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Simultaneous Removal of Copper, Zinc, and Sulfate from Coal Mine Waste in a Laboratory SRB Bioreactor Using Lactate or Ethanol as Carbon Sources

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Abstract The feasibility of inoculating coal mine waste piles with sulfate-reducing bacteria (SRB) to prevent the production of acidic leachates containing sulfate and metal contaminants was evaluated in batch and column bioreactors. The results showed that SRB growth and activity could be attained in the presence of acidic (pH 4.5) coal mine waste using lactate or ethanol as a carbon source, while no obvious growth was found at pH <3.5. Inoculation of coal mine waste in batch reactors with lactate or ethanol as a carbon source resulted in efficient neutralization and high removal of sulfate and metals. Similar results were attained in dynamic-flow columns inoculated with SRB. SEM-EDS analysis of the precipitates showed iron sulfide to be the main component. This study indicates that SRB could possibly be used to prevent or limit acidic drainage from coal mine waste piles.

Keywords Carbon source · Coal mine waste · Metal contaminants · Sulfate-reducing bacteria

Introduction

Acidic mine drainage (AMD) can be produced when sulfide minerals in coal mine waste are exposed to water and oxygen. Several methods have been reported in the literature for treatment of AMD, including microbial treatment by sulfate-reducing bacteria (SRB). This treatment is based on the fact that SRB can use organic carbon as an electron donor and the sulfate present in AMD as an electron acceptor; under anaerobic conditions, the process generates alkalinity and promotes metal precipitation as sulfides (Jong and Parry 2003). Immobilization of contaminants within coal mine waste piles using SRB has not yet been extensively studied, though it has been successfully tested in various ways (Behrooz and Borden 2012; Germain et al. 2003a, b; Jin et al. 2008a, b; Kim et al. 1999).

The choice of a carbon source for SRB activity is highly important (Costa et al. 2009; Liamleam and Annachhatre 2007). Simple organic compounds are generally used as carbon and energy sources by SRB (Neculita and Zagury 2008). Among these, lactate has been widely applied in lab-scale experiments, but its large scale application could be limited due to its high operative costs (Kaksonen et al. 2003; Pagnanellia et al. 2012). Ethanol is an attractive alternative due to its ease of availability and its relatively low cost in comparison with lactate (Costa et al. 2008). Although efficient sulfate reduction by SRB growing with ethanol as a carbon source in AMD has been reported (Greben et al. 2000; Kaksonen et al. 2003; Luo et al. 2008; Pagnanellia et al. 2012; Sobolewski 2010), the ability of SRB to immobilize metals in coal mine waste using ethanol as a carbon source has not been extensively studied. So, we evaluated the ability of SRB to immobilize metal contaminants and prevent the generation of AMD from coal mine waste in situ and compared ethanol and lactate as carbon sources for SRB. Batch and column experiments were conducted to examine whether an active SRB population could be established in coal mine waste and to evaluate the feasibility of applying ethanol or lactate as carbon source to promote SRB activity and thereby precipitate contaminants within the coal mine waste.

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Materials and Methods

Coal mine waste samples used in this study were collected from a coal mine waste pile (already dumped for 1 year) in Yangquan coal mine, Shanxi province, China. The samples were ground, passed through a 2 mm sieve, and dried at 105 °C for 12 h. The paste pH of the waste in 1:5 (w/v) solid/water suspensions, after equilibrium for 24 h, was 4.5, using a PHS-3C pH meter.

The SRB species was isolated from Jiazi river sediment at the University of Jinan. SRB was cultivated in anaerobic conditions. Widdel and Pfennig medium was used to isolate SRB in this study, since all SRB cultivated to date can be grown on a variation of this medium (Widdel and Pfennig 1981; Widdel and Hansen 1992). The basal medium had the following composition: Na_2SO_4 (3.0 g/L); KH_2PO_4 (0.2 g/L); NH_4Cl (0.3 g/L); KCl (0.5 g/L); $CaCl_2 \cdot 2H_2O$ (0.15 g/L); NaC1 (1.0 g/L); $MgC1_2 \cdot 6H_2O$ (0.4 g/L), and resazurin (0.1 %, 1.0 mL/L). Sodium lactate (10 mM), and ethanol (10 mM) were the carbon sources in the isolation experiment. To the autoclaved and cooled medium, trace element solution SL7 (1 mL/L), bicarbonate solution (30 mL/L), sulfide solution (3 mL/L) and vitamin solution (1 ml/L) were added from sterile stock solutions, as described by Hurst et al. (2007) and Widdel and Pfennig (1981). The final pH of the medium was adjusted to 7.0. SRB growth was cultivated and isolated at 30 °C in a biochemical incubator. The performance of lactate and ethanol as carbon source was assessed in terms of pH, ORP, and sulfate concentrations. Precipitation of black FeS and production of H_2S were monitored, the latter by using lead acetate paper (Linderholm et al. 2008). The SRB were sub-cultured three times on Widdel and Pfennig medium before inoculation into the bioreactors.

