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A combination of bioleaching and bioprecipitation for deep removal of contaminating metals from dredged sediment

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ABSTRACT

A linked microbial process comprising bioleaching with sulfate-oxidizing bacteria and bioprecipitation with sulfate-reducing bacteria operating sequentially was investigated to deeply remove contaminating metals from dredged sediment. The results showed that sediment bioleaching resulted in a sharp decrease in sediment pH from an initial pH ~7.6 to pH ~2.5 within 10–20 days, approximately 65% of the main heavy metals present (Zn + Cu + Cr) were solubilized, and most of the unsolubilized metals existed in residual form of sediment. The acidic leachate that resulted from sediment bioleaching was efficiently stripped of metal sulfates using a bioprecipitation reactor when challenged with influent as low as pH ~3.7. More than 99% of Zn^{2+}, 99% of Cu^{2+} and 90% of Cr^{3+} were removed from the leachate, respectively, due to the formation of ZnS, Cu_2S and CrOOH precipitates, as confirmed by SEM-EDS and XRD detection. It was also found that alkalization of bioleached sediment using $Ca(OH)_2$ excluded the risk of sediment re-acidification. The ability of the combined process developed in this study to deeply remove heavy metals in insoluble sulfides or hydroxides forms makes it particularly attractive for the treatment of different types of metal contaminants.

1. Introduction

Heavy metal contamination of sediment is one of the major environmental effects of urbanization and industrialization. As much as 300–1000 mg/kg each of Zn, Cu, Cr, Pb, and Mn is often found in river and harbor sediment because of the repeated discharge, over many years, of metal-containing industrial wastewaters and municipal sewage into aquatic ecosystems [1–3]. In some high industrial activity regions, the levels of Zn and Cd in sediment exceed more than 10,000 mg/kg and 40 mg/kg, respectively [4]. The responsibility of safely managing these contaminants together with the possibility that metals may release into the groundwater or enter the food chain through aquatic material means that the removal of heavy metals from contaminated sediment is a priority.

Bioleaching process using sulfur-oxidizing bacteria (*Acidithiobacillus* spp.), which is applied widely for metals extraction from low-grade ores, is now being extended to investigate the leaching of contaminating metals from sediment as a bioremediation technique [5]. In this process, bacteria from the genus *Acidithiobacillus* is capable of oxidizing the reduced sulfur (elemental sulfur or sulfur compounds) to sulfuric acid and thus creates acidic conditions favorable for metal solubilization from sediment [6]. Various operation parameters including inoculum density, total solids content, type of substrate and reactor configuration have been extensively studied to achieve higher metal solubilization efficiency [7–9]. This established technique has also been shown in pilot- and preindustrial-scale studies to be effective in simultaneously removing multiple metal contaminants [5,10]. For example, a solid-bed reactor with 2000 L working volume operated by Seidel et al. [5] achieved 61–81% solubilization of Zn, Cd, Mn, Co and Ni from contaminated river sediment within 21 days of bioleaching supplemented with 20 g/L elemental sulfur.

Unfortunately, there have been few commercial applications of bioleaching mediated metal-laden sediment treatment systems, even though the possibility of using this technique to remediate contaminated sediment has long been appreciated. The major reason being that the result of the bioleaching process is a complex acidic leachate rich in various metal sulfates that must be additionally decontaminated or carefully disposed of [6,8,11]. Several methods (chemical precipitation, membrane separation, solvent extraction and electrodeposition) have been recently employed, after bioleaching treatment, for the further removal of bioleached metals from acidic leachate [12–14], but high operating cost and sometimes insufficient yields of metal extraction occurring in these methods constitute major obstacles to their industrial application. There are currently few reliable remediation technologies in

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conjunction with sediment bioleaching to deal with metal contamination.

A particularly promising means to reduce the levels of contaminants in sediment bioleachate is bioprecipitation process using sulfate-reducing bacteria (*Desulfovibrio* spp.). Sulfate and metal removal by bioprecipitation is mainly based on the ability of sulfate reducer to use sulfate as the terminal electron acceptor for the metabolism of organic or inorganic substrates (e.g., alcohol, glycerol, lactate and H_2/CO_2) to produce sulfide, which readily reacts with most dissolved metals to form metal sulfide precipitates, although precipitation with hydroxides or carbonates and sorption into biomass are also possible [15–17]. In fact, this biotransformation process has been actively used for treatment of surface and groundwater contaminated with acid mine drainage. The results indicated a massive sulfate conversion and efficient metal removal without forming high amounts of metallic residue [18,19]. Moreover, a techno-economic research showed that investments and operating costs of bioprecipitation compare advantageously with current (physico-) chemical methods [20].

