



Selective recovery of soil-borne metal contaminants through integrated solubilization by biogenic sulfuric acid and precipitation by biogenic sulfide

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ABSTRACT

A hybrid process combining solubilization via sulfuric acid produced by sulfur-oxidizing bacteria with precipitation via sulfide produced by sulfate-reducing bacteria was investigated to isolate soil-borne metal contaminants as purified metal-sulfides. The highly efficient two-step acidification process involved bioproduction of sulfuric acid in a culture medium containing 30% (v/v) of sludge filtrate (SF). Soil was added to the culture after maximum acid production. Solubilization efficiencies of 95% for Zn, 76% for Cu and 97% for Cd were achieved after 16 days. At pH 1.9, 3.0 and 4.0, 99% of Cu²⁺, 96% of Cd³⁺ and 93% of Zn²⁺, respectively, were precipitated from the soil leachate by sulfide transported from sulfidogenic bioreactor via N₂ sparging, resulting in final effluent metal contents at the ppb-level. The introduction of SF did not affect the precipitation kinetics and purity of the recovered precipitates. Ultimately, 75% of Cu and 86% of Zn were recovered from the soil as pure CuS and ZnS (confirmed by SEM-EDS and XRD). These results demonstrate the potential of the integrated method for the selective production of valuable metals from metal contamination in soils.

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1. Introduction

Heavy metal contamination in soil has received worldwide attention due to rapid industrialization and urbanization. According to China's EPA, over 10% of agricultural land encompassing approximately 10,000,000 ha in China is contaminated with toxic metals because of the repeated input of metal-laden agrochemicals on farmland together with accelerated mining and metallurgical activities over many years [1]. As much as 300–1000 mg/kg each of Zn, Cu, Pb, Cr and Ni are commonly found in polluted soils [2–4], and in some cases, extremely high levels of up to 5000 mg/kg of Cr, 2000 mg/kg of Cu and 200 mg/kg of Cd have been reported [5,6]. Considering the ecological impacts of toxic metals leaching into groundwater or accumulating up the food chain through plant uptake, the removal and/or recovery of these metals from soil is a necessity.

Microbial production of sulfuric acid via sulfur oxidation has proven to be effective in leaching contaminating metals from soil

[7–10]. In this process, bacteria from the genus *Acidithiobacillus* can convert the insoluble metal compounds in soil into soluble sulfates by acidifying the soil. The soluble metals are then sequestered from the soil solids by solid–liquid separation. In addition, metal precipitation driven by sulfate-reducing bacteria (mainly *Desulfovibrio* species) has been proposed to deal with the resultant soil leachate rich in metal sulfates because the sulfide that arises from microbial sulfate reduction readily reacts with most dissolved metals to form highly insoluble precipitates [11,12]. The combination of bioproduction of sulfuric acid and sulfide provides an interesting potential for deep cleanup of soil contaminated with toxic metals. To date, there have been several successful applications of this integrated method in the detoxification of soil-borne metal contaminants at both the laboratory and pilot scales. Most notably, White et al. [13] found that a range of metals, including Cu, Ni and Mn, could be leached from contaminated soil by biogenic sulfuric acid and subsequently concentrated as a mixture of multiple metal precipitates by biogenic sulfide. Once separated, the final solid precipitates represented a ~200-fold reduction in volume compared to the untreated soil.

The role of this combined process in removing soil metal contamination has been extensively addressed, but another potential advantage of this method, the selective synthesis of high purity metal-sulfides, tends to be overlooked. A growing demand for mineral resources together with a continuous rise in metal prices makes

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efficient recovery of valuable metals more important than mere removal. However, several ions derived from soil minerals, such as Fe^{2+} , Ca^{2+} and Mg^{2+} , coexist with the valuable metals in acidic soil leachate (pH commonly < pH 3) [7], and sulfate-reducers are generally sensitive to acidic pH [14]. It is therefore difficult at low pH to attain the desired separation of the target metals in a single stage reactor system that combines simultaneous sulfate reduction with metal-sulfide formation. A two-stage precipitation system, which transports the H_2S -laden effluent or gas from a sulfate-reducing bioreactor into a separate precipitator placed downstream from the bioreactor, is advantageous over a one-stage system because it recovers the targeted valuable metals (as pure metal-sulfide) from multi-metal solutions based on their different solubilities by controlling the pH and sulfide concentration in the precipitator [15]. This two-stage metal precipitation has been actively used in the purification of metallurgical wastewater and acid mine drainage (AMD) [16–18], but there have been few reports of this method for the selective recovery of metals from soil leachate via soil acidification.

To achieve high metal solubilization efficiency, soil acidification frequently requires additional nutrients (especially phosphorus and nitrogen) and often takes as long as 20–30 days [7,19]. The challenges of this method include introducing waste nutrient sources (e.g., natural nutrient-rich sewage sludge filtrate) as the microbial growth media and developing a two-step soil acidification process where soil is added after the maximum rate of sulfuric acid bioproduction is independently obtained. Successfully overcoming these challenges should result in a more efficient process for metal solubilization from soil. However, during precipitation of AMD by the sulfide-rich effluent from sulfate-reducing bioreactors, several unexhausted macro- and micronutrients (e.g., lactate, phosphate and ammonia) present in the effluent were found to adversely affect the metal precipitation kinetics by inhibiting nucleation and crystal growth [20,21]. Sewage sludge is rich in various organic compounds and nutrient salts, and it remains uncertain whether introducing sludge filtrate into soil acidification will affect metal precipitation from soil leachate containing sludge nutrient additives or influence the purity of the recovered metals.

