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Inhibiting sulfate reducing bacteria activity by denitrification in an anaerobic baffled reactor: influencing factors and mechanism analysis

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ABSTRACT

For controlling the equipment corrosion of hydrogen sulfide produced by the microbe in oilfield system, denitrification was used to inhibit the sulfate reduction in a continuous flow anaerobic baffled reactor. The influencing factors and running effects of this process inhibition were investigated. Batch experiments were conducted to study the inhibitory mechanisms. The results indicated that SO_4^{2-}/NO_3^- ratio and relative Chemical Oxygen Demand (COD) content were the two most important environmental factors affecting the inhibitory effect. The inhibitory effect increased with decrease of SO_4^{2-}/NO_3^- ratio. The lower COD content benefited to increase the inhibitory effect. The inhibitor could act only in 1–3 chambers with the effective inhibitory time of 2.3–6.9 h. The inhibitory effect could be reflected by the system oxidation reduction potential (ORP). Denitrification predominated with the ORP in the range of −50 mV to −150 mV, while sulfate reduction predominated with the ORP in the range of −300 mV to −400 mV. Three inhibitory mechanisms were observed in the experiments: competitive inhibition for carbon source, nitrite-N inhibition, and oxidation by autotrophic denitrifying bacteria.

Keywords: ABR continuous-flow experiments; Batch experiments; Denitrification; Inhibition; Sulfate reduction

1. Introduction

Sulfate-reducing bacteria (SRB) are anaerobes which use sulfate ions as the electron acceptor for the metabolism of organic substances [1,2]. Although they have positive effect on treating high-laden sulfate wastewater, they also cause some troubles with regard to oil production [3–5]. Water is commonly used to enhance the oil recovery, but it often associates with the oilfield souring caused by hydrogen sulfide [6]. Hydrogen sulfide is a toxic and corrosive gas responsible for various

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environmental and economic problems, such as odor, reservoir souring, contamination of natural gas and oil, stabilizing undesirable oil-water emulsions, corrosion of metal surfaces, the plugging of reservoirs due to the precipitation of metal sulfides, and the consequent reduction in oil recovery [7]. Hydrogen sulfide is mainly produced by the SRB, which can use various forms of organic matters, including oil component, as the electron donor to reduce sulfate [8]. It also increases the sulfur content in mineral fuel, so it is needed to consider how to control sulfide production from the environmental and economic aspects [9].

Many methods have been used to control sulfide production, and the commonly used one has been adding broad-spectrum biocide, such as glutaraldehyde and cocodiamine [10]. However, they can cause the emergence of biocide-resistant SRB and not readily penetrate into the biofilms within the reservoirs or on the metal surfaces of industrial equipment [11]. Telang et al. have demonstrated that the SRB could resist to the high levels of cocodiamine biocides (500 mg/L) [12]. Moreover, the high concentrations of biocides might cause the equipment corrosion or kill the microorganism that offers protection against corrosion [13,14]. The vast use of biocide might also take new environmental hazard to the groundwater.

Some researchers found that the sulfide production could be controlled by the microbial methods [15]. The denitrification was commonly used to control the SRB activity. Denitrifying bacteria (DNB) could be stimulated by adding the NO_3^- or NO_2^- into the wastewater, and sulfide production could be controlled by the function of competition, symbiosis, and antagonism between SRB and DNB [16]. Many researchers have adopted this method to control the microbial sulfide production [17–20]. The main mechanisms of sulfate reduction inhibited by denitrification were as follows: (1) Competition between SRB and heterotrophic nitrate- or nitrite-reducing bacteria for common organic electron donors might result in the competitive exclusion of SRB [21,22]. (2) Nitrite (or NO, N_2O but not NO_3^-) inhibited the reduction of sulfite to sulfide by the enzyme dissimilatory sulfite reductase, the terminal enzymatic step in the sulfate reduction reaction pathway of SRB. However, many SRB have a nitrite reductase that prevented this inhibition [23,24]. (3) Nitrate- or nitrite-reducing sulfide-oxidizing bacteria (NR-SOB) used NO_3^- or NO_2^- to re-oxidize produced sulfide to elemental sulfur and sulfate, creating a sulfur cycle involving NR-SOB and SRB that resulted in net sulfide removal when insufficient organic electron donors were present to reduce all added NO_3^- [25,26]. (4) Some bacteria (such as *Desulfovibrio desulfuricans* ATCC27774) had the ability of reducing sulfite and NO_3^-, which could firstly use NO_3^- due to the thermodynamic difference, and inhibited the sulfide production [27]. (5) Addition of NO_3^- and middle production of denitrification (nitrite, NO or N_2O) could increase the system's potential. When the potential was above −100 mV, there was no hydrogen sulfide production [28]. Although there has been many studies on using the DNB to inhibit the hydrogen sulfide production, few focused on how the environmental conditions influence the inhibitory effect and how long the inhibitory effect could last.

