen fed. *Appl. Microbiol. Biot.*, **98**, 6115-6124 (2014b).

Zeng, W., Y.Z. Peng and S.Y. Wang: A two-stage SBR process for removal of organic substrate and nitrogen via nitrite-type nitrification-denitrification. *J. Environ. Sci. Hlth. A*, **39**, 2229-2239 (2004).

Zhang, T., C. Wu, Y. Liu, X. Zhou, T. Wang and H. Shi: Study on the influence of oxygen aeration as a means of controlling sulfide accumulation of sewer system. *China Environ. Sci.*, **33**, 1953-1957 (2013).