The objective of the current study was to integrate metal solubilization by biogenic sulfuric acid with metal precipitation by biogenic sulfide for the selective production of pure metal-sulfides from metal-contaminated soil from a mining site. The effectiveness of a two-step acidification using sludge filtrate as the microbial growth substrate for metal solubilization and its effects on the separate recovery of metal-sulfides by a two-stage precipitation were examined.

2. Materials and methods

2.1. Soil

Heavy metal-contaminated soil was taken from the abandoned Chun'an mine tailing region in southeastern China. Sub-surface (10–20 cm depth) soil was collected using a plastic scoop and stored in airtight polythene bags at 4 °C. The soil was air-dried and sieved through a 2.0-mm sieve to remove gravel and litter. The soil's physicochemical properties are as follows: pH 7.6 (soil:distilled water = 1:2.5), 36.3 g/kg organic matter, 2.25 g/kg total N, 0.75 g/kg total P, 17.5 g/kg total K, cation exchange capacity of 215.9 mmol/kg, 1252 mg/kg Zn, 556 mg/kg Cu, 113 mg/kg Ni, 251 mg/kg Pb, and 15 mg/kg Cd. The Zn, Cu and Cd contents were above the highest accepted levels for these elements in the Soil Environmental Quality Criteria set by China's EPA of 500, 400 and 1.0 mg/kg, respectively [22].

2.2. Microorganisms and culture media

Acidithiobacillus thiooxidans FD97 (CGMCC No. 2760) was obtained from China's General Microbiological Culture Collection Center and used in the soil acidification experiments. The sulfur-oxidizers were grown in standard mineral medium (SM) comprised of 6.0, 3.0, 0.5, 1.0 and 0.25 g/L of S^0 , $(\text{NH}_4)_2\text{SO}_4$, KH_2PO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, respectively.

Sewage sludge filtrate (SF) was collected from the primary and secondary sludge belt-filter dehydration process from the Qingdao Urban Community sewage treatment plant. The concentrations of N- NH_4 and P- PO_4 in the SF were 36 and 260 mg/L, respectively, and the total organic carbon (TOC) of the SF ranged from 250 to 280 mg/L. Soluble Zn, Cu and Cd were undetectable in the SF. The SF samples were collected in polyethylene bottles and stored at 4 °C for a maximum of 4 weeks before use. The possibility of using the sludge nutrients to partially replace SM medium for bio-production of sulfuric acid was subsequently investigated.

To determine the optimal proportion of added SF for soil acidification, its effect on the bioproduction of sulfuric acid was studied in 250-mL Erlenmeyer flasks. Viable *At. thiooxidans* FD97 (1% (v/v)) and 6 g/L of S^0 were added to 150 mL of mixed culture medium with the following different SF/SM ratios (v/v): 100/0, 70/30, 30/70 and 0/100. All flasks were incubated on a gyrotary shaker at 28 °C and 150 rpm after adjusting the pH to 4.0. The loss of water in each flask due to evaporation was replenished with distilled water based on weight loss. During incubation, samples were periodically withdrawn from the flasks for pH and sulfate content determination. Sulfate was analyzed using the photometric turbidimetry method [23].

A mixed culture of sulfate-reducing bacteria derived from several environmental sources was used to generate sulfide for the selective precipitation of the target metals from soil leachate. The predominant sulfate-reducers in this culture were vibrio-shaped bacteria with approximately 2×10^8 cells/mL cell density. A Postgate's C medium with the following composition was used to prepare their enrichment culture: 0.5 g/L KH_2PO_4 , 1.0 g/L NH_4Cl , 4.5 g/L Na_2SO_4 , 1.0 g/L CaSO_4 , 0.5 g/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5, $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, 0.06, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.06, Na_2CO_3 3.5 and 1.0 g/L yeast extract.

2.3. Description of reactor system

Soil acidification experiments were performed in a 1.0-L stirred tank reactor (STR) (Fig. 1). Mixing was achieved using a variable speed mixer operated at 250 rpm with a three blade axial impeller (2.6-cm diameter). The diffused air supply to the reactor was monitored through a regulator and flow meter. The dissolved oxygen level remained at 5 mg/L throughout the experiments.

Selective metals precipitation trials were conducted in a laboratory batch treatment system consisting of a 1.0-L CSTR sulfidogenic reactor, a 1.0-L sealed sulfide-rich solution reservoir equipped with a gas (N_2) sparging device, a 0.7-L sealed STR precipitator, a gas scrubber containing 2.0M NaOH and a 1.0-L treated effluent settling tank. Soil leachate in the STR precipitator was mixed using a variable speed mixer at 200 rpm. All reactors were operated at room temperature (25 ± 2 °C).