The aim of this study was to investigate the effect of various factors on the inhibitory effect, to determine the lasting time of the inhibitor and analyze the variation trend in each anaerobic baffled reactor (ABR) chamber through the continuous flow experiment, which would supply the theory basis for the actual application of this technology. In addition, the batch experiments were also conducted to study the inhibitory mechanisms in this process.

2. Materials and methods

2.1. Experimental apparatus

ABR with seven chambers was selected to conduct the continuous flow experiment and to determine the effective inhibiting time, as shown in Fig. 1. The effective cubage of each chamber was 4.4 L, with a total effective volume of 30.8 L. Heating apparatus was used to control the reactor tempeture at 35 ± 1℃. Influent and inhibitor were added by the peristaltic pumps. The influent flux was 46 L/d, with the hydraulic retention time (HRT) of 16.1 h.

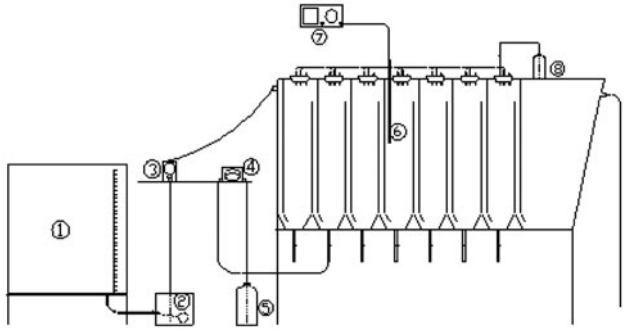

Fig. 1. Schematic diagram of continuous-flow experimental equipment: (1) Water tank, (2) gas-floating water valve, (3) water pump, (4) inhibitor adding pump, (5) inhibitor tank, (6) temperature measuring probe, (7) temperature controller, and (8) gas absorbing bottle.

Batch experiments were conducted in 500 mL culture bottles. The process was as follows: some substrate and pretreated inoculated sludge were added into bottle and diluted to the line of 400 mL with distilled water. Then, the pH of mixed liquor was adjusted to about 7.5 with 0.1 mol/L HCl or NaOH, and the anaerobic condition was obtained by blowing nitrogen gas into the bottle. Finally, the bottle was sealed with rubber stopper and cultured into the shaker (37°C, 150 rpm). Samples were taken and analyzed at 0, 2, 4, 6, 8, 10, 12, 16, 18, 20, 24, 26, 30, 44, and 50 h after the starting of experiment. For ensuring anaerobic condition, injection of 60 mL nitrogen gas into the bottles was needed prior to withdrawing 30 mL of samples for analysis.

2.2. Operational parameters

The inhibiting process could be divided into two consecutive processes: sulfate reduction stage and denitrification inhibition stage. In the former one, environmental conditions were controlled to benefit for SRB growth and make them as the dominant bacteria, with much higher SRB activity. In the latter one, the SRB activity and sulfate reduction level both decreased gradually by adjusting the environmental conditions such as SO_4^{2-}/NO_3^-, pH value, alkalinity, Chemical Oxygen Demand (COD), and inhibitory time, where the ultimate aim was to inhibit the sulfate reduction completely.

Different SO_4^{2-}/NO_3^- ratio were realized by stabilizing the influent sulfate and changing the NO_3^- added, with the value in the range of 6:0–6:6. The pH value was adjusted to 6.5 and 8.0 by adding sodium carbonate and sodium bicarbonate, with alkalinity of 600 and 1,500 mg $CaCO_3$, respectively. COD was adjusted to 1,800 and 600 mg/L by adding the sucrose.

Batch experiments were conducted to study the relationship of denitrification and sulfate reduction at different carbon contents. Experiments were conducted in six culture bottles where six levels of COD were set (120–1,200 mg/L).

2.3. Experimental water and inoculated sludge

The synthetic wastewater was used in the continuous flow experiments, which took sucrose as carbon source (1.8 or 0.6 g/L for the COD of 1,800 or 600 mg/L). About 0.9 g/L of sodium sulfate was added to obtain 600 mg/L SO_4^{2-}, while fertilizer (0.05 g/L) was added as the nitrogen and phosphorus source for microbial growth. Alkalinity was adjusted by adding sodium carbonate and sodium bicarbonate (1.3 g/L $NaHCO_3$ or 1.3 g/L $NaHCO_3$ + 0.4 g/L Na_2CO_3 for alkalinity of 600 or 1,500 mg $CaCO_3$). Wastewater was prepared every two days. $NaNO_3$ was the main component of inhibitor, which also contained 10% $NaNO_2$, 0.4% $Ni(NO_3)_2$, 0.4% $Co(NO_3)_2$, 0.4% $Cu(NO_3)_2$, and 0.2%Na_2MoO_4 in mass percent. It was confected individually according to the experiment.

