Enhanced biotreatment of acid mine drainage in the presence of zero-valent iron and zero-valent copper

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ABSTRACT

Batch laboratorial experiments were conducted to evaluate the potential of Fe\(^0\), Cu\(^0\) and sulfate-reducing bacteria (SRB) for reduction and removal of sulfate and heavy metals from synthetic acid mine drainage. The variation in solution pH indicates that the Fe\(^0\)/Cu\(^0\) bimetallic system provided favorable conditions for SRB growth and sulfate reduction. When the SO\(_4^{2-}\)/Co concentration of wastewater was 3,000 mg/L, the SO\(_4^{2-}\)/Co removal efficiency was 51.6% for the SRB system, 76.3% for the Fe\(^+\)/SRB system, and 92.0% for the Fe/Cu\(^+\)/SRB system. Moreover, Pb\(^2+\), Cu\(^2+\) and Zn\(^2+\) ions were completely removed. The results demonstrate that the Fe/Cu\(^+\)/SRB system had apparent advantages over the SRB system, especially at low pH. This study demonstrates that an Fe/Cu\(^+\)/SRB system could be a promising technology for treating wastewater containing high concentrations of sulfate and heavy metals.

Key words | reduction, sulfate-reducing bacteria, sulfide, zero-valent copper, zero-valent iron

INTRODUCTION

Acid mine drainage (AMD), generated by the biological oxidation of metal sulfides to metal sulfates in mine wastes, is characterized by low pH and high concentrations of dissolved metals and sulfates (Peng et al. 2017). AMD is extremely toxic to aquatic organisms because of its strong acidity and the solubilization of metals (Peng et al. 2017). Moreover, the sulfates can be reduced to sulfides by sulfate-reducing bacteria (SRB) under anaerobic conditions, leading to air pollution and corrosion of metal pipes. Thus, AMD represents a challenging environmental issue in the mining industry worldwide.

There exist various methods for treating sulfates in wastewater, including activated carbon adsorption (Salman 2018), biological treatment (Singh et al. 2011; Rodriguez et al. 2016), reverse osmosis (Al-Bastaki 2004), etc. The physical processes have disadvantages such as high infrastructure costs and high operating costs. Therefore, biological processes have been employed as an alternative to treat sulfate-containing wastewater (Singh et al. 2011; Rodriguez et al. 2016). Sulfates are non-toxic to organisms, but their reduction products (namely sulfides) have a highly poisonous effect on microbes including SRB (Singh et al. 2011). Thus, treating AMD by biological processes alone may be hampered by the presence of high concentrations of sulfides.

Zero-valent iron (ZVI, Fe\(^0\)), a strong reducing reagent (\(E_0 = -0.447\) V), in aqueous solution, acts as an electron donor under certain conditions and can carry out oxidation-reduction reactions without any external energy input. ZVI is expected to lower the oxidation-reduction potential (ORP), which is beneficial for the formation of an enhanced anaerobic environment for SRB (Kumar...
et al. 2015). Additionally, Fe⁰ can release from Fe⁰ to Fe²⁺ (Fe⁰ + 2H⁺ = Fe²⁺ + H₂) and thus buffer the solution pH (Liu et al. 2015) and reduce the toxicity of H₂S by the formation of FeS precipitation (Zhang et al. 2015). Fe⁰ can also lower mobility and toxicity of some heavy metals (Cu²⁺ and Cr⁶⁺) through reduction behavior. Therefore, Fe⁰ packed anaerobic bioreactors have been developed to improve the treatment performance of sulfate-containing wastewater, resulting in better sulfate reduction and metal removal (Singh et al. 2011; Zhang et al. 2011; Ayala-Parra et al. 2016). The application of Fe⁰ in anaerobic bioreactors could maintain higher organic removal and higher microbe density (Zhang et al. 2011). Moreover, it was found that Fe⁰ addition enhanced sulfate reduction and generated alkalinity (Ayala-Parra et al. 2016).

However, the reactivity of Fe⁰ tends to decline with time because of the accumulation of hydroxide precipitates and other corrosion products on the metal surface. It has been demonstrated that Cu⁰ addition could provide high reaction potential and improve reduction capacity of Fe⁰, resulting in a higher pollutant removal efficiency even in a neutral pH range (Lai et al. 2014). Thus, this study proposed an Fe/Cu bimetallic packed anaerobic system to improve sulfate removal by SRB from AMD. The validity and applicability of the system is tested and compared under different conditions.