2.4. Experimental procedures

Soil acidification: two groups of soil acidification experiments were conducted in two identical 1.0-L STR units: (i) incubation of *At. thiooxidans* FD97 with the soil (i.e., one-step acidification) and (ii) pre-incubation of *At. thiooxidans* FD97 before adding the soil once the maximum rate of sulfate formation had been achieved (i.e., two-step acidification). Each STR contained 1.5 g of S^0 , 50 mL of *At. thiooxidans* FD97, 15 g of soil and 450 mL of SF-SM medium

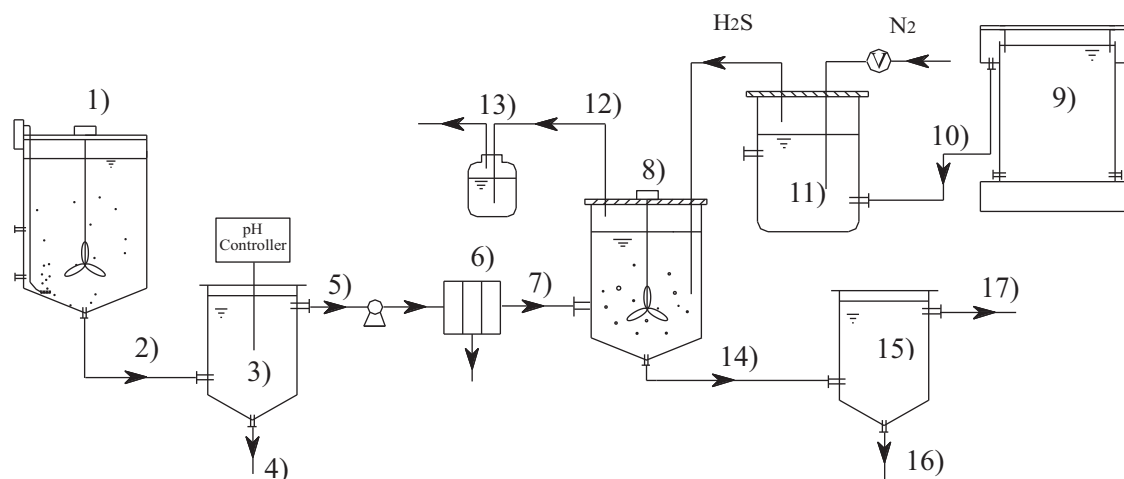


Fig. 1. Schematic representation of the combined treatment system for the selective recovery of contaminating metals from soil by biogenic sulfuric acid and sulfide. (1) Acidification reactor; (2) acidified soil suspension; (3) soil solution settling tank; (4) alkalization of soil solids; (5) soil leachate; (6) filter; (7) filtered soil leachate; (8) STR precipitator; (9) sulfidogenic reactor; (10) sulfide-laden effluent; (11) sulfide-rich solution reservoir (equipped with gas sparging using N_2); (12) H_2S ; (13) gas scrubber containing 2.0 M NaOH; (14) treated effluent; (15) effluent settling tank; (16) solid precipitates; (17) final effluent.

with a pre-determined mixing ratio. A control was not inoculated with *At. thiooxidans* FD97 for comparison. In another experiment, we demonstrated that the little to no soil acidification took place without the addition of any mineral medium or sludge filtrate over a 20-day period. All treatments and controls were done in triplicate. During soil acidification, 5 mL of the soil suspension was periodically taken from each STR for determination of pH (pHS-3D pH meter) and soluble metals (SOLAAR-M6 atomic absorption spectrometry (AAS)). The solubilization efficiencies of the metals were calculated as the ratio of the solubilized metal from acidification to the total amount of the metal in the untreated soil.

After acidification, the soil suspension was transferred to a settling tank with a working volume of 1.0 L for solid–liquid separation. The resulting metal-laden supernatant (i.e., soil leachate) was filtered through paper and then pumped into the precipitators.

Soil leachate precipitation: sulfide precipitation can be achieved in two ways: by transferring the sulfide-laden, sulfate-reducing bioreactor effluent to the precipitator (effluent recycle) or through sulfide-transfer via gas sparging (gas recycle). Gas recycle was used in this study to exclude any inhibitory effects of the impurities in the bioreactor effluent on the precipitation kinetics and to reduce any contamination of the recovered metals by these impurities. Soil leachate from the 0.7-L STR precipitator was sequentially adjusted to pH 1.9 (Period I), 3.0 (Period II) and 4.0 (Period III) with NaOH to precipitate Cu, Cd and Zn, respectively, via biogenic sulfide. The H_2S in the sulfide-rich solution reservoir (initial total sulfide concentration = 375 mg/L) was continuously sparged to the precipitator using N_2 at a flow rate of 100 mL/min. Soil leachate from the control treatment was fed into an identical precipitation treatment system to determine the effect of SF addition on the precipitation kinetics.

