



Short Communication

Treatment of acid mine drainage by sulfate reducing bacteria with iron in bench scale runs

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HIGHLIGHTS

- The real AMD containing high sulfate and heavy metals was treated effectively by SRB and Fe⁰ in an UAMB bioreactor.
- A bench scale system for AMD treatment was operated at 25 °C to low the cost of operation.
- The AMD was treated by SRB and Fe⁰ for 70 days without biomass replenishment.

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ABSTRACT

In order to treat acid mine drainage (AMD) effectively using sulfate-reducing bacteria (SRB) at high concentration of sulfate and heavy metals, Fe⁰ was added to enhance the activity of SRB. When AMD was treated by SRB and Fe⁰ at 25 °C, more than 61% of sulfate was removed and the effluent pH was improved from 2.75 to 6.20 during the operation. Cu²⁺ was removed effectively with the removal efficiency at 99%, while only 86% of Fe²⁺ was removed during the AMD treatment, without conspicuous change of Mn²⁺ in the effluent in the process.

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1. Introduction

Acid mine drainage (AMD) containing sulfate and heavy metals (Cu, Mn, Zn, Pb and Fe) with a high acidity can have a devastating effect on terrestrial and aquatic ecosystems (Elliott et al., 1998). Several methods have been used in the treatment of AMD including chemical treatment, ion exchange and membrane methods and so on (Madzivire et al., 2010). The operating costs of ion exchange and membrane methods are high, and the efficiency of chemical treatment is relatively low (Luptakova and Kusnierova, 2005) and (Hlabela et al., 2007).

Microbial remediation of AMD by sulfate reducing bacteria (SRB) is a promising alternative to the traditional methods. Sulfate is microbially reduced to hydrogen sulfide which precipitated with heavy metals (especially, Cu, Zn, and Fe) stably (Gibert et al., 2005) and (McCauley et al., 2009). A further research on the improved ability of SRB to remove heavy metals in the adverse circumstance

is required and exigent due to the complex bioreaction influenced by many factors (e.g., pH, temperature, hydraulic retention time). Iron (Fe⁰) often presents as a reducing agent in the treatment of groundwater contaminated with heavy metals because of its high reduction potential and nontoxic (Wilkin and McNeil, 2003). The system containing SRB and Fe⁰ to treat AMD provides a more effective sulfate reduction due to the enhanced activity of SRB by Fe⁰ (Yao et al., 2008) and (Lindsay et al., 2008).

The present work is to develop a system for the treatment of AMD containing high sulfate (>20 g L⁻¹) and heavy metals (Cu, Mn, Zn, and Fe) at low pH value (<2.3) by SRB enhanced by Fe⁰ in an up-flow anaerobic multiple-bed (UAMB) reactor in bench-scale continuous runs.

2. Methods

2.1. Microorganisms

The mixed culture of SRB was initially collected from activated anaerobic sludge of a wastewater treatment plant. The culture was

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Table 1
Chemical composition of medium in enrichment of SRB.

Composition	Concentration (mg L ⁻¹)	Composition	Concentration (mg L ⁻¹)
K ₂ HPO ₄	500	CaCl ₂	100
NH ₄ Cl	1000	Yeast extract	1000
MgSO ₄ ·7H ₂ O	2000	Sodium lactate	4000
Na ₂ SO ₄	500	Ammonium ferrous sulfate	1200

collected and then purged with high purity nitrogen to reduce the dissolved oxygen concentration. 3% of sludge inoculum was added into 1000 ml flask with medium (Table 1), with lactate serving as the organic carbon source and sulfate as energy source for growth. The activity of SRB was determined by the direct observation of the formation of black precipitate (ferrous sulfide). The enriched SRB culture was obtained by repeating the process for three times. All chemicals used in this study were of analytical grade and used as received.

2.2. Iron

The purity of iron powder was 90% containing approximately 2.5% Mn, 1.0% Si, 0.3% C, 0.1% S, 0.18% P and 3% hydrochloric acid insoluble materials. The average size was 255 μ m.

