

# The optimal ecological factors and the denitrification population of a denitrifying process for sulfate reducing bacteria inhibition

Chunying Li

School of Energy and Civil Engineering, Harbin University of Commerce, Harbin 150028, China.

heart.li@163.com

**Abstract.** SRB have great negative impacts on the oil production in Daqing Oil field. A continuous-flow anaerobic baffled reactors (ABR) are applied to investigate the feasibility and optimal ecological factors for the inhibition of SRB by denitrifying bacteria (DNB). The results showed that the  $\text{SO}_4^{2-}$  to  $\text{NO}_3^-$  concentration ratio ( $\text{SO}_4^{2-}/\text{NO}_3^-$ ) are the most important ecological factor. The input of  $\text{NO}_3^-$  and lower COD can enhance the inhibition of  $\text{S}_2$ -production effectively. The effective time of sulfate reduction is 6 h. Complete inhibition of SRB is obtained when the influent COD concentration is 600 mg/L, the  $\text{SO}_4^{2-}/\text{NO}_3^-$  is 1/1 (600 mg/L for each), N is added simultaneously in the 2# and the 5# ABR chambers. By extracting the total DNA of wastewater from the effective chamber, 16S rDNA clones of a bacterium had been constructed. It is showed that the Proteobacteria accounted for eighty-four percent of the total clones. The dominant species was the Neisseria. Sixteen percent of the total clones were the Bacilli of Firmicutes. It indicated that DNB was effective and feasible for SRB inhibition.

## 1. Introduction

Sulfate reducing bacteria (SRB) is the sum of bacteria with sulfate reducing capability [1, 2]. There are about 9 categories and 200 species of SRB [3, 4]. The excessive reproduction of SRB in the oilfield would lead to lots of problems [5]. The abundance of SRB had led to the increase of sulfide and suspended solid concentration, collapse caused by electric field, and other serious production accidents in Daqing Oilfield. Lots of chemicals were added to inhibit the growth of SRB [6], which caused serious impacts and damages on the oilfield production and the ambient environment. Recently, researches on the application of microbial control methods for SRB inhibition are increasing [7]. These methods are preferred as they are safe, environmental friendly and cost-effective. Ecological control of SRB is achieved based on the theories of microbial ecology. Lots of researches have discussed denitrifying process mechanism of SRB inhibition. Five major mechanisms are listed. The first was competitive inhibition of substrate. Ricardo et al. [8] had pointed out that the DNB outcompeted the SRB when acetic acid was utilized as the substrate. The energy released by the nitrate reduction is higher than that of the sulfate reduction. Also, SRB required lower oxidation-reduction potential (ORP) (-100mV) than DNB (+100mV), the reduction of nitrate is prior to sulfate reduction. The second one is the inhibition by the intermediate metabolites of denitrifying reaction. Taylor et al. [9] indicated that the inhibition effects of  $\text{NO}_2^-$  and the intermediate metabolites of denitrifying reaction (NO and  $\text{N}_2\text{O}$ ) were more effective for SRB inhibition than nitrate. Sodium nitrate and ferric