Samples from the precipitators were taken at different time intervals and analyzed for total sulfide using the methylene blue spectrophotometric method (Shimadzu UV-1601, Japan). Samples for soluble metals analysis were membrane-filtered (0.45 μm) and determined using AAS. The precipitation efficiencies of the metals were calculated as the ratio of the amount of residual metal in the treated effluent (C) to the amount of metal in initial soil leachate (C_0). The final solid precipitates formed at the end of Periods I and III were rinsed with deionized water, freeze-dried and analyzed with a Bruker X-ray diffraction (XRD) with a D8 ADVANCE model diffractometer and Cu $K\alpha$ radiation operated at 10–80° and 2θ to determine their mineralogical composition. The characteristic reflection peaks (d values) were analyzed using

International Center for Diffraction Data (ICDD) cards. The same precipitates were also analyzed on a Hitachi S-4800 scanning electron microscope (SEM) equipped with a Horiba Emax energy dispersive spectroscopy (EDS) detector operated at 15.0-kV accelerating voltage for examination of the crystal morphologies and semi-quantitative chemical analysis. The particle size distributions (PSDs) of the precipitates were measured using Malvern MS2000 laser scattering image analysis.

The final recovery efficiencies of the metals obtained from the combined process were calculated by the following equation:

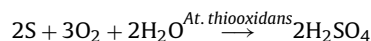
$$\begin{aligned} \text{Metal recovery (\%)} &= \frac{Me_{\text{solid}}}{Me_{\text{total}}} \times 100 \\ &= \left(\frac{Me_{\text{solubilized}}}{Me_{\text{soil}}} \times \frac{Me_{\text{precipitated}}}{Me_{\text{solubilized}}} \right) \times 100 \end{aligned}$$

Metals that existed in the soil leachate were considered to be solubilized, and the amounts of precipitated metals were calculated by subtracting the amounts of the final effluent metals from the soil leachate metals.

3. Results and discussion

3.1. Bioproduction of sulfuric acid by *At. thiooxidans* FD97 in sewage sludge filtrate

The pH and sulfate concentration were measured due to the following sulfur bio-oxidation reaction:



Changes in pH and sulfate concentration during the sulfur oxidation process with different amounts of SF added are shown in Fig. 2. The presence of 30% SF in the medium had no substantial influence on the biooxidation of sulfur to sulfuric acid because the acidification proceeded with nearly the same rate as the control to a final pH of 1.6 and a high sulfate concentration of 7000 mg/L after 16 days. Significant inhibition of sulfur oxidation was observed with 70% or 100% SF. Sulfate formation was absent for the first 6 days, and the final concentration was nearly half of the control, resulting in final pH values of 2.5 and 2.7. At day 16, enumeration of viable bacteria indicated that there were approximately 10^8 , 10^8 , 10^5 and 10^5 cells/mL in the media containing 0%, 30%, 70% and 100%

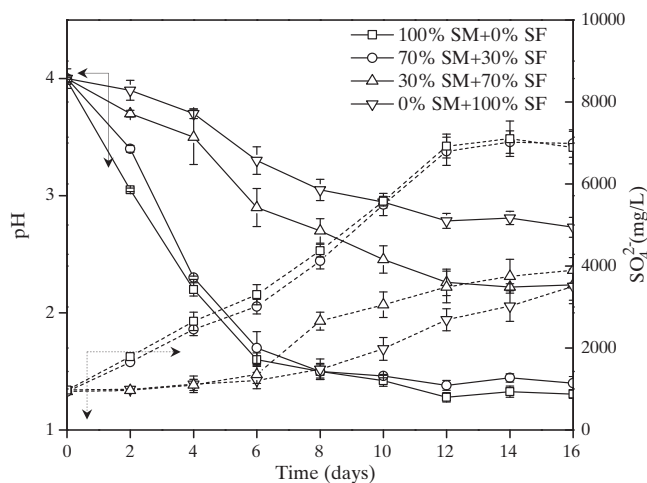


Fig. 2. Changes in pH (real line) and sulfate concentration (dotted line) during bio-production of sulfuric acid under different SF/SM ratios (SF: sewage sludge filtrate; SM: standard mineral medium).

SF, respectively. This drastic reduction in biogenic sulfuric acid at higher SF concentrations was likely correlated to the presence of high concentrations of inhibitory dissolved organic matter (DOM). Previous studies have shown that the DOM derived from anaerobically digested sewage sludge and some natural materials were markedly toxic to the growth of *Acidithiobacillus* species [24,25]. Inhibitory organic compounds can affect the sulfur-oxidizing activity of *Acidithiobacillus* by interfering with phosphates and sulfates in sulfur oxidation through biological reaction with various cations outside of the cell or by nonselectively disrupting the cell envelope or membrane [26]. Overall, the addition of 30% SF to the medium ensured efficient sulfuric acid production, and the maximum production rate ($\sim 450 \text{ mg SO}_4^{2-}/(\text{L day})$) was reached at day 4. Therefore, a mixed medium comprised of 30% of SF and 70% of SM was selected for the subsequent soil trials, and soil was added to the *At. thiooxidans* FD97 culture after 4 days of pre-incubation in the two-step acidification process.