Inoculated sludge in ABR was taken from the secondary sedimentation tank of oily wastewater treatment plant, which was cultured in static state by synthetic water composed of sucrose and sodium sulfate. Inoculated volume was 1.5 L for each chamber, which produced a MLVSS of about 5,000 mg/L in ABR.

The substrate used in batch experiments contained glucose (with COD of 120–1,200 mg/L), sodium nitrate (with NO_3^- of 200 mg/L), sodium sulfate (with SO_4^{2-} of 135 mg/L), and calcium carbonate (with alkalinity of 500 mg $CaCO_3$/L). Inoculated sludge, which had high sulfate reduction activity after three weeks' acclimation in high sulfate water, was taken from ABR and produced a MLVSS of about 2,000 mg/L in culture bottle. Sludge pretreatment was conducted to eliminate the background value of COD and sulfate, which could be realized by repeatedly centrifugal separation (8,000 rpm, 10 min) and water rinse. It was proved that the background value could be eliminated completely after three times' water rinse.

2.4. Chemical analysis

Samples taken from ABR and intermittent experiment were analyzed for pH value, alkalinity, SO_4^{2-}, S^{2-}, NO_3^-, NO_2^-, and oxidation reduction potential (ORP). The pH value was determined by a pH meter and the alkalinity was measured by the potential titration method according to the APHA standard methods [29]. Sulfate was analyzed by spectrophotometer determination [30]. Sulfide was determined by the methylene blue colorimetric method as described by Wang [30]. Nitrite was determined by the N-(1-naphthyl)-1, 2-diaminoethane dihydrochioride spectrophotometry according to the APHA standard methods [29]. NO_3^- was measured by the NO_3^- ion selection electrode ($pNO_{3\text{-}1}$-01, Shanghai REX Instrument Factory) and ORP was determined by the Pt electrode (LZW5057, HACH).

3. Results and discussion

3.1. Reactor start-up stage

Sludge cultured in static state for 1 month was equally inoculated into each ABR chamber, and syn-

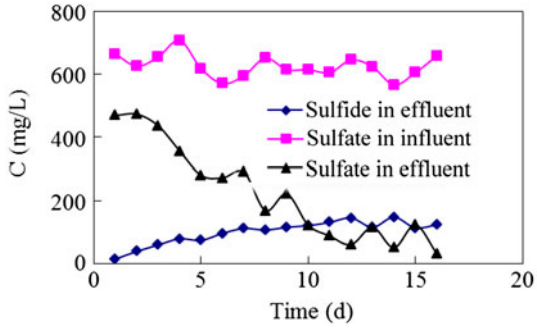

Fig. 2. Concentration profiles of sulfate in influent, and of sulfide and sulfate in effluent at the starting up stage.

thetic water was used to start up the ABR reactor. Fig. 2 shows the gradual change of sulfate and sulfide in start-up stage. After 12 days, sulfate removal efficiency reached above 80%, with the highest value of 95%, and sulfide concentration reached above 110 mg/L, which indicated the successful start-up of sulfate reduction. In the start-up stage, the other indices' changes were as follows: influent pH fluctuated in 6.15–8.93, effluent pH stabilized at 6.21–6.99, influent alkalinity was at 465–880 mg/L, effluent alkalinity was at 1,100–1,400 mg/L, ORP gradually decreased from −268 mV, and stabilized at −330–350 mV. Moreover, the sulfur content in influent was larger than the sum of sulfate and sulfide in effluent, which might be caused by three reasons. First, some sulfur was released into the air in the form of hydrogen sulfide, which was confirmed by the rotten egg stink in the lab. Second, some sulfur was produced in the ABR, which was not analyzed in this study. Finally, the sludge in the reactor might have adsorbed some sulfate or sulfide.

The quick start-up of sulfate reduction was correlated to the static state culture, which made SRB at the predominant status in one month's high-level sulfate condition. Therefore, the reactor had higher sulfate reduction ability, which provided basis for the denitrification inhibition.

3.2. Influence of environmental conditions

3.2.1. Effect of SO_4^{2-}/NO_3^- ratio

Fig. 3(a) shows the change of sulfide in each chamber at different SO_4^{2-}/NO_3^- ratios. With increasing chamber number, sulfide concentration all increased at each SO_4^{2-}/NO_3^- ratios, which indicated that SRB activity increased with chamber number. However, sulfide concentration at low SO_4^{2-}/NO_3^- ratios (6:3–6:6) was generally lower than the high ones (6:0–6:2), and