To evaluate the feasibility of growing SRB in coal mine waste and the removal efficiency of sulfate at different pH conditions, 25 g of coal mine waste was added to each conical flasks containing 150 mL of medium (containing 0.017 g/L of KH_2PO_4; 0.1 g/L of NH_4Cl; and 5 mM of ethanol), and then 5 mL of SRB inoculum was added. The initial pH of the medium was adjusted to 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, and 7.0 using 0.5 mol/L H_2SO_4 and NaOH. The batch tests were conducted at 30 °C in a biochemical incubator and pH, ORP, SO_4^{2-}, and H_2S production were monitored at day 7. No effort was made to deplete oxygen from the medium. Each test was performed in triplicate and the results were averaged.

In order to investigate the removal of sulfate and metals by SRB with lactate or ethanol as carbon sources, three batch tests were conducted with different carbon sources using sealed Erlenmeyer flasks: (1) 150 mL of sterile medium (containing 5 mM of sodium lactate) + 5 mL of SRB inoculum + 25 g of coal mine waste; (2) 150 mL of sterile medium (containing 5 mM of ethanol) + 5 mL of SRB inoculum + 25 g of coal mine waste; (3) 150 mL of sterile distilled water + 25 g of coal mine waste (blank). The pH of the mixtures was not adjusted; the initial pH was about 4.6. The batch tests were performed at 30 °C in a biochemical incubator and pH, ORP, SO_4^{2-}, Fe, Cu, and Zn were monitored every 2 days. Again, no effort was made to deplete oxygen, each test was conducted in triplicate, and the results were averaged.

The removal of sulfate, iron, and other metals from coal mine waste by SRB activity in dynamic leaching condition was assessed using glass column bioreactors (diameter 5.0 cm, height 30 cm) at elevated temperature (30 ± 2 °C) for 22 days. Three columns were each packed with 500 g of coal mine waste atop 30 g of silica sand. Column I was fed with distilled water; column II was inoculated with SRB and fed fresh medium containing lactate (5 mM); and column III was inoculated with SRB and fed fresh medium containing ethanol (5 mM). Before leaching, the column bioreactors were inoculated with SRB and added medium under anaerobic conditions for 2 days to promote bacterial growth. The experiment was irrigated with the influent (30 mL/d) fed at the top of the column and the effluent was collected at the bottom. Samples were collected from each bioreactor via a port at the base of each column every 2 days.

The pH and ORP of effluent samples were measured immediately using a PHS-3C pH/ORP meter. Sulfate concentrations were determined using the turbidimetric method (US EPA 1986: Method 9038). Fe, Cu, and Zn were measured by flame atomic absorption spectroscopy using a PE-900T model spectrometer. SRB populations were enumerated by the three-tube most probable number (MPN) assay. The assays were conducted in triplicate. The MPN tubes were incubated at room temperature for 5 days (Costa et al. 2009).

Surface morphology of the formed precipitates was examined using a Quanta FEG 250 scanning electron microscope (SEM). The SEM was equipped with an energy dispersion spectrometer (EDS, X-Max50), which was used to analyze the formed precipitates.

Results and Discussion

Batch Test Results

SRB growth was clearly evidenced by the visible formation of black precipitates (FeS) and the classic strong smell of H_2S. Sulfate reduction of 92.1 % was obtained in 2 days in the batch tests using Widdel and Pfennig medium. A pH of 7.6 and ORP of −362 mV were obtained. This demonstrates good SRB growth and confirmed that ethanol and lactate were good carbon sources.

