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Removal of sulfate and heavy metals by sulfate reducing bacteria in short-term bench scale upflow anaerobic packed bed reactor runs

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Abstract

Mildly acidic metal (Cu, Zn, Ni, Fe, Al and Mg), arsenic and sulfate contaminated waters were treated, over a 14 day period at 25°C, in a bench-scale upflow anaerobic packed bed reactor filled with silica sand and employing a mixed population of sulfate-reducing bacteria (SRB). The activity of SRB increased the water pH from ~4.5 to 7.0, and enhanced the removal of sulfate and metals in comparison to controls not inoculated with SRB. Addition of organic substrate and sulfate at loading rates of 7.43 and 3.71 kg d^{-1} m^{-3}, respectively, resulted in >82% reduction in sulfate concentration. The reactor removed more than 97.5% of the initial concentrations of Cu, Zn and Ni, while only >77.5% and >82% of As and Fe were removed, respectively. In contrast, Mg and Al levels remained unchanged during the whole treatment process. The removal patterns for Cu, Zn, Ni and Fe reflected the trend in their solubility for their respective metal sulfides, while As removal appeared to coincide with decreasing Cu, Zn, Ni and Fe concentrations, which suggests adsorption or concomitant precipitation with the other metal sulfides.

Keywords: Sulfate reduction; Arsenic; Copper; Sulfate reducing bacteria; Metal precipitation; Anaerobic water treatment

1. Introduction

Mine waters and industrial effluents may contain high sulfate and metal concentrations and pose significant disposal problems that require urgent solution to avoid serious environmental contamination. In mine waters, sulfate, metalloids (arsenic) and heavy metals such as copper, nickel, zinc and iron originate from the chemical or biological oxidation of exposed sulfide minerals. The process also generates acidity in the form of sulfuric acid which can also dissolve other minerals, releasing cations [1,2]. The metals and acid constitute acid mine (rock) drainage (AMD or ARD).

The mobility, bioavailability, and toxicological effects of heavy metals are largely dependent on its speciation. For example As(V) is less mobile and toxic than arsenite As(III), while methylated species are generally less toxic than inorganic species [3]. Both adsorption reactions and redox conditions essentially control the mobility of these chemical species [4]. Heavy metals such as Cu, Zn and Cd, and the metalloid As can be adsorbed [5] and/or co-precipitated [6] with the most abundant or reactive iron sulfide. They may also be sorbed to or released from metal oxyhydroxides depending on the redox potential and pH [7].

Established chemical treatment of contaminated waters such as AMD involves the addition of lime to raise pH and precipitate metals as hydroxides. It is, however, generally expensive and produces high sludge volumes [8]. There is increasing interest in the potential

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biotechnological applications of bacterial sulfate reduction as an alternative method for sulfate and heavy metal removal from environmental contamination particularly from mining activities [2,8–11]. Under anaerobic conditions, sulfate-reducing bacteria (SRB) oxidise simple organic compounds by utilising sulfate as an electron acceptor and generate sulfide (S^{2-}) and alkalinity. This biogenically produced sulfide can react with dissolved metals to form metal sulfide precipitates since the solubilities of most toxic metal sulfides are generally very low [9].

The treatment of mildly acidic contaminated waters using SRB in short-term bench-scale upflow anaerobic packed bed reactor (UAPB) runs was investigated. The short-term nature of the reactor experiments in this work implies that effects from aging of the material, clogging of the matrix and stabilisation of reactor performance will not be addressed, as they can only be addressed in long-term reactor experiments. The aims of the biological process were to remove heavy metals, decrease sulfate concentration and increase the pH of the water without forming high amounts of dissolved residues. In this article, we present our findings on the performance and chemistry of a bench-scale UAPB reactor.

2. Materials and methods

2.1. Column bioreactor

Sulfate reduction experiments were conducted in a bioreactor column constructed from a light grey polyvinyl chloride (PVC) pipe with an overall height of 800 mm, an internal diameter of 90 mm and a net empty working volume 4.78 ± 0.011 (Fig. 1). It was equipped with a total of seven ports used for sampling either liquid or solid material along the height of the reactor. Four 12.5 mm diameter sampling ports were equally spaced on one side of the column, including the inlet and outlet. The other three were 38 mm diameter sampling ports located equidistance directly on the opposite side of the column with respect to the inlet and outlet. The flow was dispersed with the aid of a frustum shaped cowling located at the base of the reactor (near the inlet), which also served to contain the porous media.