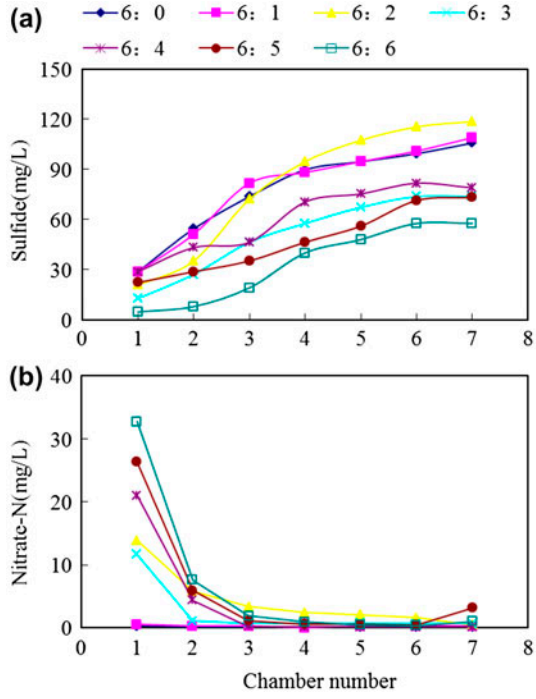

Fig. 3. Concentration profiles for S^{2-} (a) and NO_3^-–N (b) in different chamber at each SO_4^{2-}/NO_3^- ratio. (Influent COD and SO_4^{2-} concentrations were kept constant at 1,800 and 600 mg/L, respectively. NO_3^- was changed in influent to get each SO_4^{2-}/NO_3^- ratio. Samples were taken at steady period of each SO_4^{2-}/NO_3^- ratio.)

the highest reduction could reach about 50%, which indicated that denitrification could effectively inhibit sulfate reduction.

Changes of NO_3^-–N in Fig. 3(b) could reflect the reason of sulfide level increase. NO_3^-–N in each chamber decreased quickly, and reached very low at the third chamber, which led to NO_3^-–N in the latter chambers to decrease to almost zero. Denitrifying activity in the former two chambers was very high, which resulted in the relative insufficiency of NO_3^-–N in the latter chambers and decreased the denitrifying activity.

Fig. 4 shows sulfide changes in each chamber at different SO_4^{2-}/NO_3^- ratios with inhibitor added into the second chamber. The sulfide change had a similar rule at different SO_4^{2-}/NO_3^- ratios. It decreased at certain range after adding the inhibitor, and then increased gradually, which indicated that inhibitor was effective in a definite section. When the inhibitor was exhausted, sulfate reduction took up the dominant status again. Compared with sulfide changes at SO_4^{2-}/NO_3^- ratio of 6:5 and 6:6, the lowest sulfide in the former one presented in the fourth chamber, while

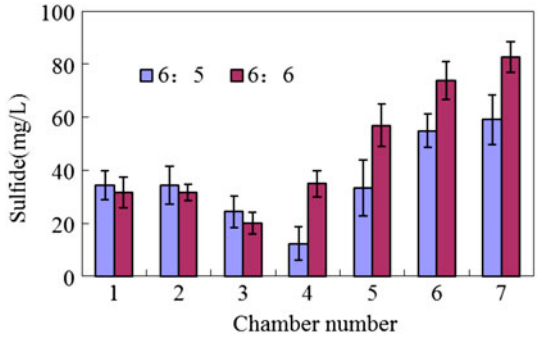

Fig. 4. Concentration profiles for sulfide at different SO_4^{2-}/NO_3^- ratio. (Operation parameters were same as Fig. 3. Inhibitor was added from the second chamber, and n referred to the testing times of water quality indices at the stable stage of reactor. $n = 5$ and 4.)

the latter one presented in the third chamber and also had a much higher subsequent increasing degree. Higher inhibitor content stimulated the higher denitrifying activity and was also quickly exhausted, which led to the decreasing of the inhibitory time, and subsequent resuming of the SRB activity rapidly.

3.2.2. Effect of pH value and alkalinity

Both SRB and DNB could grow in pH range of 6.0–8.0, but their activities were different at different pH values. The changes of sulfide and nitrite-N in different chamber at two different pH value and alkalinity levels are shown in Fig. 5.

When inhibitor was added from the second chamber, sulfide in the third chamber decreased from about 20 mg/L (ALK1) to below 10 mg/L (ALK2), which indicated that ALK2 was benefited for the denitrification. Sulfide increased in the subsequent chamber with two alkalinity levels, but increased much quickly at ALK2. Sulfide increased from 70–90 to 100–120 mg/L in the seventh chamber, which indicated that the sulfate reducing activity was much higher at ALK1 after the inhibitor was exhausted. The higher alkalinity and pH value could neutralize the acid produced in the hydrolization and the acidification process of sucrose, and provided a better growth conditions for SRB.