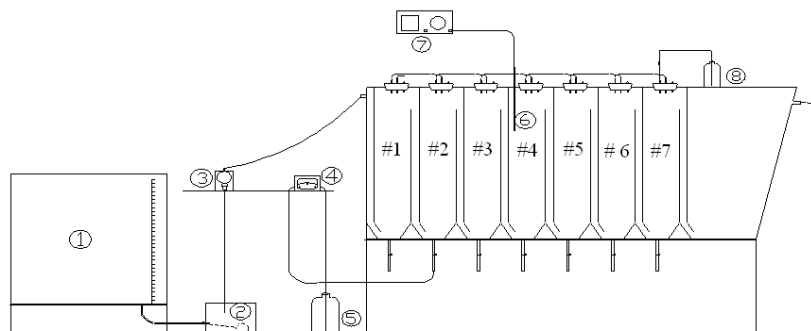
nitrate are utilized to control the production of hydrogen sulfide by inhibiting the activities of SRB [10]. It is considered that the inhibition is mainly caused by the intermediate metabolites of denitrifying reactions. The inhibition performance of ferric nitrate is more effective, if the transmission of substrate is restrained by the formulated ferrous sulfide. The third mechanism is the presence of salts with similar structure to  $\text{SO}_4^{2-}$ . Study conducted by Nemati et al. [11] indicted that salts with the similar structure to  $\text{SO}_4^{2-}$ . For example,  $\text{CrO}_4^{2-}$ 、 $\text{MoO}_4^{2-}$ 、 $\text{WO}_4^{2-}$  and  $\text{SeO}_4^{2-}$ , can inhibit SRB with an approximate effectiveness order of  $\text{CrO}_4^{2-} > \text{MoO}_4^{2-} = \text{WO}_4^{2-} > \text{SeO}_4^{2-}$ . Among all salts,  $\text{MoO}_4^{2-}$  with different dosage (0.2-200mM, 2mM and 10mM) is widely used for the inhibition of SRB [12]. Eckford et al. [13] reported that the synergistic effectiveness of nitrate, nitrite and molybdate on SRB inhibition depended on the operational parameters of the process. The fourth mechanism is the anaerobic sulfur cycle formulated in the system. A possible anaerobic sulfur cycle model presented in the oilfield is proposed by Reinsel et al. [14]. The driving force is transferred or diffused from the surface layer to deep layer of the nitrate. Jenneman et al. [15] demonstrated that the yellow matters appeared in the nitrate added environment were polymeric sulfides, which suggested the present of sulfide cycle. The fifth mechanism is the control of ORP. Gevertz et al. [16] showed that the generation of intermediate metabolites (NO and  $\text{N}_2\text{O}$ ) by denitrifying reaction enhance the ORP and thus long-term inhibition sulfate reduction was obtained. Calcium nitrate is utilized for the treatment of the putrid and smelly drain sediment [17]. The results shows that as the ORP enhanced to positive value, the odor is controlled and the 99.5 % sulfide concentration is removed. The field investigation on the feasibility of nitrate dosage for the control of hydrogen sulfide production is conducted by He et al. in the Coleville Oilfield [18]. The growth of sulphur oxidising bacteria (SOB) and DNB are enhanced. The numbers of SRB keep still or reduced. Favorable efficiencies are also achieved through the dosage of nitrate in the Skjold, the Halfdan, the Gulfaks, the Veslefrikk and the Foinaven Oilfield [19]. However, researches on the ecological factors and the bacterial community in the denitrifying process for SRB inhibition are rare. Besides, most of the previous studies focus on the inhibition of the SRB number rather than their activities.

Nowever, the inhibition of SRB through the inhibition of their activities is explored in an anaerobic baffled reactor (ABR). Indigenous microorganisms would grows, especially the DNB, could be stimulated with the dosage of N. So the sulfate reduction activities of the SRB are gradually declined. Finally, the SRB will stop the sulfate reduction reactions and thus the growth of SRB would be inhibited [20]. Thus, the inhibition strategy of SRB is converted from the simple kill of SRB to their activity inhibition. Besides, the optimal ecological factors and the microbial community structure in the ABR are also investigated.

## 2. Materials and methods

The vertical bafflers divided the ABR into seven serial chambers (Figure 1). Each chamber had a separate upflow sludge bed. Most of the sludge was in granule or floc form. The ABR was in a right-angled trapezoid shape. The effective volume of each chamber is 4.8 L, the total effective volume of the reactor is 40 L. Heating device is utilized to keep the water temperature around  $35 \pm 1$  oC. The influent and the inhibition chemicals would be fed with the peristaltic pump. The inflow is 40 L/d and the hydraulic residence time is 12 h.