The SRB grew with difficulty on a strongly acidic medium (pH 3.0 and 3.5) and no obvious decrease in the ORP or increase in pH was observed, which indicates that the strongly acidic medium inhibited the growth of the bacteria. Sulfate removal of 83.4 and 87.9 % was attained in 7 days at pH 4.5 and 5.5, respectively (Fig. 1), and medium pH increased from 4.5 to 7.1 and from 5.5 to 7.3. ORP decreased from 297 to −282 mV, and from 305 to −295 mV in the same two tests. These measurements, along with the formation of black precipitate at pH 4.5 and 5.5, and the sensorial detection of H_2S during sample collection from the liquid phase all indicate that SRB can grow well in moderately acidic media. Meanwhile, it should be mentioned that using H_2SO_4-acidified coal mine waste isn't the same as a coal mine waste that contains mineral (ferric) acidity, and differences could occur between 'naturally' weathered coal mine waste and one that has been acidified in the lab.

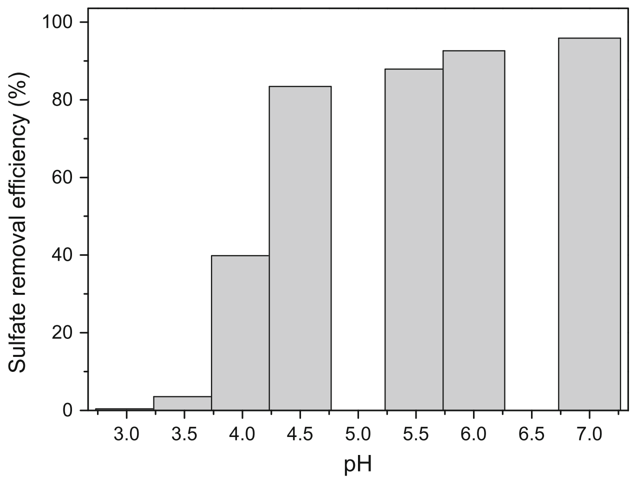

Fig. 1 Effect of pH on sulfate reduction from coal mine waste by SRB

The removal efficiencies of sulfate from coal mine waste by SRB using ethanol and lactate as carbon source are shown in Fig. 2. In the batch bioreactor using lactate as carbon source, sulfate concentrations fell 86.3 % in 7 days, with a rapid decrease during the first 3 days, followed by slower removal. The pH increased from 4.6 to 7.4 and ORP decreased from 273 to −286 mV. The batch experiments using ethanol showed a rapid removal of sulfate occurred until day 5. Final sulfate reduction (84.1 % in day 7) and operative conditions of pH and ORP (pH 7.2, ORP