Nitrite-N accumulated in the denitrifying process at both alkalinity levels. The highest concentrations both appeared in the third chamber, but it decreased quickly almost to zero in the fourth chamber. The maximal Nitrite-N concentration was in the range of 18–24 mg/L at AKL1, while it was below 7 mg/L at ALK2, which indicated that denitrification was much slower at ALK1. The change in the curve for sulfide

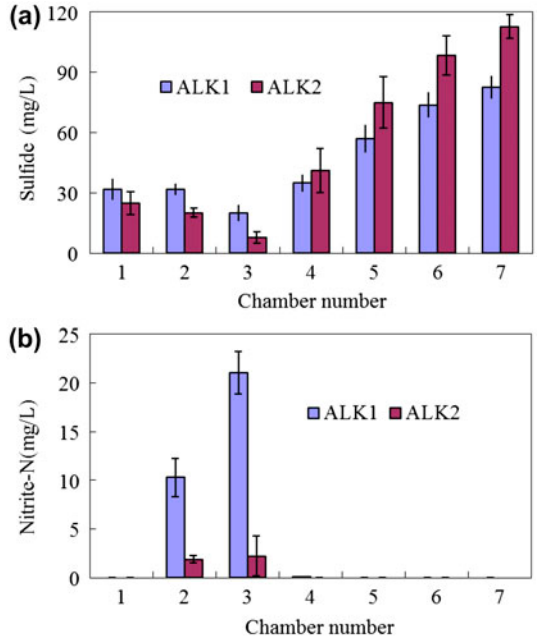

Fig. 5. Concentration profiles for S^{2-} (a) and NO_3^-–N (b) in different chamber at different alkalinity (Influent COD and SO_4^{2-} concentrations were kept constant at 1,800 and 600 mg/L, respectively, with the SO_4^{2-}/NO_3^- ratio around 6:6. Inhibitor was added from the second chamber; ALK1 was at an average of 600 mg$CaCO_3$/L with pH around 6.5, while ALK2 was at an average of 1,500 mg$CaCO_3$/L, with pH around 8.0. $n = 4$ and 7.)

and nitrite-N at two alkalinity levels were just opposite. Sulfide concentration was the lowest in the third chamber, while nitrite-N concentration was the highest, which indicated that sulfide production might be restricted by the nitrite-N.

3.2.3. Effect of carbon source content

Carbon source, used as electron donors for microorganism to conduct sulfate reduction and denitrification, is a crucial environmental factor for inhibiting sulfate reduction activity. Fig. 6 shows the change in sulfide at different carbon source content when inhibitor was added from multiple points. The sulfide both decreased after adding inhibitor, but was at different degree. When carbon source was surplus, the sulfide increased rapidly after the second point inhibition, and reached 60–80 mg/L in the seventh chamber. By comparison, when carbon source was insufficient, sulfide could not be detected in the latter chambers. When carbon source was low, COD/SO_4^{2-} (or COD/NO_3^-) decreased and made electron donors insufficient; when NO_3^- existed in the system, DNB and SRB competed for electron donors. However, NO_3^-

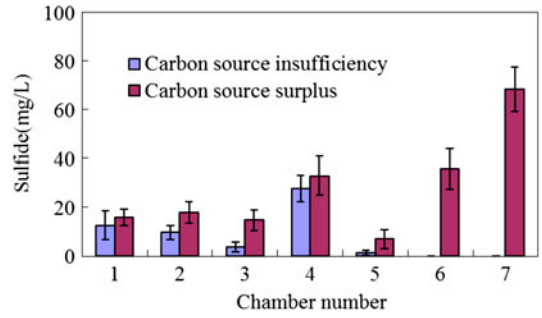

Fig. 6. Effect of carbon source content on the sulfide in different chamber (Influent pH was adjusted to 8.0, while alkalinity and sulfate in influent were kept constant at 1,500 mg$CaCO_3$/L and 600 mg/L, respectively. SO_4^{2-}/NO_3^- ratio was kept constant at 6:6. COD was adjusted to 1,800 and 600 mg/L. Inhibitor was added from the second and fifth chambers. $n = 6$ and 9.).

reduction took precedence over sulfate reduction in the anaerobic microbial treatment. Therefore, the sulfide production was inhibited. Although electron donor insufficiency could both restrict the activity of DNB and SRB, the effect on SRB was much greater.

The change in NO_3^-–N and NO_2^-–N at carbon source insufficiency is shown in Fig. 7. NO_3^-–N decreased rapidly at the first point inhibition, and DNB activity was very high. By comparison, the NO_3^-–N decreased slowly after the second point inhibition and much more nitrite-N was accumulated in the system, which indicated that carbon source was insufficient and DNB activity was low. In addition, the higher content of nitrite-N might contribute to the inhibition of SRB activity.