The entire operation of the ABR includes the start-up phase and stable operation phase. In the start-up phase, the seed sludge pre-cultured for 30 d is inoculated evenly into each chamber of the ABR. The water quality during the start-up period is presented in Table 1. In the stable operation phase, the effects of the  $\text{SO}_4^{2-}/\text{NO}_3^-$  pH value, alkalinity and COD concentration on the denitrification inhibition to sulfate reduction are investigated. Different  $\text{SO}_4^{2-}/\text{NO}_3^-$  are realized by changing the addition amount of  $\text{NO}_3^-$  at fixed  $\text{SO}_4^{2-}$  concentration. The pH value and the alkalinity are adjusted through the dosage of  $\text{Na}_2\text{CO}_3$  and  $\text{NaHCO}_3$ . Various COD concentrations are obtained by changing the added cane sugar amount (Table 1).



**Figure 1.** The ABR experimental schematic diagram.

① Water reservoir; ② Horizontal-flow valve; ③ Peristaltic pump; ④ tranquil flow device; ⑤ Dosing tank; ⑥ Temperature sensor; ⑦ Heating device ⑧ Gas collector

**Table 1.** Conditions of the stable operation phase.

Days	Na <sub>2</sub> SO <sub>4</sub> (g)	NaNO <sub>3</sub> (g)	SO <sub>4</sub> <sup>2-</sup> /NO <sub>3</sub> <sup>-</sup>	Alkalinity	COD concentration (g)	Addition position
17-26	90	14	6:1	130g NaHCO <sub>3</sub>	180	Influent
27-33	90	28	6:2	130g NaHCO <sub>3</sub>	180	Influent
34-42	90	42	6:3	130g NaHCO <sub>3</sub>	180	Influent
43-54	90	56	6:4	130g NaHCO <sub>3</sub>	180	Influent
55-70	90	70	6:5	130g NaHCO <sub>3</sub>	180	The 2# chamber
71-88	90	84	6:6	130g NaHCO <sub>3</sub>	180	The 2# or 4# Chamber
89-92	90	84	6:6	130gNaHCO <sub>3</sub> , 40gNa <sub>2</sub> CO <sub>3</sub>	180	The 2# chamber
93-112	90	84	6::6	200gNaHCO <sub>3</sub> , 40gNa <sub>2</sub> CO <sub>3</sub>	180	The 2# and 5# Chamber
113- 120	90	84	6:6	200gNaHCO <sub>3</sub> , 40gNa <sub>2</sub> CO <sub>3</sub>	60	The 2# and 5# Chamber

**Note:** The amounts in table were based on 100L; NaNO<sub>3</sub> is added separately by pers added by peristaltic creep pump

### 2.1. Water for experiment

Water for experiment is synthesized by adding 180 g cane sugar and 90 g anhydrous Na<sub>2</sub>SO<sub>4</sub>, 5g fertilizer (supplying nitrogen and phosphate for microbial growth) and 130 g Na<sub>2</sub>HCO<sub>3</sub> (adjusting pH and neutralizing acid produced in the reactions) into 100 L tap water. The main inhibitory chemical is sodium nitrate, which is delivered according to the experiment demands. The average water quality of the influent of the ABR is COD of 1800mg/L, SO<sub>4</sub><sup>2-</sup> of 900 mg/L, pH 5.5-7.5 and alkalinity of 700mg/L.

## 2.2. Seed sludge

Seed sludge is collected from a secondary sedimentation tank in a sewage treatment plant. The collected sludge is precultured with water containing cane sugar and  $\text{Na}_2\text{SO}_4$ . The seeding amount of the sludge is 1.5 L in each chamber.

## 2.3. Analysis methods

$\text{SO}_4^{2-}$  is analyzed by absorption photometry; pH is detected with an acidity meter; alkalinity is measured with the potential titration method;  $\text{S}^{2-}$  is analyzed with a sulfur ion selective electrode;  $\text{Pb}(\text{NO}_3)_2$  is detected with a volumetric precipitation method;  $\text{NO}_3^-$  is detected with a nitrate ion selective precipitation method; ORP is analyzed with a platinum electrode.