The reactor was filled with >2 mm fraction of commercially available coarse pool filter sand (density $2.62 \pm 0.12\ g\,cm^{-3}$) (Commercial Minerals Limited, Melbourne, Australia). It was pre-treated by soaking in 5% HNO_3 for 72 h to remove organic material, rinsed with distilled water and dried before use. The final pore volume was between 2110–2400 ml. Approximately 1.0 pore volume of influent substrate was pumped through the reactor to stabilise and condition the sand bed before the commencement of an experiment. The composition of the influent substrate is shown in Table 1, with lactate serving as the organic carbon source for growth. The pH of the medium was adjusted using 2 M HCl or NaOH to the required pH value. Trisodium citrate was added at $1867–18667\ mg\,l^{-1}$ to prevent metal precipitation. The influent was pumped from the medium reservoir tank to the bottom inlet of the reactor by means of a pre-calibrated variable speed peristaltic pump at $2.61\ ml\,min^{-1}$ (approximately 1.6 pore volumes per day).

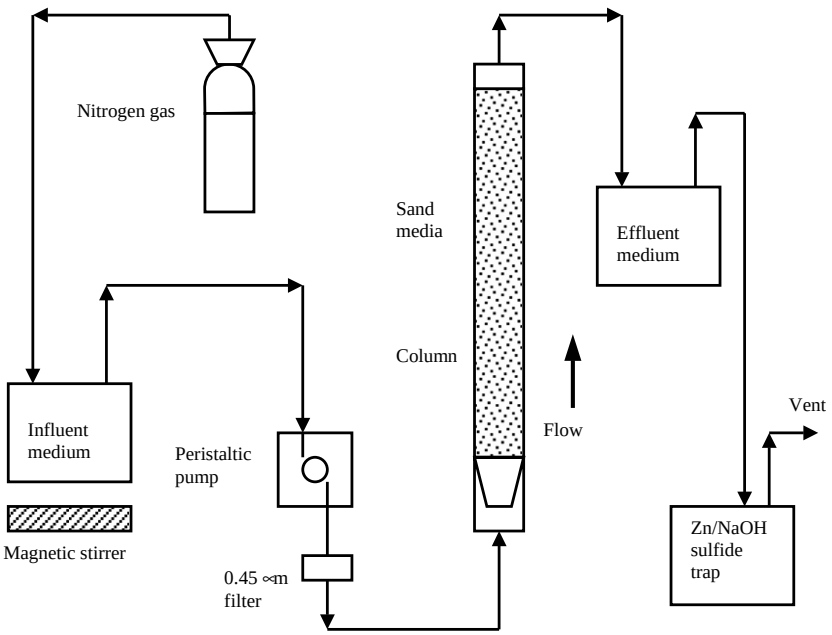

Fig. 1. A schematic diagram of the upflow anaerobic packed bed reactor setup used in this study.

Table 1
Composition of the influent substrate and column parameters

Analyte	Total concentration ($mg\,l^{-1}$)		
Al, As, Cu, Fe, Ni, Zn	50, 20, 10 and 5	ThOD[a] ($g\,l^{-1}$)	5.00
Mg	592–628	ThOD/SO_4^{2-}	2.00
$NaC_3H_5O_3$ (sodium lactate)	4690	OLR[b]($kg\,d^{-1}\,m^{-3}$)	7.43
Total P	50	SLR[c] ($kg\,d^{-1}\,m^{-3}$)	3.71
Total N	25	HRT[d] (h)	16.16
Total SO_4^{2-}	2500	Pore volume (l)	2.11–2.40
$Na_3C_6H_5O_7\cdot 2H_2O$	1867–18 667	Temp (°C)	25

[a] ThOD, theoretical oxygen demand = 96.0 × [lactate]/90.08.
[b] OLR, organic loading rate.
[c] SLR, sulfate loading rate.
[d] HRT, hydraulic retention time.

High purity nitrogen (Air Liquide) was continuously purged through the medium reservoir at a rate of approximately $1\,l\,min^{-1}$ to lower the O_2 content of the influent solution.