Accordingly, the combination of bioleaching and bioprecipitation provides a potential route for bioremediation of metal-contaminated sediment. The primary mechanisms of removing metals via these two biological processes can be summarized by the following reaction equations:

$$2S + 3O_2 + 2H_2O \xrightarrow{\text{sulfur-oxidizing bacteria}} 2H_2SO_4$$

$$\text{Me-sediment} + H_2SO_4 \rightarrow MeSO_4 + \text{sediment-2H} \quad \text{bioleaching process}$$

$$CH_2O + SO_4^{2-} \xrightarrow{\text{sulfate-reducing bacteria}} HS^- + HCO_3^-$$

$$Me^{2+} + HS^- \rightarrow MeS + H^+ \quad \text{bioprecipitation process}$$

Me^{2+} is a bivalent metal; CH_2O represents an organic substrate

Difficulties, however, may be presented by inhibition of sulfate reducer activity due to low pH of sediment leachate, with the potential for the entire remediation process to come to a halt [21]. The pH optimum for growth of sulfate reducer is between pH 5.0 and 8.0 [22], whereas the leachate generally has a pH between 2.0 and 3.5 [5,11]. Inhibition of sulfate reducer may also occur due to bioleached metals toxicity or exposure to high levels of oxygen or dissolved hydrogen sulfide. Toxic effects of hydrogen sulfide were reported at concentrations of 477–617 mg/L [23]. Until now, only limited information on the use of this combined technique for treatment of metal contamination has been available, probably because of the occurrence of these inhibitory effects. White et al. [24] first reported the combined microbial process for the removal of heavy metals, in which soil artificially contaminated by Cu and Ni was successfully treated by the sequential application of bioleaching and bioprecipitation. During the bioprecipitation process, the pH of soil leachate was carefully controlled by the addition of NaOH to maintain the reactor pH at ~6.5. This combined process was recently modified by Cabrera et al. [25] to treat synthetic ZnS–NiS–Cr_2O_3 contaminated sand, who achieved sufficient metal removal through indirect bioprecipitation process in which sand leachate containing metal sulfates was mixed with the pre-prepared H_2S-laden culture of sulfate-reducing bacteria, but this process appeared to hardly remove sulfate from the leachate. There is as yet no evidence to confirm good performance of this combined technique in treating real metal-laden sediment as previous artificially contaminated materials. Especially, the effectiveness of sulfate and metals removal via bioprecipitation at low pH (which could save costs associated with caustic addition to increase leachate pH) is still poorly understood. Furthermore, a detailed characterization of metal precipitates generated in the termination of the combined process (that may relate closely with metal final removal mechanism) has not been undertaken.

The purpose of the present work was to evaluate the performance of a hybrid process incorporating bioleaching and bioprecipitation as remediation strategies for a local dredged sediment contaminated with heavy metals. This study is divided into three parts: (i) leachability of metals from sediment by bioleaching in multi-batch tests, (ii) treatment of bioleached sediment by alkalization and (iii) sulfate and bioleached metals removal from sediment leachate, under mildly acidic conditions, by bioprecipitation.

2. Materials and methods

2.1. Sediment

The sediment used in this study was collected from the dredging process of Hai Bo River (the dredging location was close to Hang Zhou Road Bridge, western Qingdao, China). The Hai Bo River, which has a catchment area of about 27 km^2 and a total length of 6.8 km, receives many untreated and/or partially treated municipal and industrial wastewaters from uncontrolled effluents. All gravels and litters were removed from the sediment and the sediment was stored in sealed plastic bags and kept at 4 °C before use. The selected physicochemical properties of the sediment were listed as following: moisture 52.6%, pH 7.9, organic matter 3.4%, total Zn 732 mg/kg, total Cu 174 mg/kg, total Cr 206 mg/kg [Cr(VI) is undetectable in the sediment], total Ni 29 mg/kg, total Pb 26 mg/kg and total Cd 1.3 mg/kg.

2.2. Microorganisms and inoculum

A sulfur-oxidizing bacterium (*At. thiooxidans* CGMCC 2760) used in bioleaching test was obtained from China General Microbiological Culture Collection Center (CGMCC). *At. thiooxidans* was cultivated in a mineral salts medium consisting of (g/L): $(NH_4)_2SO_4$ 0.4, KH_2PO_4 3.0, $MgSO_4{\cdot}7H_2O$ 0.5, $CaCl_2{\cdot}2H_2O$ 0.25, $FeSO_4{\cdot}7H_2O$ 0.01 and elemental sulfur, 10. Bioleaching inoculum was prepared according to the acclimation procedure described by Chen and Lin [26]. Initially, 50 mL of viable growing cultures of *At. thiooxidans* was added into a 500 mL Erlenmeyer flask containing 250 mL of sediment suspension (solids content: 9.7%) and 0.9 g of elemental sulfur, then the flask was incubated in a gyratory agitator until sediment pH dropped below 2.5, as a result of sulfur oxidation into sulfuric acid. The acidified sediment thus obtained was employed as the inoculum in bioleaching reactor, and high densities of *At. thiooxidans* in the inoculum were confirmed by microscopic examination.