3.2. Soil acidification and heavy-metals solubilization

Fig. 3 displays the variations in soil pH and solubilization kinetics of the main contaminating metals present (Zn, Cu and Cd) during the one- and two-step acidification processes. As shown in Fig. 3a for the one-step process, soil acidification proceeded slowly for a period of 6 days, with the pH remaining close to its initial value (pH 6.5) before dropping rapidly and leveling out at pH 1.9 on day 24. This delay in acidification was possibly due to overcoming the cation exchange capacity and alkalinity of the soil rather than a lag period microbial activity, as there was no corresponding delay in sulfate formation, which increased steadily to approximately 6980 mg/L in the first 6 days (data not shown). It is established that the acidophilic *Acidithiobacillus* species is capable of oxidizing sulfur (into sulfuric acid) at a wide range of pH from 2.0 to 9.0 and high acidity such as $\text{pH} < 2.0$ can be achieved at the end of the biooxidation process [27], thereby creating favorable conditions for the soil acidification. In view of the soil's buffering capacity, for the bioacidification treatment with high soil concentrations, enhancement of inoculation percentage of leaching bacteria and sulfur dosage is commonly proposed to further accelerate the acidification process [7,10]. For the two-step process, a soil acidity similar to that obtained in the one-step process was reached in merely 16 days, indicating that the addition of soil after the first few days of separate incubation was a feasible method to overcome the soil's buffering

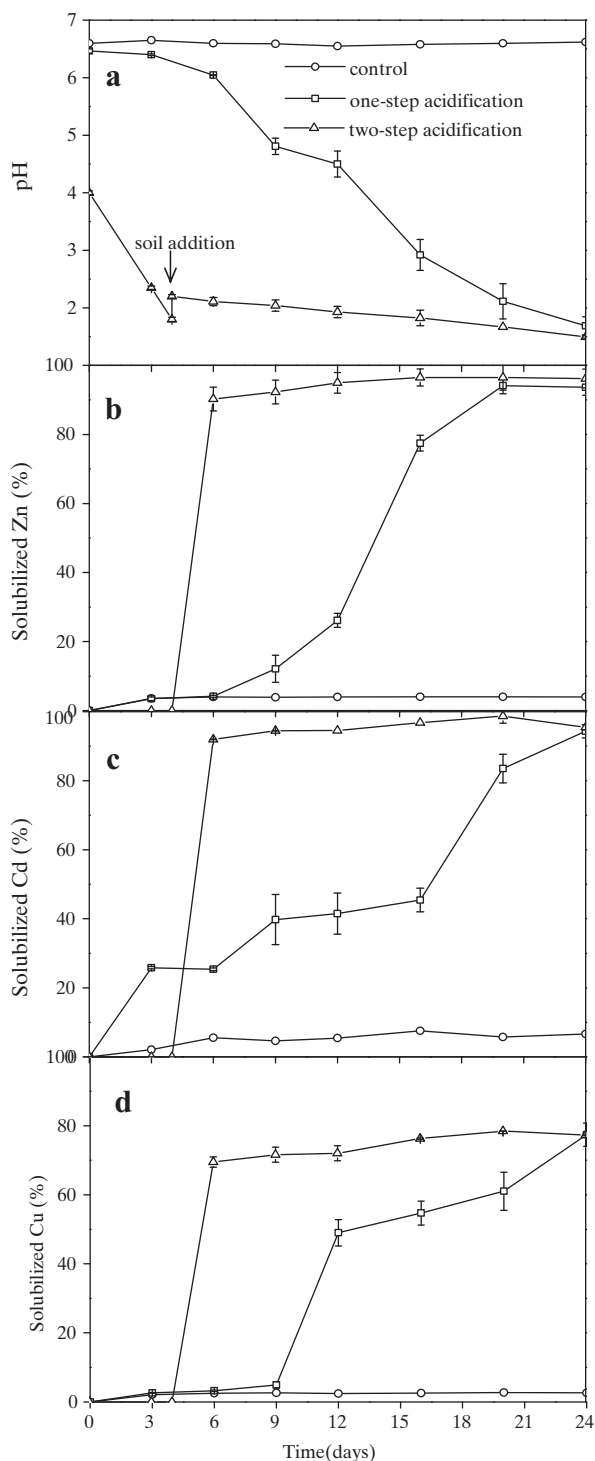


Fig. 3. Variations in soil pH (a) and heavy metal solubilization efficiencies for Zn (b), Cd (c) and Cu (d) during the one- and two-step bioacidification processes.

capacity and also a convenient way to improve the soil acidification efficiency.

It is evident from Fig. 3b–d that both the one- and two-step acidification processes proved highly efficient for the solubilization of metal contaminants from soils, but the rate of metal solubilization was much faster in the two-step process due to a faster reduction in pH. For example, the one-step process initially produced only negligible Cu solubilization, becoming significant only when the soil acidity fell below pH 4.5 (maximum at pH 1.9). In the two-step process, because an acidic pH of 2.2 that is favorable for metal