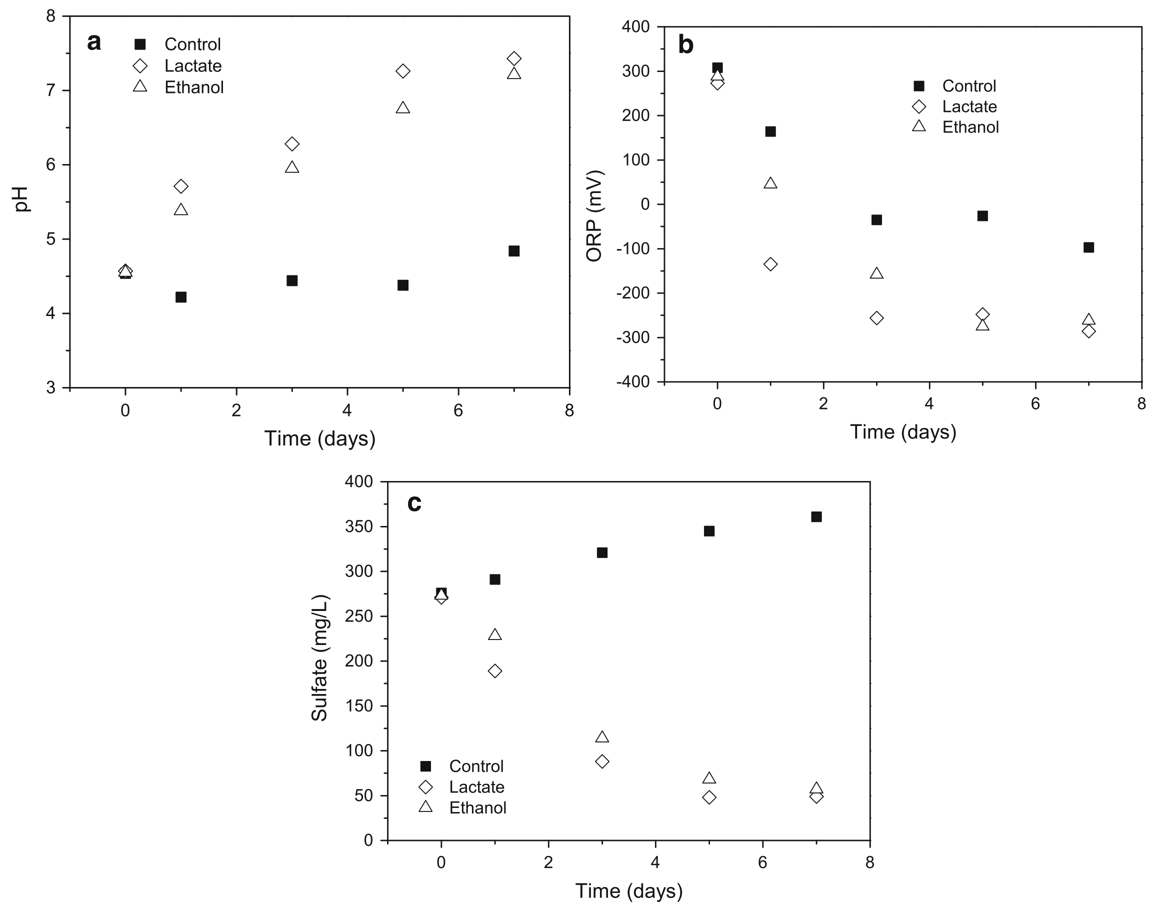

Fig. 2 Variation of pH (**a**), ORP (**b**), and sulfate (**c**) of samples from different batch bioreactors

Table 1 Concentrations of metals in batch reactors effluents (mg/L)

Metals	Control column	Lactate column	Ethanol column
Fe	11.26	0.28	1.05
Cu	0.07	BDL	BDL
Zn	0.15	0.04	0.02

BDL below detection limit

−275 mV) confirmed that the SRB were using ethanol as carbon source (Pagnanellia et al. 2012). In contrast, sulfate concentrations in the control bioreactor increased from 276 to 361 mg/L, due to dissolution and release of sulfate from the coal mine waste, and pH ranged from 4.2 to 4.8.

The ability of SRB growing on coal mine waste to remove certain metals was also investigated by comparing the metal concentrations in SRB and control bioreactors (Table 1). At day 7, the concentrations of Fe, Cu, and Zn from control bioreactor samples were 11.26, 0.07, and 0.15 mg/L, respectively. In SRB-inoculated bioreactors using lactate or ethanol as a carbon source, the concentrations of Fe dramatically decreased to 0.28 and 1.05 mg/L, Cu decreased to below detection limits, and Zn decreased to 0.04 and 0.02 mg/L, respectively. Thus, inoculation of coal mine waste in batch reactors with lactate and ethanol resulted in the efficient neutralization and high removal of sulfate (86.3, 84.1 %) and metals (Fe 97.5, 90.7 %; Cu 100, 100 %; Zn 73.3, 86.7 %). This removal was mainly attributed to the precipitation of the metals as sulfides, and phosphate, carbonate precipitates or sorption to Fe-oxy-hydroxides could also be causing metal removal (Jong and Parry 2003).

Column Study Results

Figure 3a presents the pH of the different treatments during the column experiments. Leachates from control column I were mildly acidic (pH around 4.2–5.1) throughout the experiment. As expected, the effluent pH from the SRB column II (lactate as a carbon source) increased to 7.7 in 6 days as a result of the alkalinity generated by SRB metabolism. The effluent pH from SRB column III (ethanol as a carbon source) was also enhanced to 7.3. This indicated that SRB metabolic process was not adversely affected nor inhibited by the initial low pH and that ethanol can be used as an effective carbon source for SRB in coal mine waste. The neutralization in column III was a little poorer than in column II, presumably due to the necessary conversion of ethanol into acetate before it could be used by the SRB (Liamleam and Annachhatre 2007; Muyzer and Stams 2008).