The reactor was initially filled with the influent at the desired metal concentration and inoculated with 400 ml of a mixed culture of SRB. This process preconditioned the SRB. Continuous flow was started 14 days after inoculation with each experiment conducted over a 14 day period. Duplicate experiments were conducted in a non-parallel manner. Control experiments containing no SRB were operated in the same manner as the inoculated or SRB columns. The results from the SRB columns were compared with that obtained for the controls. The sulfide concentration, the amount of sulfate removed and the concentration of dissolved heavy metals were measured along the column during the operation of the reactor as described in the analytical methods.

2.2. Isolation of SRB

The mixed SRB population was isolated from water samples collected from a wetland filter at the Woodcutters mine site in the Northern Territory, Australia. Wetland water was sampled by pushing a closed, sterile Schott bottle approximately 30 cm into the wetland filter before unscrewing the lid and allowing it to fill. Postgate's Medium B [12] was employed to isolate and grow sulfate-reducing bacteria and used for long-term storage of SRB. Batch screening of water and sediment samples for SRB was carried out by adding 12.5 ml of wetland water into a sterile 250 ml Schott bottle and completely filling it with Postgate's Medium B. The SRB were sub-cultured at least 3 times on Medium B before inoculation into the bioreactor.

2.3. Analytical and sampling methods

A total of 22 ml of water was sampled from each port of the column using a plastic syringe at pre-determined times. The sample was further divided into 3 portions. The pH and redox potentials of one portion were immediately measured in a nitrogen box, using an Activon redox/pH combined electrode (model no. AEP531). A second portion was centrifuged at 5000 rpm on a Beckman Benchtop centrifuge (Model TJ-6), then syringe filtered through a sterile 0.45 μm nitrocellulose filter (Millipore) into a polypropylene container (Sarstedt). The container was snap frozen in liquid nitrogen and stored at −20°C prior to arsenic speciation. Finally, the remaining portion was filtered through 0.45 μm and two sub-aliquots were retained for sulfide and sulfate analysis, while a third sub-aliquot was acidified with 1% HNO_3 prior to metal analysis.

Metal (^{63}Cu, ^{64}Zn, ^{60}Ni, ^{27}Al, ^{57}Fe, and ^{25}Mg) analysis was conducted using a Perkin Elmer Elan 6000 ICPMS with a Conikal concentric nebuliser (Glass Expansion, Part No. AR30-1-FC3). Total arsenic was determined using modified procedures previously described [13,14]. Briefly, the method employs a pre-reduction treatment step using a 10% HCl solution containing 0.2% KI, 0.2% ascorbic acid and $2\,\mu g\,l^{-1}$ Bi (as internal standard) to reduce all available As(V) into As(III). Arsenite was determined by replacing the KI with sodium citrate buffer (pH ~ 6.54) in the pre-reduction step. Under these conditions, As(V) is not reduced. Arsenate was calculated from the difference between total As and As(III). Arsenic analysis was determined by flow injection vapour generation inductively coupled plasma mass spectrometry (FI-VG-ICPMS) using a Perkin Elmer Elan 6000 ICPMS equipped with a Perkin Elmer FIAS 400.

Dissolved sulfide was measured immediately after sampling using the methylene blue method as described in Standard Methods for the Examination of Water and Wastewater [15] on a Hitachi U-1100 UV-Vis spectrophotometer. Water samples fixed with 1% HNO_3 were analysed for total sulfur by inductively coupled plasma spectroscopy on a Perkin Elmer Plasma 400 ICP-AES. The ICP-AES was flushed with high-purity nitrogen for about 1.5 h prior to sulfur analysis. Sulfate concentrations were calculated from the total sulfur concentrations, assuming the concentrations of other sulfur species in solution were negligible. Total bacterial count was obtained by manual methods using an improved Neubauer haemocytometer (Webber). Bacteria were viewed using an Olympus BH-2 phase contrast microscope (× 1000, Olympus Optical Co., Ltd., Japan) equipped with an oil immersion objective lens and 100 W halogen lamp (Olympus HAL-L).