2.3. Bioreactor

An up-flow anaerobic multiple-bed (UAMB) reactor, which was made of Plexiglas, consisted of two sections: a fluidized bed and a fixed one. The volume of both sections was 2150 mL. The fluidized bed, in lower section, was filled with baller rings, made of non-porous PVC. In the upper section, the fixed bed was filled with packing particles made of ceramic. It was equipped with a total of seven ports for sampling along the height of the reactor. A three phase separator on the top of the reactor was utilized effectively to separate the biogas, effluent and sludge. A detailed description of the multiple-bed reactor is available in the patent held by Kang and Feng (Kang and Feng, 2003). The constant temperature water bath was equipped with a thermostatically controlled heating element to maintain the reactor's temperature. The influent of the reactor was delivered by one of the variable-speed peristaltic pumps. The HRT adjusted by changing flow rate and recirculation was controlled using another pump.

2.4. Experiment procedure

The reactor was initially filled with synthetic wastewater and inoculated with the 3% v/v of the enriched SRB culture. The performance of the bioreactor was monitored by determination of sulfate reduction, and the steady state was achieved with the sulfate reduction ratio above 90% at 2 weeks after the inoculation. The real AMD treatment included three stages: start-up, loading operation and AMD treatment stages. During the start-up stage, the synthetic wastewater containing low concentrations of sulfate and heavy metals was fed into the system to enhance the activity of SRB.

For the first 35 days (the start-up stage), sulfate, heavy metal and acidity of influent increased step by step during five periods with an interval of 7 days (Table 2). In loading operation stage, the concentrations of sulfate and heavy metal in synthetic wastewater (Table 2) were constant and the loading rate increased with the HRT changed from 48 to 24 for adaption of SRB to the real AMD for three periods with an interval of 7 days.

At the end of loading operation stage, the performance of bioreactor was deteriorated due to high loading rate of sulfate and heavy metal. The bioreactor was recovered with the HRT increased by 96 h during 2 weeks. In AMD treatment stage, the real AMD from a copper mine in China (Table 2) was treated by SRB with Fe⁰ under continuous flow for 14 days. A small amount of lactate was supplemented as the organic carbon source in order to keep the growth of SRB and avoid the pollution resulting from the lactate. The COD of system was maintained at the small necessary amount degree during the real AMD treatment stage.

2.5. Analytical methods

The liquid samples were periodically drawn from the port of upper section once 3 days. A total of 20 mL of water was sampled a plastic syringe at pre-determined times. The supernate was collected for analysis after centrifugation of the sample at approximately 5000 rpm for 10 min. The sulfate was determined using a C200 Multiparameter Ion Specific Meter (Hanna Instruments, Italy). The pH determination was performed immediately after sample collection using an E-201-C Multi-glass electrode (Hanna Instruments, Italy). Analysis of the COD was conducted using the potassium bichromate method. Analysis of metal ions (Cu²⁺, Fe²⁺, Mn²⁺ and Zn²⁺) were conducted using a C200 Multiparameter Ion Specific Meter (HANNA Instruments, Italy) and ICS1000 Ion Chromatograph (Dionex Co.).

3. Results and discussion

The treatment of real AMD by SRB with Fe⁰ under particular temperature (25 °C) included three periods: start-up stage, loading operation stage and AMD treatment stage. The bioreactor was fed with the synthetic wastewater in the start-up stage and the loading operation stage, whereas real AMD in the AMD treatment stage.

3.1. Start-up process stage

The enhancement of SRB and maturation of biofilm were gradually established in the start-up stage due to the improved activity

Table 2a
Composition of wastewater in the start-up stage.