The inoculum used for bioprecipitation reactor was a mixed, undefined culture of sulfate-reducing bacteria derived from several environmental sources and selected for suitable properties including acid-tolerance and rapid rates of sulfate reduction. This culture was maintained in a modified Postgate's Medium C with a composition of (g/L): KH_2PO_4 0.5, NH_4Cl 1.0, Na_2SO_4 4.5, $CaSO_4$ 1.0, $FeSO_4{\cdot}7H_2O$ 0.5, $CaCl_2{\cdot}6H_2O$ 0.06, $MgSO_4{\cdot}7H_2O$ 0.06, $NaC_3H_5O_3$ 3.5 and yeast extract 1.0.

2.3. Description of reactor system

2.3.1. Bioleaching reactor

Bioleaching was carried out in a 50 L capacity centrifugal impeller reactor (CIR) made of polymethyl methacrylate. The CIR reactor consisted of main column (40 cm diameter, 50 cm height), a centrifugal-pump impeller (16 cm height), electromagnetic stirring device, and an air diffuser installed 5 cm from the bottom of the column (Fig. 1). With the rotation of the centrifugal impeller (70 rpm), a negative pressure was created in the impeller center, drawing sediment suspension from the reactor bottom through the draft tube and producing a circulation flow pattern of the sediment. Air

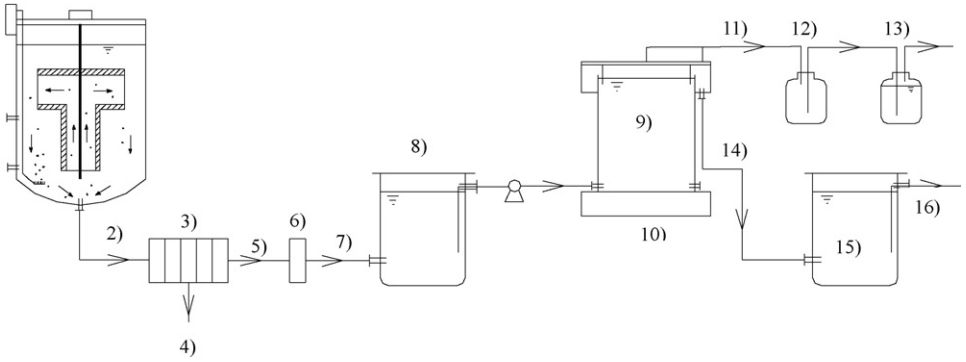

Fig. 1. Diagram of the laboratory-scale combined process for bioleaching of metals from contaminated sediment and bioprecipitation of bioleached metals from sediment leachate. (1) bioleaching reactor; (2) leached sediment; (3) sediment dehydratation using pressure filter; (4) alkalization of dehydrated sediment; (5) sediment leachate; (6) filter; (7) filtered leachate; (8) influent holding tank; (9) bioprecipitation reactor; (10) magnetic stirrer; (11) biogas; (12) safety flask; (13) biogas scrubber containing 1 M NaOH; (14) treated effluent; (15) effluent settling tank; (16) final effluent.

was sparged in bioreactor at a rate of 1.0 $m^3\ h^{-1}$ through a sintered stainless diffuser with tiny pore size to produce fine bubbles.

2.3.2. Bioprecipitation reactor

The efficiency of removing sulfate and bioleached metals from sediment leachate by bioprecipitation was studied in a bench-scale reactor. The reactor was a polymethyl methacrylate continuous-flow stirred tank anaerobic reactor (CSTR) of 0.67 L working volume. The CSTR system consisted of seven main components: influent holding tank, peristaltic pump (BT01-100, Longer Precision Pump Ltd., Baoding, China), main reactor (7 cm diameter, 12 cm height) with an internal gas–liquid–solid triphase separator, magnetic stirrer (150 rpm) and temperature controlling device (32 °C), effluent settling tank, and a volatile gas trap. Sediment leachate was pumped from the influent holding tank to the bottom inlet of the reactor by means of a calibrated variable speed peristaltic pump.

2.4. Experimental procedure

2.4.1. Sediment bioleaching

Bioleaching test can be a sequencing batch, semi-continuous or continuous mode according to various experimental purposes. In this study, all bioleaching tests were realized in a batch mode with recirculation of substances. In the first batch test, 5 L of *At. thiooxidans*-rich acidified sediment was inoculated into the CIR reactor containing 45 L of sediment suspension (solids content: 9.7%) and 150 g of elemental sulfur to allow sulfur oxidation and metals bioleaching. No temperature control was conducted (room temperature, 16–25 °C). During the bioleaching process, 15 mL of sediment samples periodically taken from the reactor were determined for pH and Eh using pHS-3D model pH meter with a Pt–Ag/AgCl electrode, and solubilized metals using a Thermo SOLAAR-M6 atomic absorption spectrometry (AAS). The solubilization efficiencies of metals were calculated as the ratio of the solubilized metal by bioleaching to total metal in the sediment before bioleaching. Zinc, Cu and Cr were studied in detail because of their relatively high contents in sediment. The change in binding form of these metals in sediment before and after bioleaching was also analyzed using the four-step procedure recommended by European Community Bureau of Reference [27] in order to investigate which metals, bound to which fractions, were solubilized during bioleaching.