All samples not analysed on the same day were stored at 4°C. All chemicals used were "analaR"reagent grade

Table 2
Typical water chemical parameters measured in the influent at the start of experiment and compared to nominal values; the detection limits of each analyte measured in the effluent water; and the percentage recoveries of arsenic species in samples spiked with appropriate concentrations of As(III) and As(V)

	pH	S^{2-}[a]	SO_4^{2-}[b,a]	Total As	As(III)	As(V)	^{60}Ni	^{63}Cu	^{57}Fe	^{64}Zn	^{27}Al	^{25}Mg
Detection limit[c]		0.31	0.45	2.31	4.28	4.50	0.08	0.13	3.71	0.22	0.14	4.25
Influent composition[a]												
Nominal	—	—	2505	10.7	—	—	10.0	11.4	12.1	10.1	10.0	620
Measured	4.52	—	2280	10.6	—	—	9.5	10.8	11.6	10.3	10.1	614

Arsenic species	As spiked[a]		As found[a]		As recovered (%)	
	As(III)	As(V)	As(III)	As(V)	As(III)	As(V)
	2	25	1.94	24.54	97	98
	2	25	2.02	25.47	101	102
	4	30	4.12	29.27	103	98
	4	30	3.97	29.87	99	99
	10	60	9.91	57.00	99	95
	10	60	10.16	62.72	102	105

[a] Concentration in $mg\,l^{-1}$.
[b] Calculated from total *S* analysis by ICP-AES.
[c] Detection limit = concentration of analyte which yields three times the standard deviation ($3 \times SD$) of the blank value ($\mu g\,l^{-1}$).

and were used without further purification. Chemical solutions were prepared with Hi-Pure water (Permutit). All plasticware and glassware were thoroughly cleaned by soaking in 1% Decon™ followed by soaking in 10% nitric acid for 48 h, rinsed several times with Hi pure water and oven dried before use.

3. Results and discussion

3.1. Nominal influent composition and quality control

In order to identify any initial precipitation of elements, the nominal concentrations of metals and sulfate in the influent water were compared to measured initial dissolved concentrations (Table 2). It was found that Mg, Cu, Ni, Fe and sulfate had been partly removed from the water phase by initial precipitation. In all cases, however, this was $<10\%$. The detection limits for the various analytes measured are also shown in Table 2. The recoveries of arsenic species in samples spiked with appropriate concentrations of the As(V) and As(III) indicated very good recoveries in the range from 95% to 105% (Table 2).

3.2. Bioreactor performance

A 14 day experimental period was chosen as early trials (data not shown) indicated that pH increased, and metal and sulfate had decreased substantially by this time in line with the aims of this work. The column was packed with sand media to increase sulfate reduction activity by providing a solid support (surface) to which SRB could adhere, since SRB tend to aggregate in areas which offer some physical protection [16]. SRB are then able to condition the immediate environment through their metabolism to form microcosms that are conducive to their survival. However, excess biomass and heavy metal precipitates can potentially be a problem by clogging the pore space in the reactor, complete clogging can be avoided by intermittent flushing of the column by increasing the influent upflow velocity. At the end of each experiment the sand media was visually examined. Black precipitates were formed in all of the experiments that were supplied with lactate. The precipitates appeared in between the sand particles and adhered onto the surface of the sand particles themselves. Estimated total bacterial counts in the water phase of the reactor were initially between 1 and 2×10^7 cells ml^{-1}. The predominant SRB in the system were vibrio-shaped bacteria. Total counts ranged from 2×10^5 to 8×10^8 cells ml^{-1} throughout the experiment.

The influent was introduced into the reactor inoculated with or without SRB for a period of 14 days at room temperature. The reactor containing no SRB served as a control. Column tests were run at influent metal (Cu, Ni, Zn, Al and Fe) and metalloid (As) concentrations of 5, 10, 20 and 50 $mg\,l^{-1}$. Generally, redox potentials dropped, pH increased and some metals were removed from the water phase. The residual

concentrations of Cu, Ni and Zn for the 5, 10 and 20 mg l^{-1} experiments were found to be below their respective detection limits as reported in Table 2. There were no substantial differences in the trends of sulfate and metal removal, which were characterised by an initial lag period of approximately 1 day, followed by a decrease in metal and sulfate concentrations (Figs. 2(c)–(e)). The data presented in Fig. 2(d) show that as the reaction proceeded, dissolved Cu concentrations for all cases decreased significantly, and that the rates of copper removal were related to the initially added Cu concentration. Similar patterns were also observed for the other metals (data not shown). In view of this, results pertaining to an influent metal concentration of 10 mg l^{-1} are discussed.