Although ORP in the control column also decreased, reaching values near −86 mV, it remained significantly higher than those of the SRB columns. The ORP reached values near −285 mV in column II and −206 mV in column III by the end of the experiment (Fig. 3b). The addition of a carbon source promoted the growth of SRB and thus the decrease of ORP (Pagnanellia et al. 2012).

The sulfate concentrations in the different column effluents are presented in Fig. 4. Sulfate concentrations in the effluents from the control column remained high, within the range of 902–2,738 mg/L, although it decreased during the experiment. The sulfate concentrations in the treatment systems inoculated with SRB decreased rapidly, which demonstrated strong sulfate reducing activity. Despite the high sulfate concentrations at the beginning of the experiment (close to 2,500 mg/L), values decreased gradually until a steady state was attained after approximately 12 days; sulfate concentrations decreased to about 72.5 and 85.6 mg/L in column II and III (corresponding to over 90 % removal), well below the maximum of 250 mg/L allowed by Environmental Quality Standards for Surface Water in China (GB3838-2002).

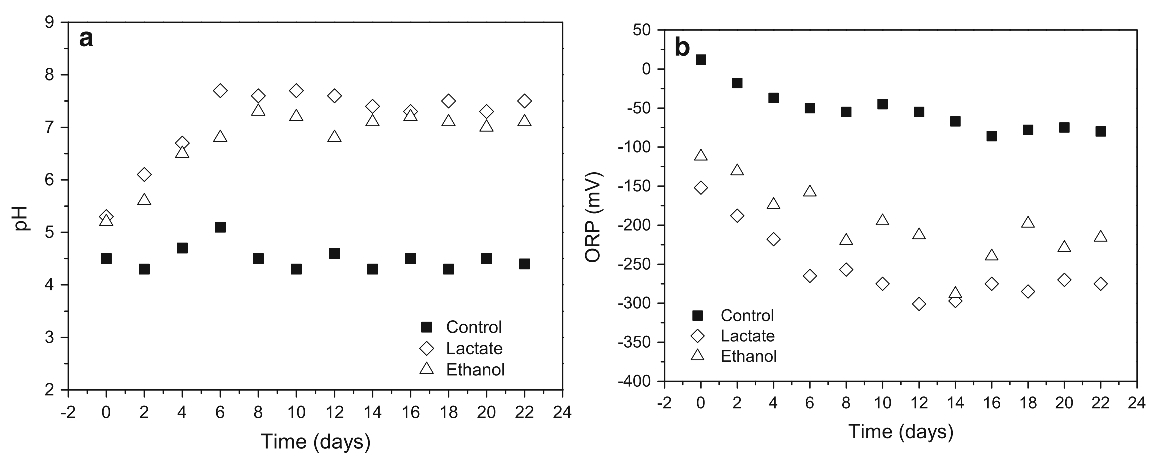

Fig. 3 Variation of pH (a), ORP (b) of effluents from different column bioreactors

Fe, Zn, and Cu concentrations in the leachates of column I (control) remained relatively high, although they decreased from 215.60 to 26.25 mg/L, from 2.53 to 0.09 mg/L, and from 0.91 to 0.07 mg/L, respectively (Fig. 5). In contrast, the concentrations of these metals were much lower in the leachates of the columns inoculated with SRB. Fe, Zn, and Cu concentrations in column II (lactate as a carbon source) remained in the range of 106.15–2.18, 0.32–0.03, and 0.12–0.01 mg/L, and those in column III (ethanol as a carbon source) remained in the range of 114.90–3.35, 0.49–0.04, and 0.23–0.02 mg/L.

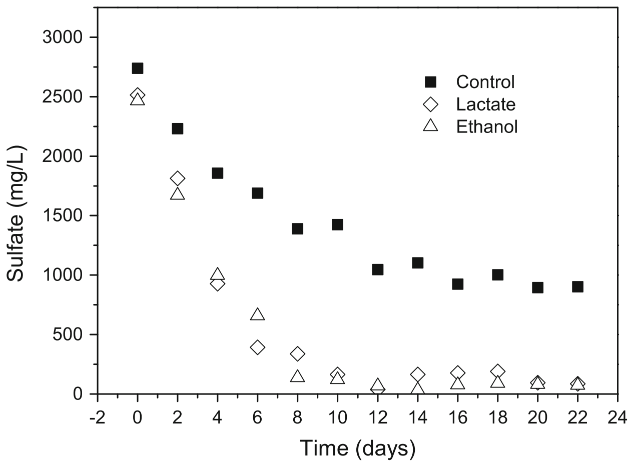

Fig. 4 Variation of sulfate concentrations of effluents from different column bioreactors