After sediment pH dropped to ~2.5, which is regarded as the indicator of termination of bioleaching [11], four batches of bioleaching tests as following were consecutively performed with the aim to examine the stabilization of bioleaching in multi-batch operations. 25 L of leached sediment with pH ~2.5 (inoculums) was circulated into next batch bioleaching test containing 25 L of fresh sediment samples and 150 g of sulfur. During these bioleaching tests, sampling and analysis were conducted according to the same procedures as previously described. Finally, leached sediment resulting from all bioleaching tests was dehydrated by a filter-press unit (Langxun Water Utilities Ltd., Hangzhou, China) to separate solubilized metals from the sediment.

2.4.2. Treatment of bioleached sediment by alkalization

Low pH, low levels of soluble metals, and large amounts of nonoxidized sulfur are common characteristics of leached sediment produced in bioleaching process, which represents a potential secondary pollution such as sediment re-acidification [5,7,8]. In this study, the dehydrated leached sediment was neutralized at pH 7.8 or 12 by adding different levels of $Ca(OH)_2$, and the resulted soil-like substrates were examined through acidogenic potential experiment described by Blais et al. [10]. Experiments were conducted in 250 mL Erlenmeyer flasks containing 100 mL of deionized water and 10 g of leached sediment (moisture, 66%) previously neutralized with $Ca(OH)_2$. The samples were incubated at 28 °C and 180 rpm in a gyratory shaker for 14 days. During the incubation process, the pH, Eh, and soluble metal concentrations were periodically measured.

2.4.3. Sediment leachate bioprecipitation

Sediment leachate coming from bioleaching tests was pre-filtered through Whatman 41 filter paper for removal of large-size suspended solids to minimize the connecting tubing of peristaltic pump blocking. Subsequently, the resulted filtrate was added to the influent holding tank, and some nutrients ($NaC_3H_5O_3$, 20 g/L, yeast extract 1 g/L, NH_4Cl, 1 g/L and KH_2PO_4 0.5 g/L) were supplemented in response to the measured sulfate content in the filtrate to achieve a constant 1.8 chemical oxygen demand (COD)/SO_4^{2-} ratio favorable for sulfate reduction entering the bioprecipitation reactor [28]. Finally, control of pH was carried out by the addition of 1 M NaOH to maintain the influent pH at ~3.7 because our previous studies found that below a pH of 3.5, the bioreactor was less successful. The run influent fed to the bioreactor with pH ~3.7 contained Zn^{2+} 54.6 mg/L, Cu^{2+} 10.7 mg/L, Cr^{3+} 5.6 mg/L, and SO_4^{2-} 7240 mg/L.

The bioprecipitation reactor, containing 600 mL of medium C with 7.2 g/L Na_2SO_4 at pH ~4.2 and 70 mL of a mixed culture of sulfate-reducing bacteria (volatile suspended solids content, 9.2 g/L), was operated in batch mode for the first 3 days to obtain a steady anoxic condition and start up microbial sulfate reduction rapidly. Once ~40% reduction of sulfate was achieved, continuous-

flow was started with a flow rate of 15 mL/h using the run influent (sediment leachate) at pH ~3.7.

Reactor influent and effluent samples were periodically measured for pH, sulfate, sulfide and soluble metals concentrations. Sulfate was analyzed by the photometric turbidimetry method [29]. Sulfide was determined by spectrophotometer (Shimadzu UV-1601, Japan) according to the methylene blue method [30]. Samples for the determination of soluble metals were filtrated through 0.45 μm membrane and analyzed using AAS. The removal efficiencies of sulfate and metals were calculated as the difference of influent and effluent sulfate and soluble metal concentrations, respectively.

At the completion of bioprecipitation test, the precipitates, collected from the reactor and the effluent settling tank, were rinsed with deionized water, freeze-dried, and determined by a Bruker X-ray diffraction (XRD) using a D8 ADVANCE model diffractometer with a Cu Kα radiation operated at 10–80° and 2θ to determine the major mineralogical composition. The characteristic reflection peaks (d values) were analyzed using International Center for Diffraction Data (ICDD) cards. The morphology and elemental composition of the precipitates were examined by 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. The particle size distribution (PSD) of the precipitates was measured with a Malvern MS2000 laser scattering image analysis.

3. Results and discussion

3.1. Sediment acidification and heavy metals solubilization during bioleaching

In bioleaching, acidification is a key parameter because the pH determines the rate and level of metal solubilization [6]. Following inoculation of sulfur-oxidizing bacteria and addition of sulfur, acidification proceeded slowly for a period of ~3 days with sediment pH remaining close to its initial value of pH 7.6 before dropping rapidly, leveling out at pH 2.4–2.7 at day 20. The initial delay in acidification could be attributed to the necessity of overcoming the cation exchange capacity and alkalinity of the sediment. Me solubilization, defined as the sum of Zn, Cu and Cr solubilization, was also initially negligible, becoming significant only once the acidity of leached sediment had fallen below pH 5.0 and then increasing to a maximum (~65%) that occurred at pH ~2.5. In addition, it is evident from Fig. 2a that a consecutive bioleaching process by circulating pH < 2.5 leached sediment was a feasible method to overcome the buffering capacity of sediment and also a convenient way to inoculate *Acidithiobacillus* species in practical operation, since for the subsequent four batches tests sediment acidification proceeded with nearly the same rate to the final pH of ~2.5 and the target metals was correspondingly solubilized to a high degree.