MATERIALS AND METHODS

Materials

Iron shavings (Fe⁰) and copper shavings (Cu⁰) were collected from a local machining factory. The shavings used had a purity of greater than 99% and a grain size range of 14–18 mesh. The shavings (1 g) were mixed with 10 mL of 10% (v/v) HCl solution for 2 min and were then rinsed with nitrogen-sparged deionized water, and then rinsed in acetone. The shavings were dried with N₂ inside the anaerobic glove box before use.

The mixed SRB population was enriched from activated sludge obtained from the anaerobic digester at a local sewage treatment plant. For this, 5 g anhydrous Na₂SO₄ was added into a 1-L bottle containing 1 L of anaerobic sludge. After 7 days of incubation at 37 °C, 50 mL of sludge was transferred to another 1-L bottle containing 950 mL of autoclaved Postgate’s B medium (Postgate 1984), and then incubated at 37 °C for another 7 days. This procedure was repeated 7 times to enrich SRB. The resultant culture was centrifuged (8,000 g, 10 min), washed twice with physiological saline, and centrifuged again as indicated above to collect the pellet (namely the final consortium). Light microscopy and scanning electron microscopy (SEM) showed that motile vibrios dominated the population. The presence of SRB was further confirmed by the observation of the precipitation of black iron sulfide. The SRB consortium was stored under N₂ at 4 °C, at the College of Life Science, Longyan University. The 16S rRNA gene based clone libraries demonstrated that the SRB consortium mainly contained Desulfomicrobium spp., Desulfobulbus spp., and Desulfovibrio spp.

Experimental procedures

In this work, an artificial AMD was prepared by using Na₂SO₄, Pb(NO₃)₂, Cu(NO₃)₂, and ZnCl₂. The stated concentrations of SO₄²⁻, Pb²⁺, Cu²⁺ and Zn²⁺ ions were 5,000, 50, 50 and 50 mg/L, respectively, in the wastewater prepared (Peng et al. 2017). To provide inorganic nutrient and organic substrate for SRB, the AMD was supplemented with K₂HPO₄ (0.5 g/L), NH₄Cl (1.0 g/L), CaCl₂ (0.1 g/L). Chemical oxygen demand (COD) was adjusted as required with glucose.

Batch tests were conducted in 1-L glass bottles, to test the effect of various parameters on the removal of sulfates. The bottles had one inlet for probe insertion and two outlets for sampling and gas discharge. The bottles were connected with 500 mL aspirated bottles (gas holder) filled with 2.0 M NaOH solution to trap the H₂S generated during incubation.

SRB consortium (about 5 g) was transferred to bottles with 900 mL artificial AMD. The headspace was flushed with N₂/CO₂ gas (80:20, v/v) to exclude oxygen and bottles were sealed with butyl rubber septa to exclude oxygen permeation. Fe⁰ and Cu⁰ were added to the mixture at a final concentration of 2 and 0.2 g/L, respectively. The bottles were then incubated at 100 rpm and 37 °C. There were four treatments with three replications as follows: (1) Fe/Cu;
SRB; (3) Fe + SRB; (4) Fe/Cu + SRB. In each treatment, the volume of AMD was identical (900 mL).

**Analysis methods**

Liquid samples were obtained from each bottle using a plastic syringe at a pre-determined time. The supernatant was collected for analysis after centrifugation at 8,000 rpm for 10 min. Sulfide, sulfites, and sulfate were determined by ion chromatography (C196-E039A, Shimadzu, Japan) with a Shim-pack IC-A3 chromatographic column. Metal ions were measured using atomic absorption spectrophotometry (Shimadzu AA6300G, Kyoto, Japan) with a graphite furnace atomizer (GFA-EX7i). Microbial populations were enumerated following the completion of the batch experiments. Relative populations of SRB and iron reducing bacteria (IRB) were determined using the most probable number (MPN) technique (Cochran 1950).

**RESULTS AND DISCUSSION**

**Effect of Fe\(^0\) and Cu\(^0\) on sulfate reduction**

The performance of the systems was analyzed by taking into account the efficiency of sulfate reduction when the COD concentration of AMD was 3,600 mg/L, and the initial pH was 7.0. The effect of Fe\(^0\) and Cu\(^0\) on sulfate reduction is shown in Figure 1. As shown, no sulfate reduction occurred in the case of Fe/Cu. This indicates that sulfate could not be reduced by Fe\(^0\), and the adsorption of sulfate by Fe\(^0\) or Cu\(^0\) was negligible. The standard Gibbs free energy change (\(\Delta G_0\)) for the reaction \(4\text{Fe}^0 + \text{SO}_4^{2-} + 8\text{H}^+ \rightarrow 4\text{Fe}^{2+} + \text{S}^2/\text{C}_0 + 4\text{H}_2\text{O}\) is about 1,430 KJ/mol > 0. Thus, this reaction cannot take place spontaneously.