## 2.4. Construction of 16SrDNA clone library and sequence submissions to GenBank

The constructions of 16SrDNA clone library refer to the previous literature. The nucleotide sequences of 16S rDNA of the DNB have been deposited in the GenBank database under accession numbers from DQ450402 to DQ450424.

# 3. Results and discussion

## 3.1. The performances of ABR during the rapid start-up phase

Stable performance of ABR is achieved rapidly with the abundant reproduction of SRB and the production of hydrogen sulfide. The removal efficiency of  $\text{SO}_4^{2-}$  and  $\text{S}^{2-}$  concentration are important indexes for the reactor operation. In the start-up phase, the concentrations of sulfate and  $\text{S}^{2-}$  changed gradually. The removal efficiency of sulfate in the ABR reached 80 % after 10 d and ultimately reach 95%. The  $\text{S}^{2-}$  concentrations exceed 110 mg/L . Thus, the reactor is successfully started. The other indexes of the influent and effluent are shown in Table 2.

**Table 2.**Water quality of the influent and the effluent during start-up period .

	Influent	Effluent
pH	6.15-8.93	6.21-6.99
Alkalinity (mg/L)	465-880	1100-1400
$\text{SO}_4^{2-}$ (mg·L <sup>-1</sup> )	560-660	31-468
$\text{S}^{2-}$	Undetected	Gradually increased from none to 110-1 47 mg/L
ORP	Undetected	Gradually declined from -268 mV to 330-350 mV

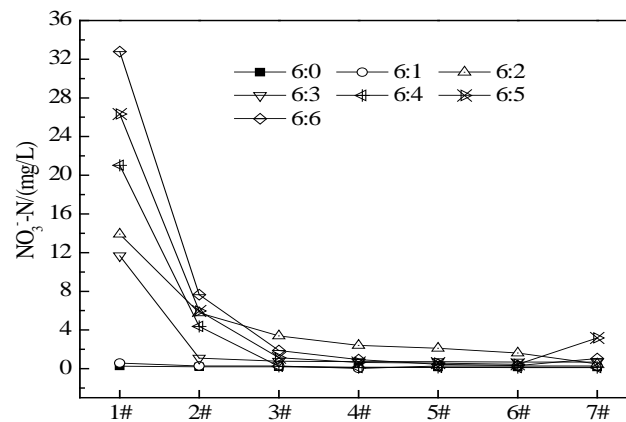
## 3.2. The performances of ABR during the stable operation phase

The inhibition of SRB in the ABR is achieved through the regulation and the control of external ecological factors and the addition of inhibiting chemicals. The effects of the  $\text{SO}_4^{2-}/\text{NO}_3^-$ , pH value, alkalinity and COD concentration on the denitrification inhibition to sulfate reducing are investigated. Based on these investigations, the feasibility and the optimal operational ecological factors of the denitrification inhibition to sulfate reducing are explored.

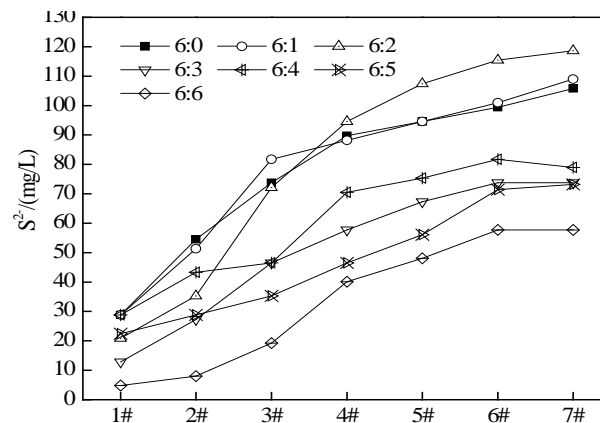
## 3.3. The regulation and control of $\text{SO}_4^{2-}/\text{NO}_3^-$ concentration ratio