3.2.4. Effect of inhibitory time

When the inhibitor was added from the influent, NO_3^- could not effectively act on the whole system, so the effective action time was to be considered. ABR was adopted in this experiment, which was plug flowed from influent to effluent. It was independent between each chamber, and the HRT of each chamber was 2.3 h. The different adding position implied different inhibitory time. Fig. 8 presents the changes of sulfide in each chamber when the inhibitor was added from the single second chamber or from both the second and fifth chambers. When inhibitor was added from the second and fifth chambers, sulfide content decreased rapidly, and the lowest concentration appeared in the third and fifth chambers. Compared with the single-point inhibiting condition, sulfide decreased in a greater degree under multiple-points inhibiting condition. Sulfide in the seventh chamber decreased from 100–120 mg/L at single-point inhibition to 60–80 mg/L at multiple-points inhibition with an average decrease of 36.4%.

It could also be concluded that the inhibitor could only act in 1–3 chambers (in Fig. 6), with the effective action time of 2.3–6.9 h, which was related to the sludge activity and the adjustment of operational parameters.

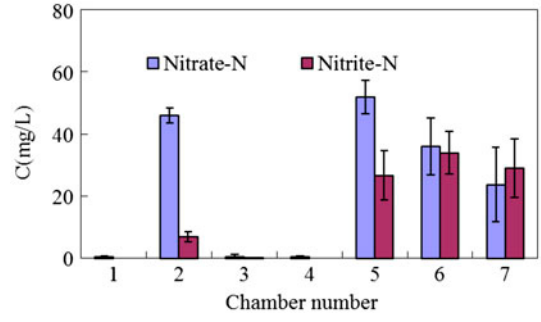

Fig. 7. Concentration profiles for NO_3^-–N and nitrite-N at two points adding inhibitor (Influent COD was kept constant at 600 mg/L and other parameters were the same as Fig. 5. Inhibitor was added from the second and fifth chambers. $n = 9$.)

3.3. Relation of effluent ORP and inhibitory effect

Variation of effluent ORP and sulfide with time is shown in Fig. 9. In the day of 0–113 d, the effluent sulfide content was very high, and the effluent ORP was between −300 mV and −400 mV. Judged from the ORP value, the system belonged to sulfate reduction stage [31]. The denitrification did not inhibit the sulfate reduction effectively (or did not inhibit the SRB activity). In the day of 114–120 d, the effluent ORP

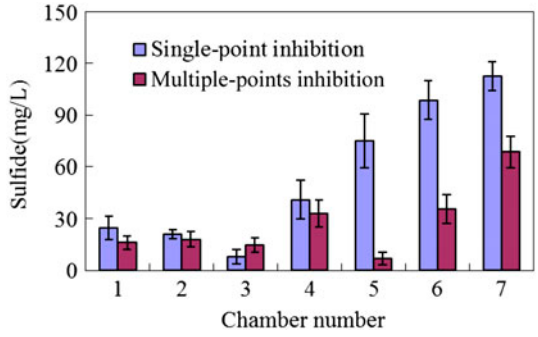

Fig. 8. Concentration profiles for sulfide at different inhibitor adding location (Influent pH was adjusted to 8.0, while alkalinity, sulfate, and COD in influent were kept constant at 1,500 mg$CaCO_3$/L, 600 and 1,800 mg/L, respectively. SO_4^{2-}/NO_3^- ratio was kept constant at 6:6. Single-point inhibitor was added from second chamber, while multi-point inhibitor was added from second and fifth chambers. $n = 7$ and 9.).

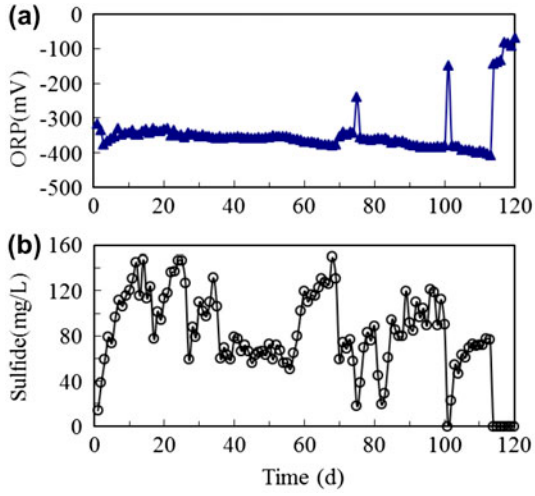

Fig. 9. Changes of sulfide and ORP with time in the effluent of ABR continuous-flow experiment.

