Experimental study on AMD treatment by SRB biodegradation in a UASB reactor

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Abstract. According to the water quality characteristics of acid mine drainage (AMD), sulfate removal from simulated AMD by sulfate reducing bacteria (SRB) was improved in a UASB bioreactor. In the meanwhile, the influences of C/S ratio, pH, temperature, and HRT on sulfate removal rate were analyzed to obtain the optimal operation parameters. The results show that under the optimal operation conditions of C/S = 3.0, pH = 6.5, T = 35 ℃, and HRT = 10 hours, the maximum removal rate of sulfate (86.3%) is reached with an influent sulfate concentration of 2 000 mg/L and an effluent sulfate concentration of 274 mg/L which satisfies the local mine drainage discharge standard (500 mg/L). Different from other traditional desalting technologies, the reduction of salinity is realized in this study by removing sulfate from AMD during SRB biodegradation process.

1. Introduction
Acid mine drainage (AMD) is acidic solution generated by the oxidation of sulfide minerals during mining activities. Taking pyrite (FeS₂), the most abundant sulfide mineral in Earth’s crust, as an example, when pyrite (FeS₂) is exposed to air, the oxidation of pyrite can be briefly described as follows [1]:

\[
\begin{align*}
\text{FeS}_2 + 7.5\text{O}_2 + 3.5\text{H}_2\text{O} & \rightarrow \text{Fe(OH)}_3 + 2\text{SO}_4^{2-} + 4\text{H}^+ \\
\text{FeS}_2 + 14\text{Fe}^{3+} + 8\text{H}_2\text{O} & \rightarrow 15\text{Fe}^{2+} + 2\text{SO}_4^{2-} + 16\text{H}^+ \\
\text{FeS}_2 + 3.5\text{O}_2 + \text{H}_2\text{O} & \rightarrow \text{Fe}^{2+} + 2\text{SO}_4^{2-} + 2\text{H}^+
\end{align*}
\]

(1) (2) (3)

As the above equations shown, the oxidation of pyrite promotes the generation of \( \text{SO}_4^{2-} \) and \( \text{H}^+ \). That’s the reason why AMD is characterized by extremely high content of sulfate (>1 000 mg/L) and low pH (2 to 4) [2]. Except sulfate, AMD also contains various ions like potassium, sodium, magnesium, calcium, iron, copper, aluminium, phosphate, chromium, nickel, and zinc [3, 4, 5]. Thus, AMD has been a serious environmental problem for mining area [6, 7].

There are two conventional methods for AMD treatment to improve pH, remove heavy metal and sulfate, which are active and passive methods [8]. The active method includes adding alkaline chemicals, ion exchange, adsorption, and membrane technology [9]. The passive method includes constructed
wetlands, chemical treatment with limestone drains and biological treatment with sulfate reducing bacteria (SRB) [10, 11].

Among all the conventional technologies, SRB biodegradation is feasible and cost effective and has become an attractive option to treat AMD [12, 13]. Sulfate reducing bacteria (SRB) is a group of strict anaerobic bacteria that use organic matters as the electron donor and sulfate as the electron acceptor [14]. Under anaerobic conditions, SRB converts sulfate to sulfide ($S^{2-}$) and generates alkalinity to neutralize the acidity of solution [13, 15]. In the meanwhile, the dissolved metal ions react with sulfide ($S^{2-}$) to form metal sulfide precipitates which have very low solubility [16].

In this research, a laboratory-scale SRB biodegradation system with a UASB reactor was set up to study the performance of sulfate removal by SRB biodegradation technology and analyze the impacts of COD/SO$_4^{2-}$ (C/S), pH, temperature, and HRT on the removal rate. In the end, The optimal operation conditions were proposed as a reference for practical engineering.

2. Materials and methods

2.1. Simulated AMD

The simulated AMD was prepared by modifying Postgate’s Medium C [17] based on the water quality of AMD collected from a coal mine in Shandong province of China. It is consist of KH$_2$PO$_4$ (0.5g/L), NH$_4$Cl (1.0g/L), Na$_2$SO$_4$ (1.8g/L), CaCl$_2$•2H$_2$O (0.06g/L), MgSO$_4$•7H$_2$O (2.0g/L), yeast extract (1.0g/L), FeSO$_4$•7H$_2$O (0.004g/L), sodium citrate (0.3g/L), and glucose (depend on the requirements of experiments). The pH is adjusted to specific values (from 4.5~8.0) with NaOH and HCl solutions. The temperature is kept constant (from 20~45°C) by a temperature control system.

2.2. Enrichment of SRB

The active sludge is collected from a wastewater treatment plant. In order to reinforce the dominance of SRB, the sludge is domesticated for 40 days. After SRB domestication, the SRB is sealed and conserved at 4°C.