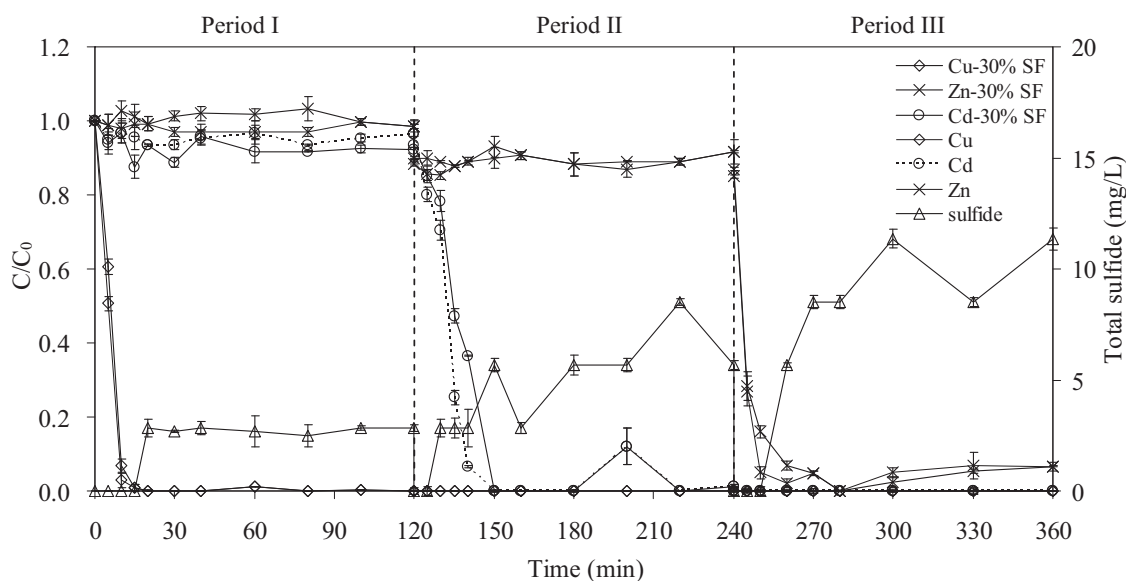


Fig. 4. The efficiencies of the selective metal precipitation and aqueous total sulfide concentrations in the sulfide precipitator at pH 1.9 (Period I), 3.0 (Period II) and 4.0 (Period III).

dissolution had been achieved prior to soil addition, Cu solubilized immediately from the soil, and the period needed for its maximum solubilization was shortened substantially to 16 days. However, despite the distinct difference in solubilization rate, the ultimate yields of each solubilized metal were similar at the end of the one- and two-step processes (95% for Zn, 76% for Cu and 97% for Cd) because the soil acidity was identical by the end of the experiment.

3.3. Separate precipitation of metals from soil leachate by biogenic sulfide

The sulfide produced from the sulfidogenic bioreactor was transported to the precipitator containing soil leachate by N_2 to sequentially precipitate Cu, Cd and Zn at pH values of 1.9, 3.0 and 4.0, respectively. In Period I, Cu was selectively precipitated over Zn and Cd at pH 1.9. Following transport of H_2S -laden gas to the precipitator, the soil leachate progressively turned into a turbid suspension, and significant brownish-black precipitates formed. The results shown in Fig. 4 demonstrate that the treatment system efficiently precipitated Cu over other metals, as evidenced by the decrease in the soluble Cu concentration from 12,600 to 126 $\mu\text{g/L}$ within 10 min, and soluble Cd and Zn concentrations were unchanged during this period. The precipitation rate of 1240 $\mu\text{g Cu}/(\text{L min})$ was comparable to that of the SF-free soil leachate (1260 $\mu\text{g Cu}/(\text{L min})$), suggesting a marginal influence of the 30% SF present on the Cu precipitation kinetics. Similar observations were also recorded in the sulfide precipitation processes of Cd (Period II) and Zn (Period III). Low levels of interfering substances present in the soil leachate due to the low amounts of SF added could be a plausible explanation for this negligible inhibition of metal precipitation.

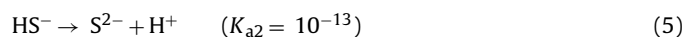
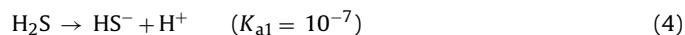
At the end of Period I, a drop in pH of the slight soil leachate from 1.9 to 1.7 and a sulfide-laden solution pH rise from 8.1 to 8.4 were noted, which was mostly likely due to H^+ being released from solution for the formation of H_2S (Eq. (1)) and acid accumulation from the formation of Cu-sulfides (Eq. (2)). The same pH changes were also reported in the precipitation of Cu over Zn from a Cu–Zn AMD by biogenic sulfide [18].



On the other hand, we found that the sulfide level in the precipitator was initially undetectable, becoming appreciable only after Cu precipitation was complete and then elevating to a maximum (2.85 mg/L). This relatively low sulfide level that was constant in the later stage of Period I was related to sulfide volatilization under the conditions of low pH and continuous N_2 bubbling through the precipitator. Furthermore, it is interesting that after complete Cu precipitation, Zn and Cd did not precipitate in spite of the exposure to detectable concentrations of sulfide. One possible factor contributing to these observations could be that the sulfide concentrations produced did not reach the levels required to initiate Zn or Cd precipitation. Using the $\log K_{sp}$ value of ZnS (-23.8), for example, the required total sulfide concentration to initiate Zn precipitation can be calculated as follows:

$$K_{sp} = [Zn^{2+}][S^{2-}] \quad (3)$$

where $[Zn^{2+}]$ and $[S^{2-}]$ are the activities in equilibrium. The activity coefficients were calculated using the Davies equation [28]. Using the dissociation constants (Eqs. (4) and (5)) and the $[S^{2-}]$ calculated from Eq. (3), the total sulfide concentration required to initiate Zn precipitation can be calculated by Eq. (6) [29]:



$$\text{Total sulfide} = [S^{2-}] \left[1 + \frac{[H^+]}{K_{a2}} + \frac{[H^+]^2}{K_{a1}K_{a2}} \right] \quad (6)$$

These calculation results confirm that, under this experimental condition, the sulfide concentrations were lower than the minimum sulfide concentrations (9.89 mg/L) required to initiate precipitation of 35.6 mg/L Zn^{2+} at pH 1.9.