All curves in Figure 1 follow a similar pattern, namely a time lag was required by the microflora to adapt to the change in environment, after which the sulfate was reduced at accelerating rates. This lag phase was minimum at Fe/Cu + SRB. At the end of the experiment, the residual \(\text{SO}_4^{2-}\) concentrations across various treatments decreased in the order Fe/Cu > SRB > Fe + SRB > Fe/Cu + SRB (Figure 1). After 60 days, the residual \(\text{SO}_4^{2-}\) concentrations were 1,450, 710 and 240 mg/L for systems SRB, Fe + SRB and Fe/Cu + SRB, respectively, corresponding to removal ratios of 51.6%, 76.3% and 92.0%, respectively. The reduction rate of sulfate declined quickly after 10 days and then gradually reached a plateau. These results indicate that sulfate reduction can be enhanced by the presence of Fe\(^0\). Briefly, cathodic H\(_2\) is produced when Fe\(^0\) corrodes anaerobically (Equations (1) and (2)) (Ayala-Parra et al. 2016):

\[
\text{Fe}^0 + 2\text{H}_2\text{O} \rightarrow \text{Fe}^{2+} + \text{H}_2 + 2\text{OH}^- \quad (1)
\]

\[
\text{SO}_4^{2-} + 4\text{H}_2 + \text{H}^+ \rightarrow \text{HS}^- + 4\text{H}_2\text{O} \quad (2)
\]

H\(_2\) can then serve as an electron donor for stimulating the anaerobic biotransformation of reducible contaminants. Additionally, there is evidence for direct electron transfer from Fe\(^0\) to microbes based on SRB accelerating the rate of Fe\(^0\) corrosion beyond the abiotic rate (Venzlaff et al. 2015). Moreover, adding Fe\(^0\) could reduce the toxicity of sulfides to microbes by the formation of FeS precipitation (Zhang et al. 2011).

Figure 1 also demonstrates that adding Cu\(^0\) can further promote sulfate reduction by SRB relative to Fe + SRB. Cu\(^0\) can improve the reduction capacity of Fe\(^0\) due to the high reaction potential, and higher pollutant removal efficiency could be obtained even in the neutral pH range (Fan & Ma 2009). Two types of catalytic mechanisms of...
the bimetallic reductants have been proposed (Lai et al. 2014): (a) indirect reduction by the atomic hydrogen absorbed on the surface of bimetallic reductants, and the transition metal additives can facilitate the generation of surface-bond atomic hydrogen, (b) direct reduction on the catalytic activity site by accepting electrons from the oxidation of Fe⁰, and the transition metal additives can increase the oxidation of Fe⁰ through the formation of infinite galvanic cells.

**Effect of COD/SO⁴²⁻ ratio on sulfate reduction**

In this work, glucose was provided as the sole organic substrate for microbial growth and sulfate reduction, and the relevant reaction is as follows:

\[ \text{organics(COD)} + \text{SO}_4^{2-} + \text{H}_2\text{O} \rightarrow \text{H}_2\text{S} + 2\text{HCO}_3^- \]  (3)

The COD mass used for the desulfurization was calculated based on stoichiometry that 0.67 g COD is required to reduce 1 g of SO⁴²⁻; meanwhile, 1.042 g of alkalinity is produced (as CaCO₃) (Vossoughi et al. 2005). Thus, in subsequent experiments, the effect of COD/SO⁴²⁻ ratio on SO⁴²⁻ reduction was investigated by varying the COD concentration of wastewater, while the SO⁴²⁻ concentration was kept at 3,000 mg/L.

Figure 2 shows the plots of the COD/SO⁴²⁻ ratio and sulfate removal in the bottles after 60 days of incubation. From data presented in Figure 2 it can be observed that the COD/SO⁴²⁻ ratio has an apparent effect on sulfate reduction. The removal efficiency of SO⁴²⁻ significantly increased with increasing the COD/SO⁴²⁻ ratio from 0.3 to 1.2. This demonstrates that the wastewater COD could not satisfy the SRB requirement for growth and sulfate reduction at low COD/SO⁴²⁻ ratio, and SO⁴²⁻ could not be efficiently reduced to sulfide by SRB because of the deficiency of electron donors. When the COD/SO⁴²⁻ ratio was varied between 1.2 and 2.0, sulfate removal remained relatively stable at 51.6–92.2%. In this study, a similar situation was observed among various systems: namely higher sulfate reduction in Fe⁰-amended systems did not result in a higher organics requirement. This indicates that cathodic H₂ could serve as an electron donor for sulfate reduction by SRB (Equation (1)).