2.3. Bioreactor set up and operation

A laboratory-scale SRB biodegradation system was set up to treat AMD (Fig. 1). The whole system is consist of a UASB bioreactor, an influent tank containing simulated AMD, an effluent collection tank, an air collection system and peristaltic pumps. The UASB bioreactor was operated towards upflow direction and equips a peristaltic pump to realize internal circulating and a temperature control system to keep the temperature constant. The volume of the UASB bioreactor is 15L. The internal circulating rate is 75 mL/min. During the stable operation period, the flow rate of influent is 25 mL/min. N$_2$ gas (0.005L/min) is introduced at the bottom of UASB to stripping H$_2$S away to the exhaust collection system.

Figure 1. Schematic diagram of laboratory scale UASB reactor.
Four main influencing factors studied in this research is C/S, pH, temperature(T), and hydraulic retention time (HRT). Their influences on sulfate removals were analyzed by changing influent COD from 1 000 mg/L to 10 000 mg/L, pH from 4.5 to 8.0, temperature from 20 to 45 ℃, and HRT from 6 hours to 48 hours. The operation conditions were shown in Table 1.

Table 1. Operation conditions for experiments.

| Influencing factor | Influent COD, mg/L | Influent SO₄²⁻, mg/L | C/S   | T, °C | pH    | HRT, h |
|-------------------|-------------------|---------------------|-------|------|-------|-------|
| C/S               | 1000~10000        | 2000                | 0.5~5.0 | 35   | 6.5   | 10    |
| pH                | 6000              | 2000                | 3.0   | 35   | 4.5~8.0 | 10    |
| T                 | 6000              | 2000                | 3.0   | 35   | 6.5   | 10    |
| HRT               | 6000              | 2000                | 3.0   | 35   | 6.5   | 6~48  |

*pH of the solution in UASB bioreactor.

2.4. Analysis methods
The analytical methods used in this research is shown in Table 2[17].

Table 2. Analysis methods.

| Index | Method                        |
|-------|-------------------------------|
| COD   | Potassium Dichromate Titration|
| SO₄²⁻ | Barium Chromate Spectrophotometry|
| pH    | pH meter                      |

The performance of SRB was evaluated by sulfate removal efficiency(ƞ) which is calculated by the following equation [17, 18].

\[ \eta = \frac{(C_t - C_0)}{C_0} \times 100\% \]  

Where,
\[ \eta \] = sulfate removal
\[ C_t \] = the sulfate concentration after t hours (mg/L)
\[ C_0 \] = the sulfate concentration in the influent (mg/L)

3. Results and discussion

3.1. Effect of C/S on sulfate removal
The influent C/S ratio affect the metabolic phase-separation and the stability of the reactor [19]. Theoretically, SRB need a C/S ratio of 0.67 to completely reduce sulfate [12]. In the fact, another bacteria, methane product bacteria (MPB), competes with SRB for organic matters. As a result, the C/S needed is much larger than the theoretical value [19].

In this C/S experiments, the influent SO₄²⁻ concentration was kept constant (2 000 mg/L), while the influent COD concentration changed from 1 000 mg/L to 10 000 mg/L. Thus the C/S ratio obtained was from 0.5 to 5.0.
The effect of C/S on sulfate removal rate is shown in Fig. 2. The sulfate concentration in the influent is 2 000 mg/L. The results shows that the effluent sulfate concentration decreases rapidly from 1 829 mg/L to 274 mg/L and the removal rate of sulfate increases from 8.6% to 86.3% with the C/S increasing from 0.5 to 3.0. Then a steady state is reached when C/S is above 3.0. At the steady state, average effluent sulfate concentration and average removal rate are 218 mg/L and 89.1%, respectively. It is indicated that COD added is not enough to reduce sulfate when C/S is below 3.0. Conversely, too high C/S(>3.0) can ensure a high sulfate removal rate, but causes high chemical cost and residual COD in the effluent[20]. Thus, the optimal COD dosage is three times of the sulfate concentration.

3.2. Effect of pH on sulfate removal
SRB is sensitive to pH [21]. pH is one of the main factors that can affect the activity of bacteria [22]. The effect of pH on sulfate removal rate was studied at different pH (4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0). The influent sulfate concentration was 2 000 mg/L.