Because of the respective electron receptors for the SRB and DNB's reduction process, the concentrations of  $\text{SO}_4^{2-}$  and  $\text{NO}_3^-$  are crucial for the SRB and DNB's activity . The regulation and control of  $\text{SO}_4^{2-}/\text{NO}_3^-$  are significant for the SRB inhibition through denitrification process. The concentrations' variations of S and N indicate that along with the flow of the water and the extension of the reaction time, the sulfide concentration increase stepwise at different  $\text{SO}_4^{2-}/\text{NO}_3^-$  (Figure 2 , Figure 3). The total sulfide concentrations are lower at lower  $\text{SO}_4^{2-}/\text{NO}_3^-$  (6:3-6:6) than that at higher  $\text{SO}_4^{2-}/\text{NO}_3^-$  (6:0-6:2). It indicates that the sulfate reduction can be inhibited by denitrification with the addition of sufficient nitrate. The reason for higher sulfide concentration at higher  $\text{SO}_4^{2-}/\text{NO}_3^-$  is the rapid decline of the nitrate concentration along with the flow of water (Figure 3). The nitrate concentration in the 3# chamber of the ABR is extremely low and can not be detected in the

subsequent chambers. It suggests that the unfavorable SRB inhibition at higher  $\text{SO}_4^{2-}/\text{NO}_3^-$  is caused by the shortage of nitrate.



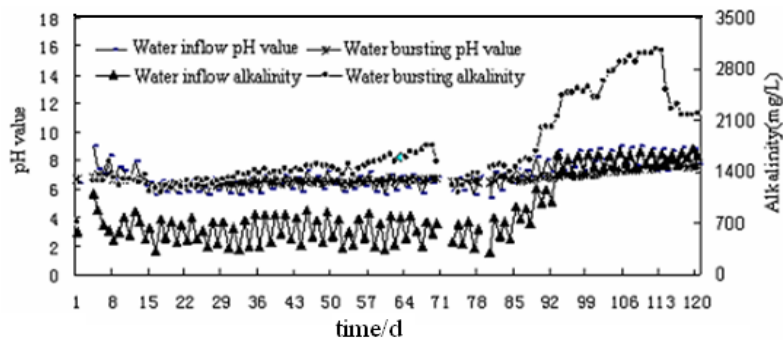
**Figure 2.** Sulfide concentration variations in each chamber under different  $\text{SO}_4^{2-}/\text{NO}_3^-$