After Cu precipitation, the pH of soil leachate was successively elevated to 3.0 (Period II) and 4.0 (Period III) through NaOH addition to sequentially precipitate Cd and Zn, respectively. The initial pH adjustment precipitated approximately 5% of Zn and 1% of Cd as their respective hydroxides. In Period II, approximately 96% of the incoming Cd was precipitated within 30 min, and the soluble Cd concentration in the soil leachate was reduced from approximately 420 to 15 $\mu\text{g/L}$. Zn precipitation did not occur in this period. Sulfide precipitation of Zn was initiated after 240 min (Period III). Similar to Cd, the Zn concentration in the precipitator also decreased

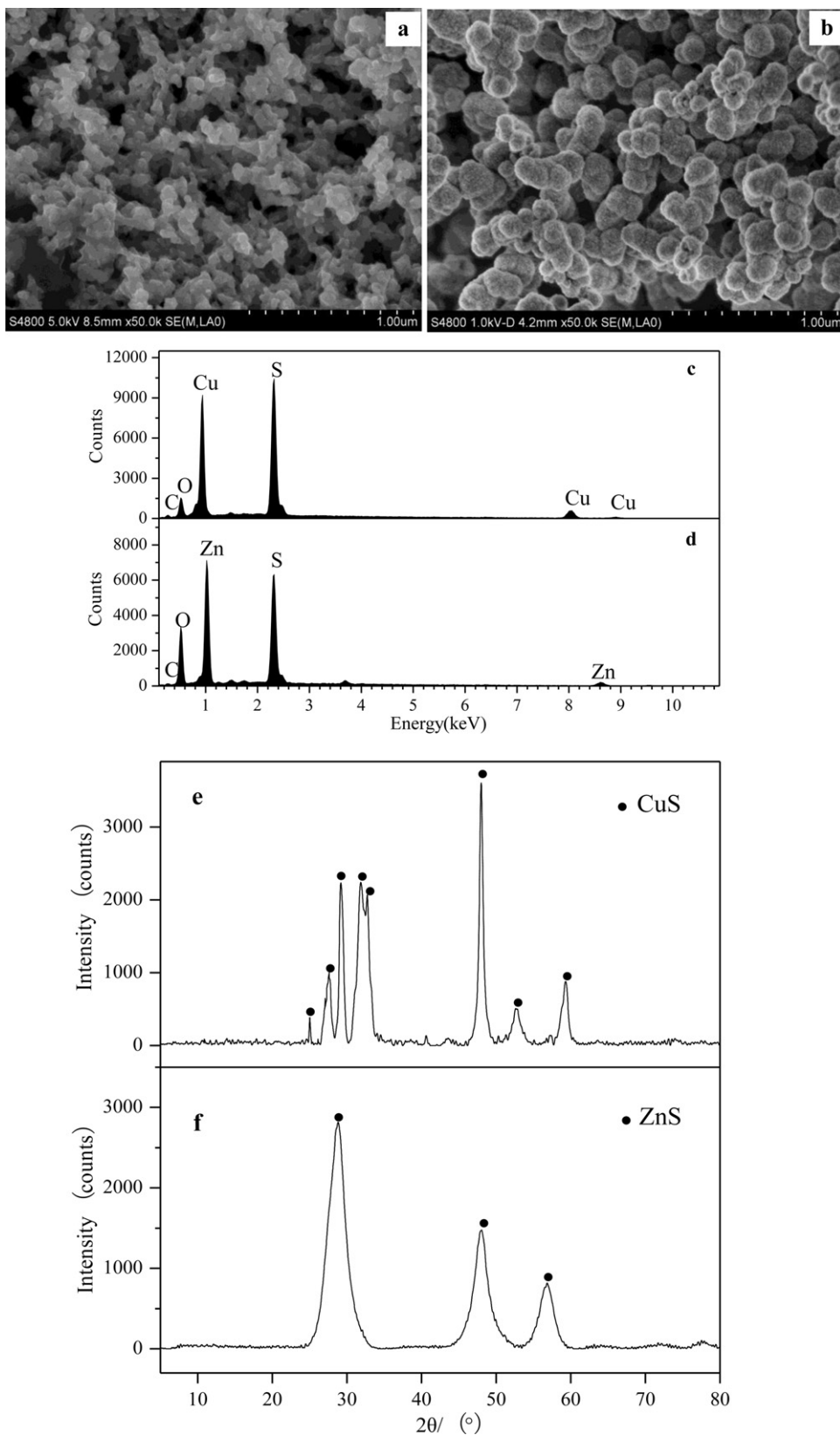


Fig. 5. SEM image, EDS spectra and XRD pattern of Cu and Zn precipitates collected from the sulfide precipitation process. (a and b) SEM of Cu- and Zn-precipitates, respectively; (c and d) EDS of Cu- and Zn-precipitates, respectively; (e and f) XRD of Cu- and Zn-precipitates, respectively.