Several studies have been conducted to find the optimum COD/SO⁴²⁻ ratios for SRB to reduce sulfate but the results were diverse. The best COD/SO⁴²⁻ ratio was found to be 5.0 with sludge as the organic substrate (Al-Ani 1994), whereas SRB was predominant for a ratio below 1.7 using natural or synthetic substrates (Prasad et al. 1999). When complex organics were used as substrate, higher optimum ratios were likely obtained because not all the carbon present was utilized by SRB. Glucose is an excellent carbon source for microbes, thus a low COD/SO⁴²⁻ ratio was demonstrated in this study.

**Effect of initial pH on sulfate reduction**

The effect of initial pH on sulfate reduction was tested with pH decreasing from 7.0 to 3.0, when the concentrations of SO⁴²⁻ and COD were respectively 3,000 and 3,600 mg/L, and the temperature of the system was 37 °C. Figure 3 shows the changes of sulfate removal and effluent pH in the bottles after 60 days of incubation across the three systems. As shown, initial pH exerted an apparent effect on sulfate removal across various systems. In the SRB system, sulfate removal gradually declined with the initial pH decreasing from 7.0 to 4.0, and an apparent decrease of sulfate removal was observed with the decrease of pH from 4.0 to 3.0 (Figure 3(a)). In contrast, the influence of initial pH on sulfate removal in the Fe + SRB and Fe/Cu + SRB systems...
was slighter compared with that in the SRB system (Figure 3). The sulfate removal of the SRB, Fe + SRB and Fe/Cu + SRB systems were 32.5%, 63.5% and 78.4% respectively with the initial pH at 4.0. Sulfate removal was only 23.6% in the SRB system with pH 3.0 (Figure 3(a)). Sulfate removal reached 58.2% and 74.3% in the Fe + SRB and Fe/Cu + SRB systems respectively under the same pH condition (Figure 3(b) and 3(c)). The optimal pH for SRB growth was around 6–7, whereas the performance of the Fe0-amended systems was good for sulfate removal when the initial pH was above 4.0.

A similar behavior of the effluent pH influenced by the changed initial pH was observed in the three systems (Figure 3). The effluent pH in the SRB system was above 6.2 with the initial pH above 4.0. When the initial pH decreased to 3.0, the effluent pH was about 5.5 (Figure 3(a)). In the Fe + SRB and Fe/Cu + SRB systems, effluent pH was above 6.5 with the initial pH from 3.0 to 7.0. The result of initial pH effect on sulfate removal and effluent pH suggests that Fe0 could increase the pH of the systems to improve the SRB activity. In this study, the increase of effluent pH after incubation was primarily due to the release of bicarbonate alkalinity during sulfate reduction (Equation 3), which is beneficial for acid neutralization and pH buffering (Singh et al. 2011). Additionally, in Fe0-amended systems, alkalinity can be generated by anoxic corrosion of Fe0 (Equation (1)) and sulfate reduction (Equation (2)), as both processes involve the consumption of hydrogen ions. Therefore, the pH values of the effluent from the Fe + SRB and Fe/Cu + SRB systems were higher than that from the SRB system.

![Figure 3](https://iwaponline.com/jwrd/article-pdf/8/4/447/482412/jwrd0080447.pdf)

Figure 3 | Effect of initial pH on sulfate removal and effluent pH of the three systems after 60 days of incubation. Experimental conditions were: [SO4\(^{2-}\)]\(_0\) = 3,000 mg/L, [COD]\(_0\) = 3,600 mg/L, T = 37 °C.