Black precipitate was observed on the bioreactor wall and silica sand located at the bottom of the SRB column bioreactors, while no black precipitate was observed in the control column. SEM analysis shows that the precipitates are composed of small variably-sized micro-particles (Fig. 6a). EDS spectra presented in the same figure show that the precipitates mainly consist of iron and sulfur (Fig. 6b), consistent with FeS precipitation. EDS spectra of Cu and Zn were not found, probably due to their low concentration in the coal mine waste.

SRB quantification showed that the addition of either lactate or ethanol achieved considerable MPN. The maximum MPN for the lactate column was about 8×10^5 CFU/mL, while the maximum MPN for the ethanol column was about 5×10^5 CFU/mL. This indicates that an active SRB population can be established in coal mine waste piles with either lactate or ethanol as a carbon source. Column tests

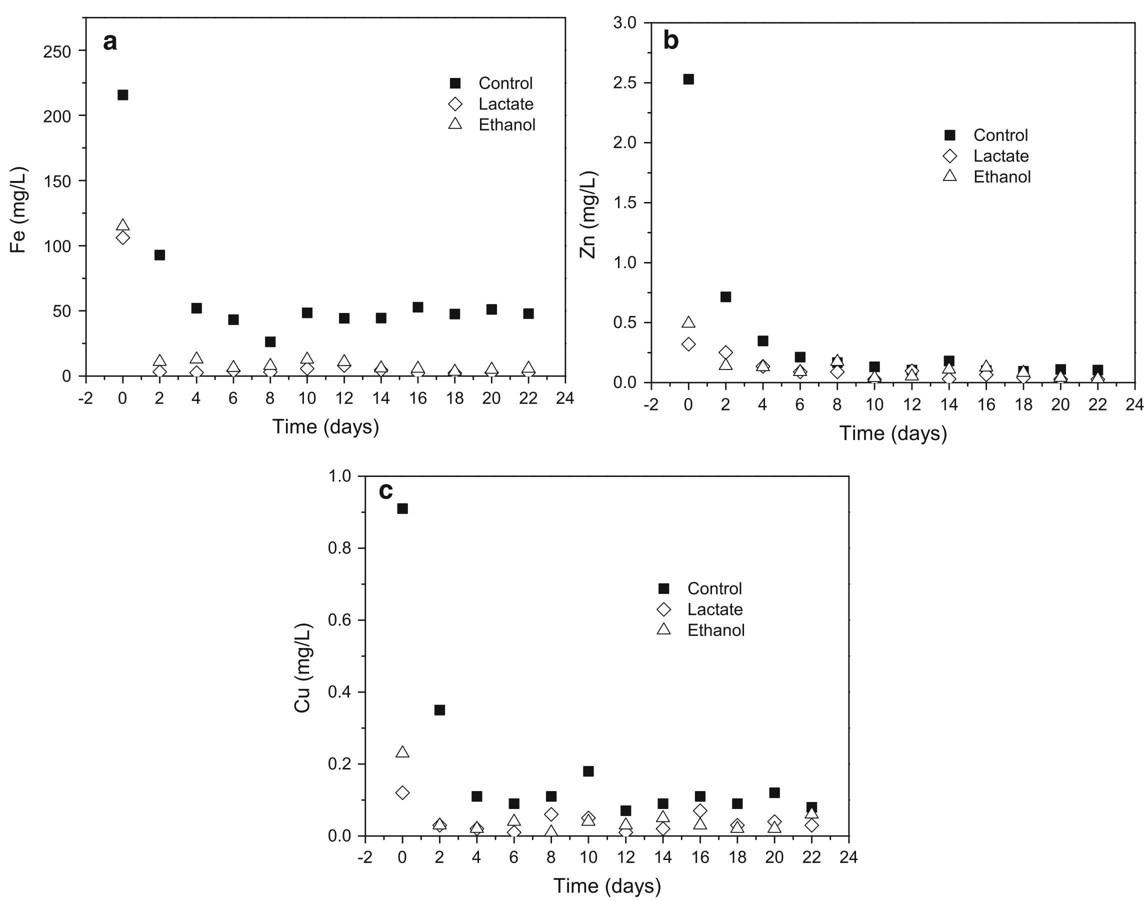

Fig. 5 Variation of Fe (**a**), Zn (**b**), and Cu (**c**) of effluents from different column bioreactors