rapidly over time even though the initial concentration of Zn was approximately 75 times higher than that of Cd. The average Zn concentration in the soil leachate decreased from approximately 35,600 to 350 $\mu\text{g}/\text{L}$ after 40 min, equivalent to a 99% Zn

precipitation efficiency. Unfortunately, the Zn precipitation efficiency unexpectedly decreased from 99% to 93% over the remainder of the experimental period. The reappearance of soluble Zn in the later stage of Period III (even at high sulfide concentrations of

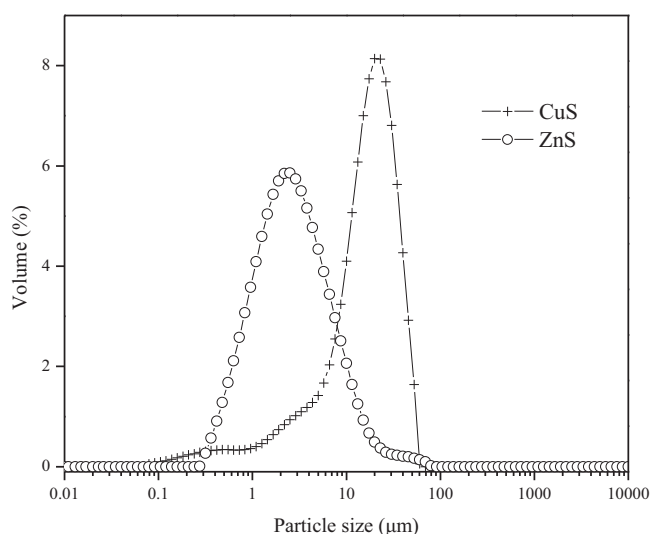


Fig. 6. Particle size distribution of Cu (+) and Zn (o) precipitates collected from the sulfide precipitation process.

approximately 12 mg/L) could be due to the formation of small and/or colloidal Zn-sulfide particles with the particle size between 1 nm and 1 μm (e.g., $\text{Zn}(\text{HS})^+$ and $\text{Zn}(\text{HS})_2^0$) [30] that had passed through the 0.45- μm membrane and would have been included in the soluble Zn fraction. The PSD analysis revealed that 2.8% of the Zn particles were smaller than 0.45 μm (Fig. 6).

3.4. Characterization of metal precipitates

The recovery and reuse potential of metal precipitates depends on their purity and settling tendency. In this study, the solid Cd precipitates were not recovered from Period II because of extremely low concentrations. SEM-EDS analysis of the precipitates of Cu and Zn collected from Period I (III) indicated significant formation of micrometer-sized particles with compacted structures containing copper, zinc, sulfur, oxygen and carbon with a metal/sulfur ratio of approximately 1.0 (Fig. 5). The minor presence of carbon and oxygen (CO_2) likely resulted from the sulfidogenic bioreactor and were transferred along with H_2S to the precipitator by gas sparging. This would not affect the purity of the recovered precipitates due to the considerable difference in the K_{sp} constant between Cu (Zn)-sulfides and Cu (Zn)-carbonates. XRD analysis further confirmed that these two precipitates had a fine crystallinity and consisted primarily of CuS (ZnS). The PSD of the CuS particles was narrower compared to that of the ZnS particles (Fig. 6). The mean and mode (highest % differential volume) of the PSD for CuS were 16 and 18 μm , respectively, whereas the mean and mode of the PSD for ZnS were 3 and 3 μm , respectively, indicating that the CuS particles had superior settling quality. The characteristics for copper- and zinc-precipitates collected from the precipitation treatment of the SF-free soil leachate had the same characteristics as described above (data not shown). The PSD of metal precipitates is commonly determined by the precipitation kinetics, i.e., the competition between nucleation and crystal growth [31]. It is well documented that at low sulfide concentrations, the crystal growth rate of metal-sulfide particles is larger than the nucleation rate, resulting in large sulfide particles [32]. Further attempts should be made to promote the growth of the sulfide crystals over nucleation by manipulation of the supersaturation level to form larger particles that favor sedimentation of solid precipitates from soil leachate and for proper dewatering of the solids.

Based on the experimental data from the metal solubilization and precipitation processes, approximately 75% of Cu and 86%

of Zn were ultimately recovered as purified CuS and ZnS from the soil by the integrated method. The results compare favorably to those of other treatment systems designed for cleanup of metal-contaminated soils, in terms of metal removal efficiency, the effluent metal concentration and the reuse potential of the recovered metals. Comparative economical study on treatment of highly metal-contaminated soils with high solids concentrations by this proposed microbial method and other chemical methods (e.g., soil washing using mineral acids and precipitation of metal ions using commercial sulfides) is currently underway.

In summary, a combination of biosolubilization and bioprecipitation appears to be a potentially efficient route for selectively recovering valuable metal resources from metal-contaminated soils. In the future field application, where soil and soil water conditions permit, e.g., a homogenous soil and freedom from possible groundwater contamination, the biosolubilization component of the combined system could possibly be carried out in situ although conditions allowing this would be really infrequent. In other cases, several ex situ options, e.g., heap leaching or slurry reactors, could be used to solubilize metals and feed the sulfide precipitation component.

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