Period	Day	SO ₄ ²⁻ (mg L ⁻¹)	COD (mg L ⁻¹)	Cu ²⁺ (mg L ⁻¹)	Fe ²⁺ (mg L ⁻¹)	Mn ²⁺ (mg L ⁻¹)	Zn ²⁺ (mg L ⁻¹)	pH
I	0–7	500	1300	20	55	10	15	4.0
II	8–14	1200	3000	48	132	24	36	3.8
III	15–21	1800	4000	72	198	36	54	3.5
IV	21–28	2400	5000	96	264	48	72	3.25
V	29–35	3000	6150	120	330	60	90	3.0

Table 2b

Composition of wastewater in the loading operation stage.

Period	Day	SO ₄ ²⁻ (mg L ⁻¹)	COD (mg L ⁻¹)	Cu ²⁺ (mg L ⁻¹)	Fe ²⁺ (mg L ⁻¹)	Mn ²⁺ (mg L ⁻¹)	Zn ²⁺ (mg L ⁻¹)	pH	HRT (h)
I	36–42	3000	6150	120	330	60	90	3.0	48
II	43–49	3000	6150	120	330	60	90	3.0	36
III	50–56	3000	6150	120	330	60	90	3.0	24

Table 2c

Component and concentration of the real AMD.

pH	COD (mg L ⁻¹)	SO ₄ ²⁻ (mg L ⁻¹)	Cu ²⁺ (mg L ⁻¹)	Fe ²⁺ (mg L ⁻¹)	Mn ²⁺ (mg L ⁻¹)
2.75	<100	20,800	230	545	190

of SRB by domestication of SRB to the waste water. HRT was maintained at 48 h to avoid the significant abrasion of SRB biofilm due to collision of liquid flow. The sulfate and metals were increased incrementally and pH was gradually decreased with five periods and temperature was 25 °C for SRB adaption to the new wastewater system.

3.1.1. Sulfate reduction and effluent pH

The removal of sulfate in discharge shown in Fig. 1 was effective from period I to III. Trace quantities of sulfate in charge was found with effluent pH above 6.7. It was observed that effluent sulfate sharply increased and changed uncertainly with average values at about 100 mg L⁻¹ and 150 mg L⁻¹ in period IV and V, respec-

tively. The sulfate reduction rate increased with the growing sulfate load, and it was 59.4 mg L⁻¹ h⁻¹ at last period. It is shown in Fig. 1 that the effluent pH decreased with the increasing sulfate load, and pH of discharge was at a range from 6.45 to 7.05. Even though the influent pH was below 3.0 at period V, the effluent pH was above 6.45.

3.1.2. Heavy metals removal

As shown in Fig. 1 and Table 3, the removal efficiency of Zn²⁺ stayed constant for all concentrations throughout the process. The removal of Zn²⁺ was effective and stable with effluent of Zn²⁺ below 1.0 mg L⁻¹ and the maximum of removal rate at 1.9 mg L⁻¹ h⁻¹. In period I, the effluent Cu²⁺ was only about 5 mg L⁻¹ due to lower influent concentration of Cu²⁺. It is observed that the effluent concentration of Cu²⁺ weakly fluctuated in the second period due to the deficient sulfite, and more sulfite was required for a desirable effluent Cu²⁺. The removal efficiency of Cu²⁺ grew with the increasing influent of Cu²⁺ after period II with the concentration of effluent Cu²⁺ below 5 mg L⁻¹. The removal of Cu²⁺ reached a better level with the effluent Cu²⁺ declining, resulting from the increasing amount of sulfite with the growing sulfate load. The remove rate of Cu²⁺ was hold at 2.5 mg L⁻¹ h⁻¹, similar with the loading rate at the end of the process. The high removal degree of Cu²⁺ and Zn²⁺ could be achieved due to a similar value of removal rate and loading rate.

The concentration of Fe²⁺ and Mn²⁺ in discharge increased with the growing metal loading. It was obtained that the removal efficiency of Fe²⁺ was maintained from 83% to 93% throughout the process. The removal efficiency of Mn²⁺ was constant low, only 50%. At period V of the start-up stage, the effluent Mn²⁺ reached a high level at 30–40 mg L⁻¹.