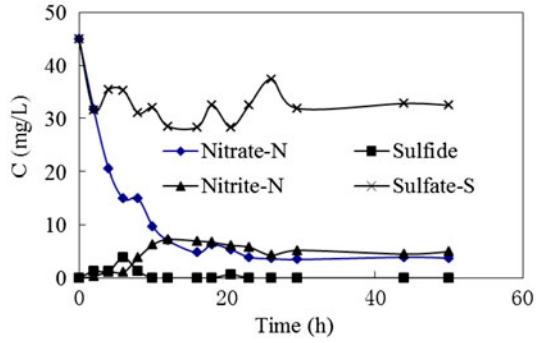

Fig. 10. Various indices changed with time in the reaction system (Initial pH was adjusted to 7.5, with ALK of 500 mg$CaCO_3$/L. SO_4^{2-} and NO_3^- concentrations were added at 135 and 200 mg/L, respectively, with SO_4^{2-}-S/NO_3^-–N ratio around 1:1. Glucose was used as carbon source, with COD of 120 mg/L.)

was at −50 mV to −150 mV, which was the ORP section of the microbial denitrification and mainly conducted denitrification. At this stage, sulfate reduction was inhibited effectively and sulfide in the effluent was below the detection limit. The inhibitory effect could be reflected by the effluent ORP. When ORP was at −50 mV to −150 mV, denitrification predominated; but when ORP was at −300 mV to −400 mV, sulfate reduction predominated.

3.4. Mechanism analysis

Three inhibitory mechanisms: competitive inhibition for carbon source, nitrite-N inhibition, and oxidation by autotrophic DNB, were observed in the batch experiments.

3.4.1. Competitive inhibition of DNB and SRB on carbon source

Growth conditions of DNB and SRB were very similar. When they existed in the same environment, compared with sulfate reduction, denitrification predominated both on the thermodynamics and kinetics aspects. Therefore, DNB was prior to use the substrate, and the effect was quite obvious with the carbon source insufficiency.

Fig. 10 presents the water quality index variation with time in the mixed system at the COD of 120 mg/L. NO_3^-–N decreased obviously, with some NO_2^-–N accumulation. However, denitrification was not conducted completely. NO_3^-–N and NO_2^-–N stabilized at about 4 and 5 mg/L, respectively, which indicated that the carbon source was seriously insufficient. On the contrary, sulfate reduction was almost on the stagnant state. Only a little amount of sulfide was produced in the initial state, and then it disappeared quickly. Sulfide was not detected in the system until the completion of reaction, and sulfate stayed at the stabilized state all the time, which indicated that SRB activity was very low. However, DNB had a better activity, which proved that DNB had a stronger competitive ability at the carbon source insufficiency.

3.4.2. Oxidation of autotrophic nitrate reducing bacteria

Autotrophic nitrate reducing bacteria (ANRB) are the kinds of DNB that could use inorganic substance as electron donors to reduce NO_3^- into nitrogen gas. Some ANRB, such as *Thiobacillus denitrificans* and sulfide-oxidizing bacteria, could use sulfide as electron donors and reduce NO_3^-, the reaction equations are as follows [32]:

$$2NO_3^- + 5S^{2-} + 6H_2O \rightarrow 5S^0 + N_2 + 12OH^- \quad \Delta G^{0\prime} = -1,168.4\ \text{KJ} \tag{1}$$

$$1.25S^{2-} + 2NO_3^- + 2H^+ \rightarrow 1.25SO_4^{2-} + N_2 \uparrow + H_2O \quad \Delta G^{0\prime} = -972.8\ \text{KJ} \tag{2}$$

Both the above two reactions are exothermic, and could be conducted spontaneously. However, it demanded that the system contains little organic matter content and abundant inorganic carbon source as energy source.

Fig. 11 presents the sulfide concentrations with time at different COD conditions. The sulfide concen-

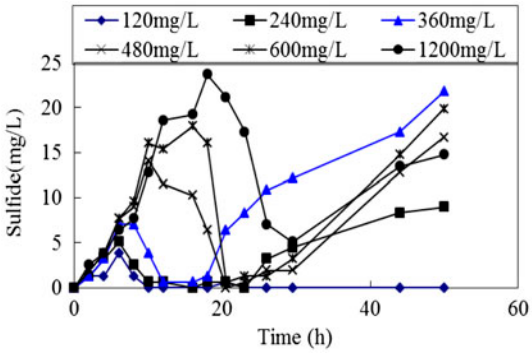

Fig. 11. Concentration profiles for sulfide with time at different carbon source content. (Initial conditions were the same as Fig. 10, and carbon source content indicated with COD concentration.)

trations firstly increased to peak value, then decreased to a much lower value, and finally reincreased (except COD = 120 mg/L). Moreover, with COD content increasing, the peak value increased gradually, and peak occurrence time also postponed. This process could be divided into three stages: carbon source abundance stage, denitrifying inhibition stage, and sulfate reduction stage. In the first stage, SRB and DNB could grow simultaneously due to the initial surplus COD as carbon source, where sulfide was accumulated in the system indicating that SRB was not inhibited by DNB. The duration time of this stage was related to the COD content. In addition, the accumulated sulfide in this stage also might be the result of discharging the metabolized production of residual sulfate inside the microbial cells, because the sludge pretreatment only removed the sulfate outside the cells. In the second stage, with the consumption of organic carbon source, denitrification predominated gradually. Both heterotrophic and autotrophic denitrification bacteria were active in the system. Therefore, it could not only inhibit the SRB activity by competing organic carbon source, but also could consume the accumulated sulfide by using as substrate or conducting chemical reaction. In the final stage, due to the completion of denitrification, the inhibition disappeared and the SRB activity restarted. It began to reduce sulfate again, and sulfide accumulated in the system once more.