**Figure 3.** Nitrate concentration variations in each chamber under different  $\text{SO}_4^{2-}/\text{NO}_3^-$

### 3.4. Regulation and control of pH value

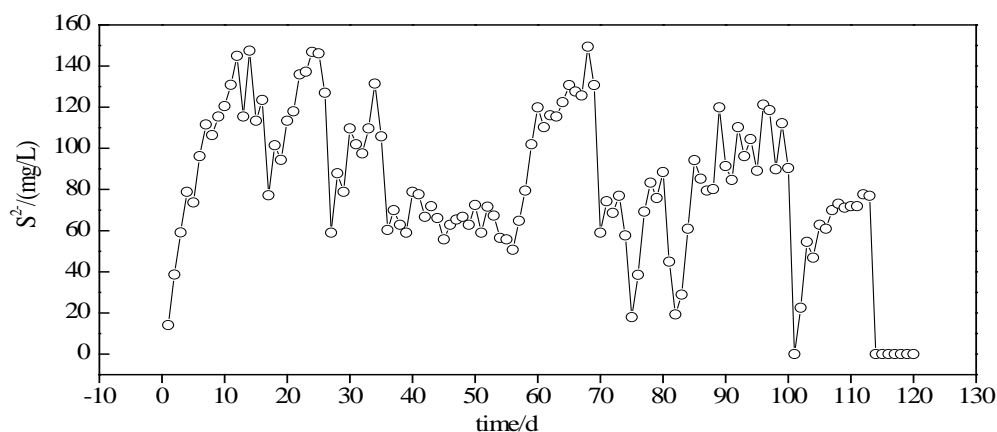
When having one of the ecological factors, pH is very important on the bacterial growth. Variations in pH value would cause different degree of impacts on the SRB and DNB in the ABR. Besides, the alkalinity would neutralize the acid produced in the incipient acidification period and thus maintain the pH value. With the addition of  $\text{NaHCO}_3$  and  $\text{Na}_2\text{CO}_3$ , the influent pH is 5.5-7.3 and the alkalinity was 400-900 mg/L (Figure 4). The pH value of the effluent ranges from 6.2 to 6.7, and the alkalinity increases with the decrease of  $\text{SO}_4^{2-}/\text{NO}_3^-$ . When the  $\text{SO}_4^{2-}/\text{NO}_3^-$  decrease from 6:1 to 6:5, the alkalinity of the effluent increase from 1100 mg/L to 1700 mg/L and remain stable when fixed conditions are provided. Extra alkalinity is produced with the addition of  $\text{NaNO}_3$  and the subsequent denitrification. The ABR is resistant to certain pH and alkalinity variations. The production of extra alkalinity by the ABR is verified as the alkalinity of the effluent is always higher than that of the influent. This is in accordance with the theory that the sulfate reduction and the denitrification will generate alkalinity. The enhancement in pH value would increase the activities of SRB but reduce that of DNB. The enhancement range of effluent alkalinity is 200 mg/L higher to that of the influent. Also, the effluent nitrate concentrations also increase compared to the former phase, which are strongly supported that the activity of the SRB will be increased but that of the DNB would be reduced.



**Figure 4.** Alkalinity and pH's changes in ABR reactors.

### 3.5. Concentration variations of effluent sulfate and sulfion

The concentrations of effluent sulfate and sulfion are important parameters for the evaluation of the SRB inhibition efficiency. The influent sulfate is kept stable around 600 mg/L. The trend for the concentration variations of effluent sulfate and sulfion is reverse, which is in accordance with the conservation of sulfur. When the  $\text{SO}_4^{2-}/\text{NO}_3^-$  is 6:1-6:4, the  $\text{S}^{2-}$  concentration reduced from 120 mg/L to 60 mg/L (Figure 5). It suggested that the regulation and the control of  $\text{SO}_4^{2-}/\text{NO}_3^-$  could influence the efficiency of sulfate reduction inhibition. The sulfion concentrations gradually increase with the flow of the water on different experimental stage. The possible explanation is that the activity of DNB dropped as  $\text{NO}_3^-$  is almost depleted in the back of the ABR. Most of the  $\text{NO}_3^-$  is consumed in the front of the ABR and thus the SRB returned its predominance at the following chamber of the ABR. When the  $\text{SO}_4^{2-}/\text{NO}_3^-$  is 6/5, the sulfion concentrations rapidly increase to more than 100 mg/L at the end of the ABR. It shows that SRB is predominant in the back of the reactor. Growth of DNB is almost lagged with the shortage of  $\text{NO}_3^-$ .



**Figure 5.** The time course of effluent sulfide concentration.

## 4. Conclusions

(1) The  $\text{SO}_4^{2-}/\text{NO}_3^-$  and COD are important ecological factors for the inhibition of sulfate reduction by dinitrification. The best performances of sulfate reduction are obtained with the  $\text{SO}_4^{2-}$  to  $\text{NO}_3^-$  concentration ratio 1/1. Lower COD concentrations are beneficial for sulfate reduction inhibition. Obvious inhibition of DNB on SRB are detected in the 3# chamber of the ABR. The effective action sections are 2# and 5# chamber, with the effective time of 6 h. Thus, the addition of indicators should be conducted in different sections to assure the inhibition efficiency.



(2) In the continuous-flow ABR, when the effluent COD concentration decrease to 600 mg/L and the nitrate is added at the 2# and 5# chamber simultaneously with the  $\text{SO}_4^{2-}/\text{NO}_3^-$  of 1/1, the sulfate reduction is completely inhibited. Thus, the inhibition of SRB by denitrification is feasible.

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