In start-up stage, the removal of metals by the precipitation with sulfite depended on the solubility products of these metal sulfide precipitates. It has been reported that the solubility products of ferrous sulfite ($K_{sp} = 6.3 \times 10^{-18}$) and manganese sulfide ($K_{sp} = 2.5 \times 10^{-13}$) were to some extent higher than those of soluble metal concentrations. By contrast, a high level removal of Zn²⁺ and Cu²⁺ was established due to the low solubility products of zinc sulfide ($K_{sp} = 2.93 \times 10^{-25}$) and copper sulfide ($K_{sp} = 6.3 \times 10^{-36}$) with independent of solution pH value.

3.2. Loading operation stage

In start-up stage, the performance of bioreactor was stable, and the biofilm attaching on fillers was gradually mature. It is reasonable that the performance of bioreactor could reach a higher level due to the mature. In order to obtain a better removal of sulfate and heavy metals, the load of sulfate and heavy metal was increased by the decreasing HRT in the loading operation stage.

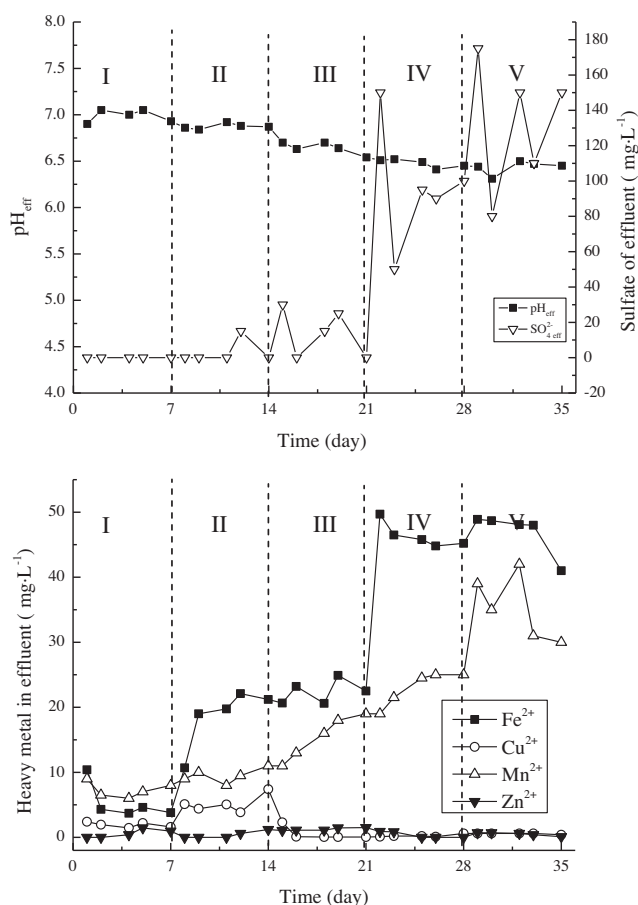


Fig. 1. The alteration of sulfate, effluent pH and heavy metals during the start-up stage.

Table 3

Sulfate and metals removal results at the start-up stage.

Period	Removal efficiency (%)					Removal rate ($\text{mg L}^{-1} \text{h}^{-1}$)				
	Sulfate	Fe	Cu	Mn	Zn	Sulfate	Fe	Cu	Mn	Zn
I	>99.9	93.1	92	20.0	93.7	10.4	1.07	0.38	0.04	0.29
II	>99.9	83.9	84.6	54.2	96.7	25.0	2.31	0.85	0.27	0.73
III	>99.9	88.6	99.9	47.2	97.2	37.5	3.66	1.5	0.35	1.09
IV	95.8	82.9	99.4	47.9	>99.9	47.9	4.56	1.99	0.48	1.50
V	95	85.5	99.7	50	99.9	59.4	5.88	2.49	0.63	1.87

3.2.1. Sulfate removal and effluent pH

It is shown in Fig. 2 and Table 4 that the effluent sulfate was 150 mg L^{-1} in period I, then increasing to 500 mg L^{-1} in period II with the stable removal of sulfate. In period II, the effluent sulfate gradually increased 1500 mg L^{-1} with the HRT at 24 h after 19 days. The removal efficiency was above 80% in period I and II, whereas decreased by 50% at the end of period III. The removal rate showed a same pattern during this process, and it was $68.6 \text{ mg L}^{-1} \text{h}^{-1}$ in period II and decreased by $63.3 \text{ mg L}^{-1} \text{h}^{-1}$ in period III. It was noticed that the removal rate showed a decreasing trend even in the next stage due to the deteriorated performance of bioreactor.