From the changes in total content and binding form of each metal before and after bioleaching (Fig. 2b), it was found that the extent to which a metal is solubilized was highly related to its chemical form in the original sediment. Take Zn for instance, Zn (56%) was predominantly associated with the acid-soluble form and with sulfides/organic matter, and these forms of metals were easily leached from the sediment at low pH created by bioleaching process—a contributing factor to its high solubilization level in this study. These observations were consistent with those reported by other authors [26,31,32].

On the other hand, we noted that the metal removal determined from solid sediment before and after bioleaching was somewhat lower than the Me solubilization calculated from the concentrations of soluble metals in bioleaching suspension. Similar results were also observed by Löser et al. [8], who ascribed this discrepancy to the loss of sediment mass owing to the dissolution of mineral components (mainly CaO, MgO, Al_2O_3) under long-term acidic treatment condition. Some researches argued that controlling the pH of leached sediment at ~3.0 was capable of reducing the dissolution of mineral components evidently and, simultaneously, marginally disturb metals solubilization [8,32].

3.2. Bioleached sediment treatment by alkalization

Land application is recognized by numerous researchers as the most economical way for final disposal of bioleached sediment as it combines the recycling of plant nutrients and sediment disposal at the same time [6]. However, the presence of residual sulfur in leached sediment can be slowly oxidized to sulfuric acid

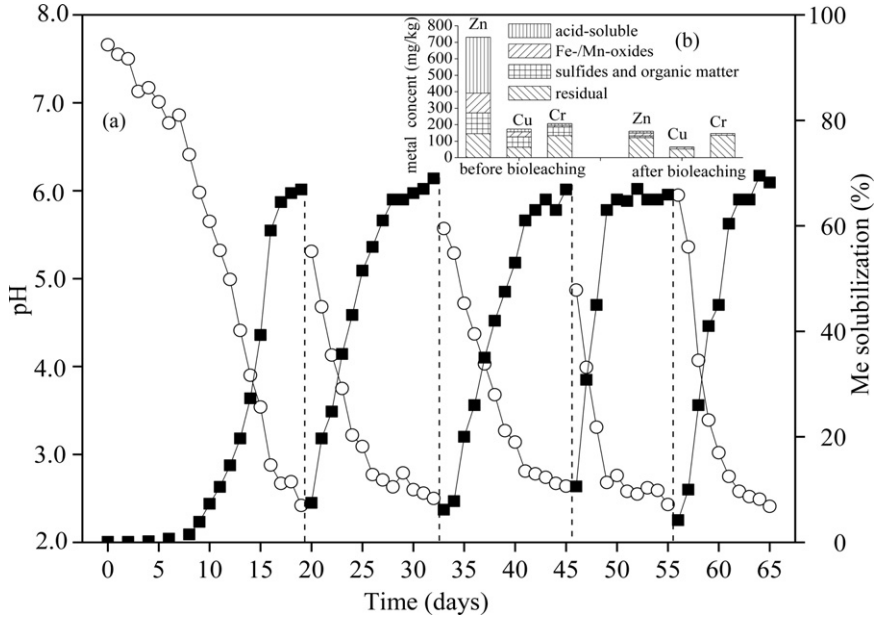

Fig. 2. Bioleaching of heavy metal-contaminated sediment. (a) pH (○) and metal solubilization (■) (Me = sum of Zn, Cu and Cr), and (b) binding forms of Zn, Cu and Cr before and after sediment bioleaching.

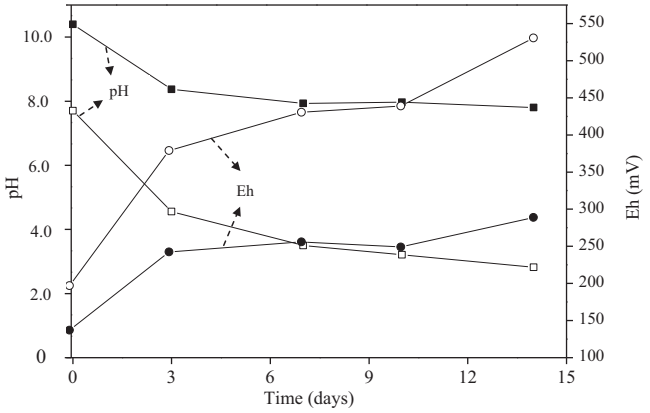

Fig. 3. Changes in pH (pH 7.8 □; pH 12 ■) and Eh (pH 7.8 ○; pH 12 ●) of the alkalized bioleached sediment with different alkalic levels during 14 days of incubation process.