As displayed in Fig. 3, with the increasing of pH, the effluent sulfate concentration decreases rapidly first from 1 691 m/L to 274 mg/L and then increases slightly to 772 mg/L. Correspondingly, the sulfate removal rate increases rapidly first from 15.5% to 86.3% and then decreases slightly to 61.4%. A sharp decrease in sulfate removal from 76.3% to 39.2% is observed when reducing pH from 6.0 to 5.5. That’s because the activities of SRB is inhibited by too high or low pH and SRB is hard to survive. In addition, high pH is not conductive to the stripping of H$_2$S [12]. For instance, high concentration of protons may cause high diffusion pressure on the cell membrane of microorganism [23, 24]. The pH range suitable for the metabolism of SRB is between 6.0 to 7.0 with an average sulfate removal rate of 81.1%. The optimal pH for SRB biodegradation is 6.5 with the maximum removal rate of 86.3%.

As mentioned before, the pH of AMD is typically 2 to 4 which is too low for SRB. Although, SRB can produce alkalinity [25] and increase pH from 3.0 to about 7.0[26, 27]. It is still suggested to maintain the pH in the reactor around 6.5 by adding HCl or NaOH [28].

Figure 2. Effect of C/S on sulfate removal rate.

Figure 3. Effect of different solution pH on sulfate removal rate.
3.3. Effect of temperature on sulfate removal

According to the living temperature, SRB can be divided into two types, namely mesophilic bacteria and thermophilic bacteria [29, 30]. Almost all of the SRB founded in real engineering belongs to mesophilic bacteria which has an optimal temperature of approximately 30°C [12].

In this study, the operation temperature was changed from 20°C to 45°C while the other parameters were kept constant. The influent sulfate concentration was 2 000 mg/L. As displayed in Fig. 4, the effluent sulfate concentration decreases from 1 758 mg/L to 274 mg/L and the sulfate removal rate increases from 12.1% to 86.3% when the temperature rises from 20°C to 35°C. Then effluent sulfate concentration increase from 274 mg/L to 1890 mg/L and the sulfate removal rate decreases sharply from 86.3% to 5.5% when the temperature rises from 35°C to 45°C.

Figure 4. Effect of temperature on sulfate removal rate.

Therefore, the maximum removal rate is reached at the optimal temperature of 35°C, and it is 5°C higher than the theoretical temperature. It can be explained that the sulfate removal rate of SRB does not just depend on temperature, but is also affected by other microorganism existing with SRB [12]. When the solution temperature is higher than 35°C, the activities of SRB is inhibited because the protein structure may occur irreversible damage under high temperature.

3.4. Effect of HRT on sulfate removal

Hydraulic retention time (HRT) is determined by flow velocity. The effect of HRT on the removal rate of sulfate was investigated by varying HRT from 6 hours to 48 hours and the results is shown in Fig. 5. As displayed in Fig. 5, the effluent sulfate concentration decreases from 876 mg/L to 139 mg/L and the corresponding sulfate removal rate increases from 56.2% to 93.1% with increasing HRT. A nearly stable stage is reached with an average effluent sulfate concentration of 214.8 mg/L and an average removal rate of 89.3% when HRT is higher than 10 hours. It is because that short HRT (<10 hours) may not allow adequate time for SRB to reduce sulfate and neutralize acidity. Besides, short HRT may result in biomass being washed out of the reactor. However, long HRT (>12 hours) may deplete the substrate and lead to low hydraulic conductivity and short-circuiting [31]. In this study, the optimal HRT is found to be 10 hours.
Figure 5. Effect of HRT on sulfate removal rate.

4. Conclusion
In this study, the maximum removal of sulfate by SRB was optimized in terms of C/S ratio, pH, temperature, and HRT in a UASB reactor. The influences of C/S ratio, pH, temperature, and HRT on sulfate removal rate were analyzed and the optimal operation conditions were proposed for real engineering.

(1) With C/S increasing from 0.5 to 5.0, the sulfate removal rate increases rapidly from 8.6% to 86.3% and keeps a steady value of 89.1% when C/S is above 3.0.

(2) With pH in the reactor increasing from 4.5 to 8.0, the sulfate removal rate increases rapidly first from 15.5% to 86.3% and then decreases slightly to 61.4%.

(3) With temperature increasing from 20°C to 45°C, the sulfate removal rate increases gradually from 12.1% to 86.3% and then decreases sharply to 5.5%.

(4) With HRT increasing from 6 hours to 48 hours, the sulfate removal rate increases sharply from 56.2% to 93.1% and then reaches to a nearly stable stage with an average removal rate of 89.3% after 10 hours.

(5) The optimal operation parameters are C/S = 3.0, pH = 6.5, T = 35°C, and HRT = 10 hours. Under the above operation conditions, a maximum sulfate removal rate of 86.3% is reached and the effluent sulfate concentration is 274 mg/L with satisfy the local mine drainage discharge standard (500 mg/L).

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