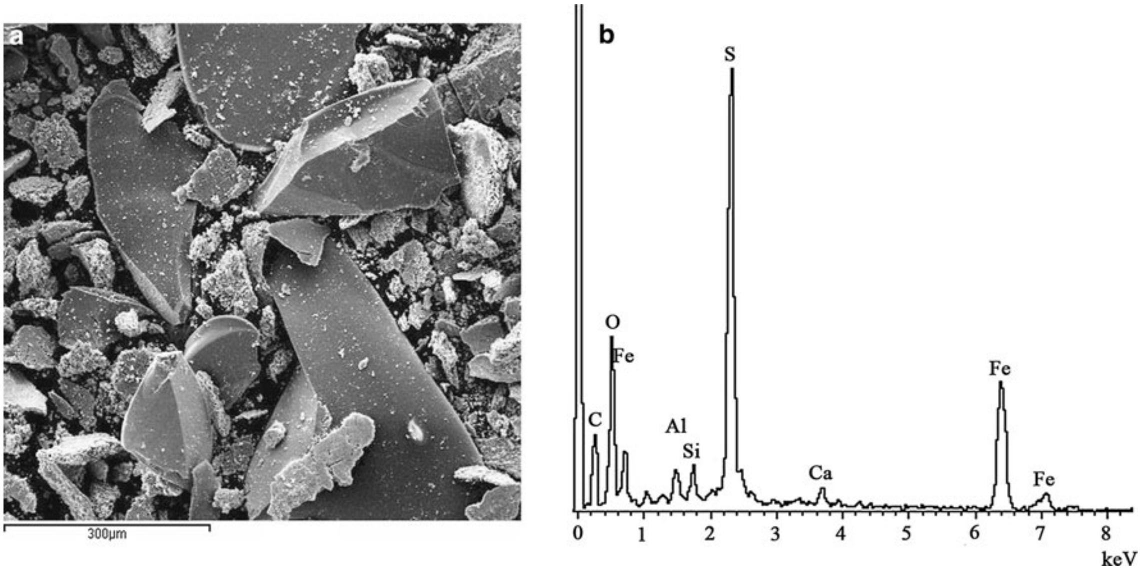

Fig. 6 SEM micrograph (**a**) and EDS spectra (**b**) of the precipitates from SRB bioreactors

with ethanol confirmed that SRB can use this substrate to remove sulfate and metals efficiently, offering a valid alternative to lactate substrates.

This research indicated that the generation of acidic leachate from coal mine waste piles could be limited or prevented by adding organic carbon to coal mine waste piles and promoting the activity of SRB. Moreover, the addition of organic carbon to coal mine waste piles can contribute to the formation of anaerobic conditions that can inhibit the activity of the iron-oxidizing species that play an important role in sulfide oxidation and subsequent generation of acidic leachate (Kleinmann and Crerar 1979).

In addition to organic carbon materials, agricultural and municipal wastes (e.g. animal manure, municipal compost, peat, wood chips, straw, sawdust, leaf compost, dairy waste) have been used as low-cost solid carbon source for AMD treatment (Chang et al. 2000; Gibert et al. 2004; Zagury et al. 2006). Agricultural and municipal wastes can also serve as nutrient additives to promote revegetation of mine waste piles (Halofsky and McCormick 2005). So the combined utilization of SRB and organic wastes to prevent the production of acidic leachate from coal mine waste will be investigated in next experiment, and attention will be paid to other potential contaminants, such as arsenic, selenium, and manganese since their chemical speciation and bioavailability are affected by a low ORP environment.

Conclusions

The results showed SRB growth and activity could be attained in the presence of acidic coal waste (pH 4.5) using lactate or ethanol as a carbon source, but that no obvious growth was found in strongly acidic condition (pH <3.5). Inoculation of coal mine waste in batch reactors and dynamic flow column reactors with lactate and ethanol as carbon source resulted in efficient neutralization and high removal rates of sulfate and metals. This demonstrates that removal of sulfate and heavy metals by inoculating coal mine waste pile with SRB could potentially be a novel alternative to traditional passive treatment of acid leachate from coal mine waste.

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