under acid conditions and thus limits its use as soil amendment. Based on the variations in sulfate concentration before and after bioleaching (data not shown), we estimated that ~53% of elemental sulfur added in bioleaching reactor was not oxidized in this study. The leached sediment was therefore alkalized by the addition of $Ca(OH)_2$ for reducing the risk of sediment re-acidification. The acidogenic potential experiment demonstrated that the alkalization treatment with the addition of 5% $Ca(OH)_2$ to leached sediment elevated the pH to the alkalic value of ~8.2 and stabilized the Eh at ~280 mV during 14 days of shaking incubation (Fig. 3), which represented a low residual potential for acidification due to the fact that microbial sulfur oxidation could hardly proceed under a high alkalic pH > 8.0 condition. Furthermore, it was observed that alkalizing to pH ~8.2 immobilized all the residual bioleached metals, and that the metal content in the eluate laid below the detection limits of the AAS method. The practicability of alkalization treatment was also exhibited by Seidel et al. [5] who concluded that exposing the alkalized bioleached sediment (pH ~7.0) to the weather for 450 days neither re-decreased sediment pH nor re-solubilized heavy metals.

More recently, some new forms of sulfur sources, e.g., recyclable molded sulfur (sulfur prills and pieces) and biogenic hydrophilic sulfur, have been prepared successfully in Canada, Germany and Taiwan to enhance sulfur availability for bioleaching bacteria and reduce the re-acidification risk in the bioleaching process [7,33].

3.3. Sulfate and bioleached metals removal from sediment leachate by bioprecipitation

In previous studies, it was found that the bioprecipitation reactor used in the present study was capable of supporting sulfate reduction and metals removal under mildly acidic conditions (pH 3.5–5.6) from a synthetic wastewater containing high levels of Cu^{2+} (20–32 mg/L), Zn^{2+} (45–60 mg/L), Cr^{3+} (5–10 mg/L), and sulfate (4500–6500 mg/L) [28]. The performance of bioprecipitation of sediment leachate at pH ~3.7 in terms of sulfate removal, sulfide production, and the effluent pH over 23 days incubation is illustrated in Fig. 4. During the first 3 days of pre-incubation at pH ~4.2, the redox potential (Eh), initially at ~200 mV, decreased to ~−180 mV, density of total bacterial counts (mainly vibrio-shaped bacteria) increased from $\sim 2 \times 10^7$ to $\sim 10^8$ cells mL^{-1}, and ~40% of the influent sulfate was removed, all of which suggested the formation of a favorable condition for sulfate conversion. From day 4, the leachate (pH ~3.7) coming from sediment bioleaching was continuously fed into the bioreactor. From data presented in Fig. 4, it was demonstrated that the influent adjustment did not substantially interfere with the microbial sulfate conversion process, as it was not reflected by any decline in sulfate removal. In general, at pH ~3.7 the bioreactor was able to sustain a sulfate reduction rate of 3 kg SO_4^{2-} m^{-3} d^{-1}, removing ~60% of influent sulfate, produce a variable effluent sulfide concentration with a mean value of ~145 mg/L, and raise the effluent pH to ~6.9. The incomplete sulfate reduction at excess COD/SO_4^{2-} levels was likely due to competition with other anaerobic bacteria (methanogens and acetogens) for the electron donors [34]. Optimization of sulfate reduction at low pH for enabling the electron flow in the bioreactor to exclusively direct toward sulfide production and promote the dominance of SRB over other microbe is still needed in future study.