3.3. Redox potential and pH

A drop in the pH was observed at the beginning of the operation, and steadily increased in all SRB column experiments (Fig. 2(a)). The drop in pH values was probably caused by the introduction of the influent at a lower pH. A simultaneous increase in redox potential (Eh) was observed, due to the introduction of the influent at a higher Eh value (Fig. 2(b)). The pH and Eh of the effluent from the control remained constant, conversely the effluent pH and Eh from the SRB column reached approximately 7.2 and −218 mV in 4 days, respectively. The gradual increases in pH and decrease in Eh seen after day 1 was indicative of an adaptation period by SRB to new conditions. Similar increases in pH were observed when acidic leachate from pyritic mining wastes were treated in anaerobic reactors filled with spent mushroom compost [11] and in packed-bed reactors filled with mine gob materials [9].

3.4. Sulfate reduction

Sulfate concentrations in the effluent from treatment systems inoculated with SRB decreased rapidly while there was no decrease in the control reactor (Fig. 2(e)), demonstrating that the SRB inoculated column promoted strong sulfate-reducing activity. The effluent from the SRB column showed greater than 6 fold decrease in sulfate, reaching a minimum of 357 mg l^{-1} sulfate by day 7. The sulfide concentration in the effluent ranged between 15 and 134 mg l^{-1} throughout the 14 day period. Similar values were reported by Christensen et al. [1] using an anaerobic packed-bed reactor to treat acid mine water. Trends in sulfide concentrations, however, were observed through this period. Increasing SRB activity caused higher sulfate reduction rate, resulting in gradually increasing sulfide concentration. A large drop in sulfide concentration after day 2 was probably due to metals precipitating as insoluble metal sulfides and adsorption of sulfide onto the walls of the reactor. Days 2–4 showed a less dramatic decrease in sulfide concentration, but remained constant thereafter.

The extent of sulfate removal measured between 0.5 and 7 days, decreased with increasing initial concentrations of metals (Fig. 2(f)), indicating that sulfate reduction at higher metal concentrations was still occurring but at a lower rate compared with influent at lower metal concentrations. This was attributed to two factors. One possible factor was that the rate of sulfate removal was lowered in cases of higher metal concentrations due to the reduction in SRB metabolic activity as a result of metal toxicity on the SRB. It has been previously reported that the consumption of sulfate by *D. desulfuricans* is significantly slower in the presence of Cu(II) [17]. Song et al. [18] reported a sulfate removal IC_{50} for Cu(II) (concentration causing 50% inhibition of SRB sulfate removal efficiency) of 156 mg l^{-1}. Contrastingly, Sani et al. [17], who used a single SRB strain and a specific metal toxicity medium containing constituents that did not result in any abiotic precipitation of metal ions, reported an IC_{50} for Cu(II) of 1.02 mg l^{-1}. These studies suggest that metal toxicity and inhibition in SRB systems are strongly influenced by the chemical and physicochemical properties of the surrounding SRB environment. At an initial metal concentration of 10 mg l^{-1}, the highest average sulfate reduction rate was about 475 mg l^{-1} d^{-1} and compares well with methods previously used to treat AMD [9,11]. The constantly changing flow paths through areas of high and low sulfate reduction activity in the column likely caused the non-uniform distribution of sulfate throughout the column. Therefore, the second factor contributing to the observed effect could be that the high levels of metal sulfide precipitation caused partial blockage of the sand-bed, leading to mass transfer limitations which are more severe in the cases of higher metal concentrations.