3.4.3. The middle production inhibition of denitrification

Many researchers had proven that the intermediate products of denitrification, such as NO_2^-, NO, and N_2O, had an obvious inhibitory effect on the microbial activity [23,24]. According to the experimental phenomena, the effect of nitrite on the SRB activity was studied.

Fig. 12 shows the relation of sulfide and nitrite-N at different COD content. The variation trends were just opposite at any COD condition. When

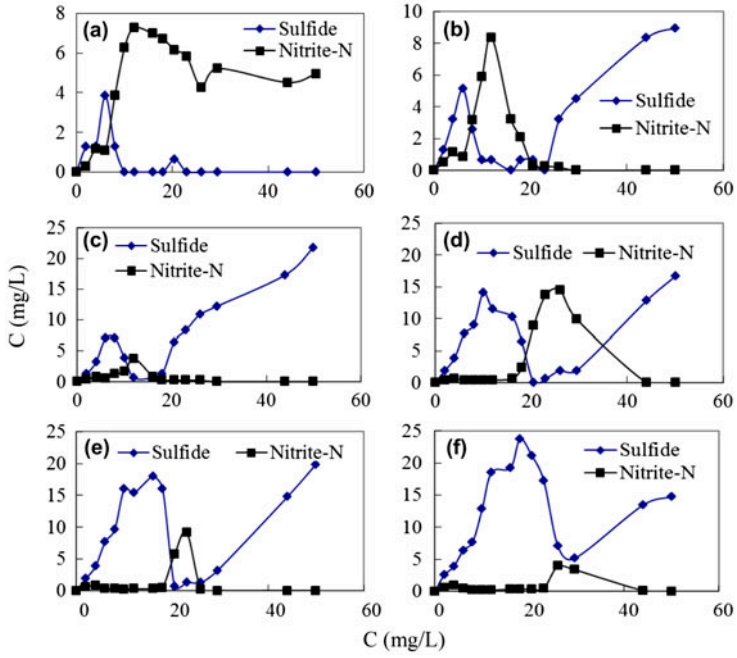

Fig. 12. Relation of nitrite-N and sulfide at different carbon source content (Initial conditions were same as Fig. 10 except COD. COD of graph A–F was adjusted to 120, 240, 360, 480, 600, and 1,200 mg/L, respectively.)

nitrite-N reached a constant value, sulfide concentrations started to decrease and SRB activity was inhibited. With the consumption of nitrite-N in the denitrification process, sulfide started to increase and SRB activity resumed, which indicated that the existence of nitrite-N inhibited the SRB activity, and the inhibitory effect disappeared with the nitrite-N consumption. In addition, when the nitrite-N concentration was above about 2 mg/L, the sulfide production presented a decreasing trend and SRB activity was inhibited. Myhr et al. found that 0.7/0.98 mg/L nitrite-N could partially and completely inhibit the dominant bacteria (*Desulfomicrobium hypogeium*, S2552) in the batch experimental system; however, 1.68 mg/L nitrite-N could completely inhibit the production of 14.4 mg/L sulfide in the continuous column [20]. In the study of Reinsel et al., in situ production of 7.98 mg/L NO_2^-–N from NO_3^- or external addition of 12.04 mg/L NO_2^-–N could inhibit H_2S production [28]. The inhibitory concentration of nitrite-N on SRB activity was shown to be different in each research, which was related to the SRB species, sludge source, inoculated amount, environmental conditions, and sulfide concentration.

4. Conclusions

According to the above analysis, the following conclusions could be obtained:

(1) SO_4^{2-}/NO_3^- ratio and COD relative content were the two most important environmental factors observed in the process of denitrifying inhibition. With the decreasing of SO_4^{2-}/NO_3^- ratio, inhibitory effect increased. The lower COD content was beneficial to improve the inhibitory effect.
(2) The inhibitor could act only in 1–3 chambers and the effective inhibitory time was 2.3–6.9 h, which was related to the sludge activity in the reactor and adjustment of the operational parameters.
(3) The condition of pH value 8.0 and alkalinity 2,500 mg/L had negative effect on the denitrifying inhibition, but the affecting extent was not very significant.
(4) The system ORP could reflect the effect of denitrifying inhibition. When ORP was at −50 mV to −150 mV, denitrification predominated; but when ORP was at −300 mV to −400 mV, sulfate reduction predominated.
(5) Three inhibitory mechanisms were observed in the study: competitive inhibition for carbon source, nitrite-N inhibition, and oxidation by autotrophic DNB.

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