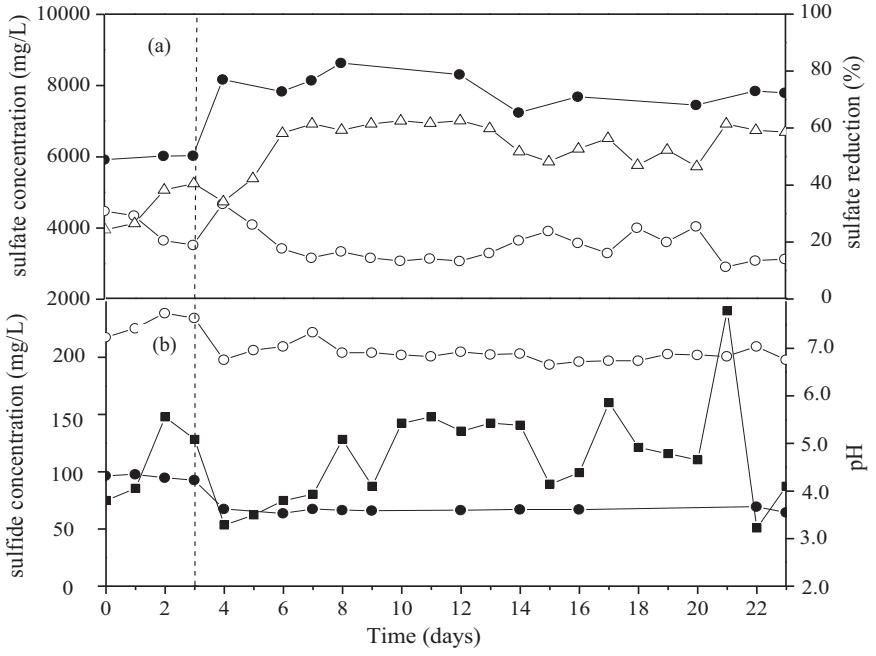

Fig. 4. Sulfate reduction efficiency (a), effluent sulfide concentration and reactor pH (b) in the bioprecipitation reactor. influent sulfate (●), effluent sulfate (○), sulfate reduction efficiency (Δ), effluent sulfide (■), influent pH (●), effluent pH (○).

Table 1
Comparison between heavy metals bioleaching–bioprecipitation efficiencies in this study and those obtained in previous studies.

Treated material	Metal content in contaminated material (mg/kg)	Metal bioleached (%)	Metal content in leachate (mg/L)	Metal precipitated (%)	References
Artificially contaminated soil	Zn 142	91	Zn^{2+} 1.76	97.1	White et al. [24].
	Cu 88	91	Cu^{2+} 4.29	93.9	
	Ni 109	94	Ni^{2+} 6.22	86.8	
	Cr 132	90	Cr^{3+} 5.3	92.6	
Industrial site contaminated with Cu and Ni	Cu 5240	69	–	–	
	Ni 1507	68			
Artificially contaminated sand	ZnS 4000	98	Zn^{2+} 35.8	27.7	Cabrera et al. [25].
	NiS 1000	33	Ni^{2+} 1.2	44.1	
	Cr_2O_3 1700	17	Cr^{3+} 2.59	2.1	
Contaminated river sediment	Zn 732	78	Zn^{2+} 54.6	99.9	Present work
	Cu 174	63	Cu^{2+} 10.7	99.8	
	Cr 206	29	Cr^{3+} 5.6	90.3	

Comparison between the amount of sulfate reduced and the sulfide concentration measured in the effluent indicated that dissolved sulfide levels were substantially underestimated based on the stoichiometry of sulfate reduction. This result was attributed to three factors. One possible factor was due to the formation of various metal sulfides. It has been previously reported that during the bioprecipitation process the amount of sulfide lost from metal precipitates accounted for ~7.6% of the total sulfur mass [35]. The second factor contributing to the observed effect could be loss of volatile H_2S (under acid conditions) from the solution. Thirdly, the observation that the inside of the PMMA column was dark gray/black implied that some of volatile H_2S diffused into the walls of the column, leading to the further loss of dissolved sulfide in the effluent.

Table 1 gives the removal efficiencies of bioleached metals in the bioprecipitation reactor during the stable runs. The average results obtained in this study compare favorably with those of other bioreactor systems designed for treatment of acidic bioleachate laden with metal sulfates in terms of sulfate reduction rate, metal removal efficiency, and metal levels in the influent and final effluent. The present reactor proved highly efficient for the removal of the target metals to the low ppb range. The average concentrations of soluble Zn^{2+}, Cu^{2+}, and Cr^{3+} in the effluent were reduced from ~54,600 to ~10 μg/L, from ~10,700 to ~17 μg/L, and from ~5600 to ~543 μg/L, respectively. These values are equivalent to metal removal efficiencies, 99% of Zn^{2+}, 99% of Cu^{2+}, and 90% of Cr^{3+}, respectively. The final effluent concentrations of these metals were able to meet Chinese criteria for environmental discharge. The relatively lower Cr^{3+} removal was presumably because Cr could not form stable sulfides in the presence of water and thus Cr^{3+} removal during the bioprecipitation process might result from other precipitation forms (e.g., hydroxides or carbonates) with higher solubility products as compared to sulfide precipitation. Our subsequent XRD analysis confirmed the presence of CrOOH in the final precipitates (Fig. 6).

3.4. Characterization of metal precipitates

SEM-EDS analysis for the precipitates collected from the bioprecipitation reactor at the completion of the entire treatment process indicated significant formation of minerals with compacted structure containing potassium, calcium, sodium, magnesium, silicon, copper, zinc, chromium, iron, and sulfur (Fig. 5). PSD data showed that the d_{10}–d_{90} (the mid 80% range) particle size of the precipitates was between 20–73 μm, which was favorable for their subsequent settling and dewatering. XRD analysis revealed that the produced precipitates had a fine crystallinity and consisted primarily of SiO_2, Na_2S, $CaSO_4$, FeS_2, ZnS, Cu_2S, and CrOOH (Fig. 6). Precipitation of iron in the bioreactor is not surprising given the low solubility products of its metal sulfide (10^{-18} for FeS). Minor accumulation of sodium and calcium in these precipitates was possibly because of incompletely washing with deionized water prior to SEM-EDS and XRD detection. The characteristics for the samples withdrawn from the effluent settling tank were similar to the results for the precipitates from the reactor (data not shown). These findings suggested that in this study Cu^{2+} and Zn^{2+} precipitation with sulfides and