The effluent pH of the reactor was close to 6.3 throughout the loading operation stage although the feed pH was decreased to about 3.0 in period I and II as illustrated in Fig. 2. In period III, the effluent pH was 5.2 after 19 days with a constantly decreasing trend.

3.2.2. Heavy metals removal

The development of heavy metals in effluent in loading operation was similar with that in start-up stage. Although the concentration of heavy metals in effluent remained a stable level, the removal of different metals was changeable. The removal efficiencies of Cu^{2+} and Zn^{2+} were higher than those of many other metals. During the loading operation, the concentration of Cu^{2+} and Zn^{2+} were 0.2 mg L^{-1} and 0.5 mg L^{-1} respectively, which was suitable for the discharge standard. Although the performance of bioreactor was deteriorated resulting in a decreasing sulfate removal, the higher removal efficiency of Cu^{2+} and Zn^{2+} both held at 99% in period III due to the sufficient sulfite to precipitate with Cu^{2+} and Zn^{2+} . By addition, the removal rate of Cu^{2+} and Zn^{2+} increased with the growing load and reached $4.99 \text{ mg L}^{-1} \text{h}^{-1}$ and $3.72 \text{ mg L}^{-1} \text{h}^{-1}$ respectively after an adaption, as similar as the loading rate of each ion.

The concentration of Fe^{2+} in effluent was $40\text{--}50 \text{ mg L}^{-1}$ in period I of loading operation stage. In the beginning of period II, it sharply increased to 220 mg L^{-1} , and then decreased to 50 mg L^{-1} at the end of period II. An increasing effluent concentration was found at 300 mg L^{-1} after 19 days in period III with only 10% of Fe^{2+} removed. The reason for this increased concentration of Fe^{2+} in effluent was due to the difficult to precipitate ferrous with sulfite in low pH. The trend of Mn^{2+} removal was amazingly similar to the result of the start-up stage with a constant concentration in effluent at 30 mg L^{-1} and low removal efficiency at 50%. It was suggested that Mn^{2+} removal was generally less effective than other metals removal throughout this study.

3.3. AMD treatment stage

At the end of the loading operation stage, the deteriorated performance was established due to the higher loading rate. The bioreactor performance was improved after 2 weeks with a longer HRT about 96 h. In the AMD treatment stage, the bioreactor was used to treat real AMD from a copper mine at 25°C .

The concentration of sulfate decreased from $20,800 \text{ mg L}^{-1}$ in influent to 8200 mg L^{-1} in effluent with 61% of sulfate reduction, when the loading rate was $217 \text{ mg L}^{-1} \text{h}^{-1}$ and the reduction rate was $131 \text{ mg L}^{-1} \text{h}^{-1}$. The combination of SRB and Fe^0 can improve the pH from 2.75 to 6.20 during this stage.

The change of heavy metals removal was reported in Table 5. The better removal of Cu^{2+} than other metals was observed with the influent concentration of 230 mg L^{-1} decreasing by 0.2 mg L^{-1} . As a result, 99% of Cu^{2+} was removed and the removal rate was $4.9 \text{ mg L}^{-1} \text{h}^{-1}$. The removal efficiency of Fe^{2+} was still at 86.2%, and the concentration was decreased from 545 mg L^{-1} in influent to 75 mg L^{-1} in effluent with removal rate at $2.4 \text{ mg L}^{-1} \text{h}^{-1}$. Mn^{2+} removal during the stage was typically low and 53% of Mn^{2+} was removed and removal rate was $1.04 \text{ mg L}^{-1} \text{h}^{-1}$. The results showed that the effluent pH and Cu^{2+} in effluent were coincident with the emission standard. The removal rates of three metals were much lower than the maximum removal capability, so a