Metal removal

To confirm the positive effect of Fe0, heavy metals removal was monitored during the first 96 h of reaction in the Fe, SRB, Fe + SRB and Fe/Cu + SRB systems with Pb\(^{2+}\), Cu\(^{2+}\) and Zn\(^{2+}\) concentrations of 50, 50 and 50 mg/L, respectively (Table 1). In the Fe system, the concentration of Zn\(^{2+}\) was almost unchanged, since Zn\(^{2+}\) could not be reduced by Fe0; however, the Pb\(^{2+}\) and Cu\(^{2+}\) removal was almost 100% after 72 h, indicating the reduction and precipitation of Pb\(^{2+}\) and Cu\(^{2+}\). In the SRB, Fe + SRB and Fe/Cu + SRB systems, the removal ratios of Pb\(^{2+}\), Cu\(^{2+}\) and Zn\(^{2+}\) were 100% after 48 h. At the end of experiments, the residual ratios of Fe0 were 34.3% and 28.5% in the Fe + SRB and Fe/Cu + SRB systems, while the residual ratio of Cu0 was 92.6% (data not shown).
It was remarkable that the results of SRB, Fe + SRB and Fe/Cu + SRB systems were identical, indicating that the content of sulfides was sufficient for precipitating with metals. The addition of Fe⁰ could enhance the pH value of the solution and the sulfate reduction capacity of SRB in anaerobic systems, leading to the accelerated removal of heavy metals. Metal-sulfide mineral precipitation and reduction/precipitation were the two dominant mechanisms of metal removal in this study. However, other mechanisms, such as (oxy)hydroxide precipitation, co-precipitation and surface complexation, may be important (Yue et al. 2013). The contribution of individual routes to metal removal will be further explored in the following work.

Microbial activity

Figure 4 shows the populations of SRB and IRB in samples collected after 60 days. The number of SRB populations ranged from 5.2×10⁶ to 1.4×10⁷ MPN/mL, while no evidence of both SRB and IRB growth was observed in the sample collected from the Fe system. Additionally, no IRB was detected in the SRB system, since no Fe⁰ was applied in this case. Populations of IRB ranged from 2.4×10⁴ to 2.3×10⁵ MPN/mL, and the MPN value was approximately 2 orders of magnitude lower than that of SRB (Figure 4).

The SRB populations are consistent with calculated sulfate reduction efficiency (Figure 1). Although H₂(g) concentrations were not measured, increments in pH were indicative of anaerobic Fe⁰ corrosion which produced H₂(g) (Equation (1)). SRB growth could be enhanced with the addition of H₂ as an electron donor (Ayala-Parra et al. 2016). Improved sulfate reduction efficiency in the presence of Fe⁰ may therefore correspond to higher relative populations of H₂ oxidizing SRB.

Sulfur element balance

Sulfate conversion was analyzed with the contents of different forms of sulfur. Figure 5 shows the S balance in the three systems. The proportion of sulfate converted into gas H₂S was 20.6–45.4%, whereas aqueous sulfide in the effluent accounted for 6.3–21.5% of influent sulfate. About 5.7–36.8% of sulfate was reduced to S²⁻ and then precipitated with metals such as Fe, Pb, Cu and Zn. About 8–48.4% of sulfate was left in the effluent. Moreover, 3.5–3.8% of sulfate

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**Table 1** The concentration of heavy metals (mg/L) with time in the four systems under the conditions: [SO₄²⁻]₀ = 3,000 mg/L, [COD]₀ = 3,600 mg/L, pH₀ = 7.0, T = 37 °C

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dissipated through other processes such as microbial assimilation, oxidation of sulfide into elemental sulfur, etc. Apparently, the addition of Fe⁰ greatly reduced the content of aqueous sulfide, which may lessen the inhibitory effect of sulfide on SRB.

Economic analysis

A rough economic analysis was performed on the operating costs, such as the costs of reagents and the costs of energy. It should be noted that this analysis is just an approximate tool to differentiate the trends in the operating costs associated with the use of combined treatment. A rigorous economic analysis should consider initial investment, prices at plant scale, maintenance and labor costs and so forth. Table 2 shows that the total reagent and power costs were $0.95/m³. The cost of an advanced and complete treatment is always up to $5–7/m³ for industrial wastewater (Oller et al. 2011), which is higher than that in the present study. This may be because the investments in equipment and construction, maintenance and labor costs were not included in the operating cost calculations in the present study.

CONCLUSIONS

The results of operating conditions on sulfate reduction in four systems indicate an enhanced activity of SRB by adding Fe⁰/Cu⁰. The highest sulfate reduction efficiency of 92.0% was obtained with the feed sulfate loading rate of 3,000 mg/L in the Fe/Cu + SRB system, which was significantly higher than that of SRB and Fe + SRB systems. The presence of Fe⁰ could increase solution pH and decrease solution toxicity through the formation of metal sulfide precipitate. The sulfate reduction of the Fe + SRB system could be further enhanced by adding Cu⁰. This study demonstrates that the Fe/Cu + SRB system could be a promising technology for treating wastewater containing heavy metals and sulfate.

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