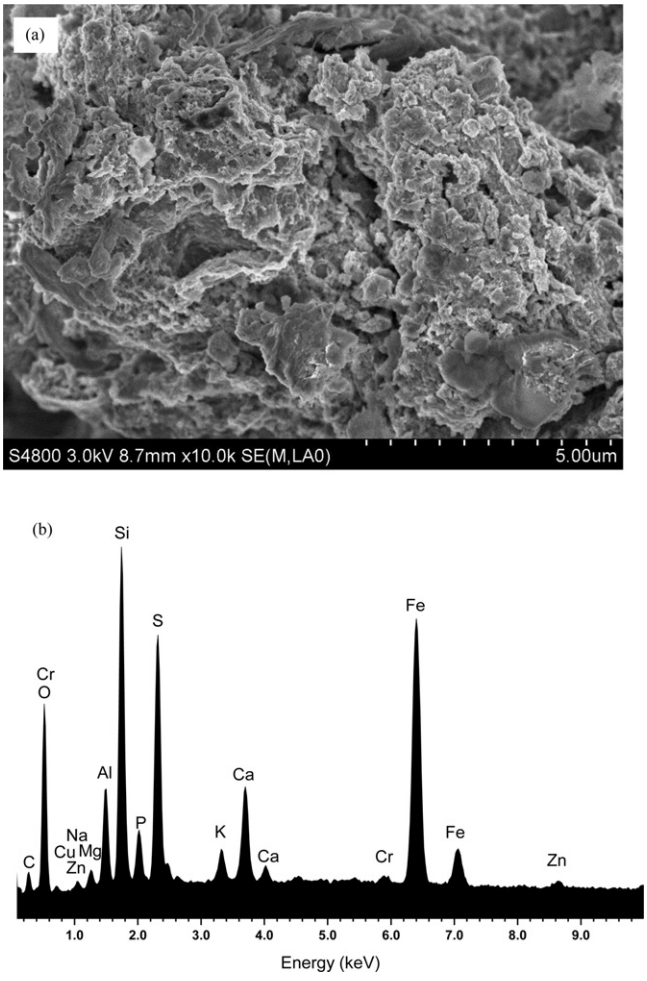

Fig. 5. SEM image (a) and EDS (b) of the precipitates collected from the bioprecipitation process.

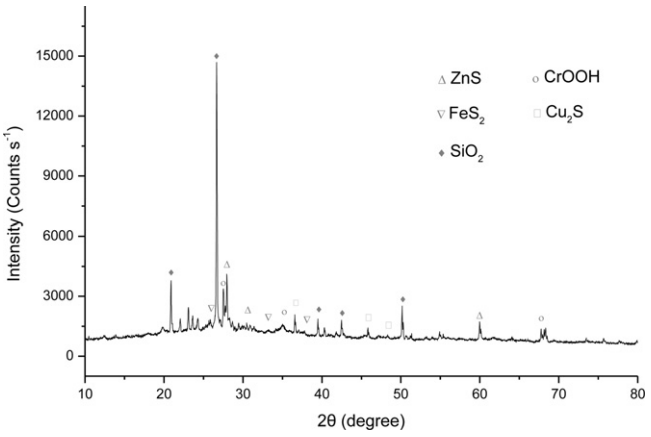

Fig. 6. XRD pattern of the precipitates collected from the bioprecipitation process.

Cr^{3+} precipitation with hydroxides were the primary mechanism for their respective removal. Nevertheless, other potential mechanisms for metal removal, such as sorption into biomass, could still not be ruled out as a result of the complexity of bioprecipitation process and diversity of the leachate constituent. In fact, in another experiment, we found that EPS secreted from sulfate-reducing bacteria cells could sequester readily Cu^{2+} in solution with maximum adsorption capacity (q_m) of 2000 mg (Cu^{2+})/g(EPS) to form insoluble EPS-Cu(II) complexes, indicating that during the bioprecipitation process the role of biosorption activity in metal removal did exist and were capable of improving, to a certain extent, metal removal efficiency.

The laboratory experiments presented herein have demonstrated that the harnessing and combination of bioleaching using sulfur-oxidizing bacteria and bioprecipitation using sulfate-reducing bacteria is technically effective in leaching and concentrating a range of contaminating metals, including Zn, Cu and Cr, from dredged sediment. The next step of this work should particularly demonstrate the economic potential of the proposed process (including cost of energy, metallic residues disposal cost, and the cost required to build the bioreactors). Likewise, some efforts, such as bioconversion of the residual organic COD (mainly acetate and other volatile fatty acids) to methane by alkaline anaerobic digestion treatment via methanogens, can possibly be further made in order to ensure organic COD present in the treated effluent to meet direct discharge criteria. In addition, some attempts, e.g., the possibility of organic wastes used as carbon and energy source for the bioprecipitation process, can also be carried out.

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