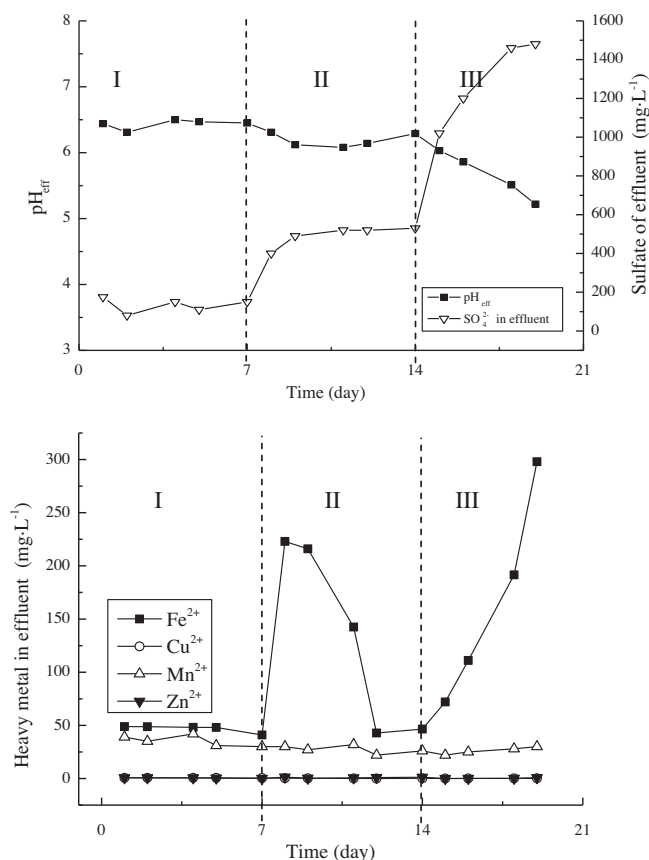


Fig. 2. The alteration of sulfate, effluent pH and heavy metals during the loading operation stage.

Table 4

Metals removal results at the loading operation stage.

Period	Removal efficiency (%)					Removal rate (mg L ⁻¹ h ⁻¹)				
	Sulfate	Fe	Cu	Mn	Zn	Sulfate	Fe	Cu	Mn	Zn
I	95.0	85.5	99.7	50	99.9	59.4	5.88	2.49	0.63	1.87
II	82.3	85.9	99.8	56.7	98.7	68.6	7.89	3.33	0.94	2.47
III	50.7	9.7	99.8	50	99.1	63.3	1.33	4.99	1.25	3.72

Table 5

Metals removal results of the effluent at the AMD treatment stage.

Metals	Concentration (mg L ⁻¹)	Removal efficiency (%)	Removal rate (mg L ⁻¹ h ⁻¹)
Fe ²⁺	75.0	86.2	4.90
Cu ²⁺	0.2	99.9	2.40
Mn ²⁺	90.0	52.6	1.04

better removal of the heavy metals could be established with a longer HRT.

The removal of sulfate and heavy metals were deteriorated in the AMD treatment, indicating of a bad performance of bioreactor due to inappropriate operation. The effluent of bioreactor would be discharged directly by further treatment, which will be reported in the future.

4. Conclusions

The real AMD was treated by SRB and Fe⁰ at 25 °C in the current study. The sulfate decreased to 8200 mg L⁻¹ in discharge with 61% of sulfate reduction. The effluent pH was improved from 2.75 to 6.20 during the operation. 99% of Cu²⁺ was removed with the effluent concentration at 0.2 mg L⁻¹, and Fe²⁺ was decreased from 545 mg L⁻¹ to 75 mg L⁻¹ in effluent. Mn²⁺ removal was typically low with removal efficiency of Mn²⁺ at 53%.

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