3.5. Sulfur mass-balance

The amount of sulfate in water samples from the 10 mg l^{-1} experiment is shown in Table 3. All calculations and comparisons of sulfate were done on an elemental sulfur (S) basis. This data highlights several observations. First, that a nominal 761 mg l^{-1} sulfur was introduced into the system (S_{in}), but only 124 mg l^{-1} was detected in the column effluent (S_{out}) after the completion of the experiment at day 14. Secondly, the difference is partly due to the formation of heavy metal sulfide precipitates, which accounted for only 3.5% of the total sulfur budget. Thirdly, dissolved sulfide measurements ($S_{diss} = S^{2-} + HS^{-} + H_2S$) may have substantially underestimated the amount of sulfate reduced. For example, although sulfide analysis was conducted immediately after sampling, the highly volatile nature of sulfide itself means that some sulfide was possibly lost as a result of air oxidation in transferring samples from the

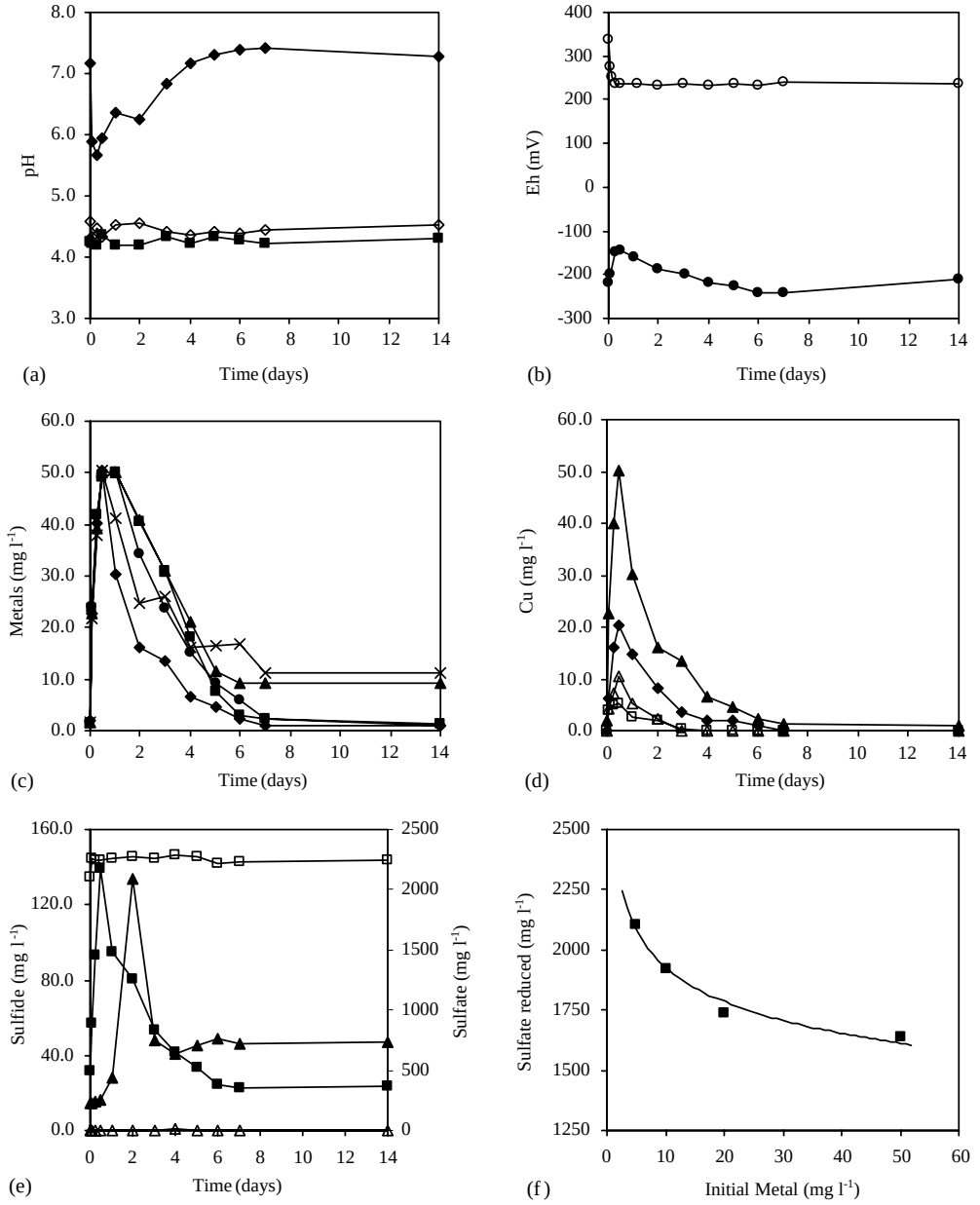

Fig. 2. Variations in (a) pH/SRB (♦—♦), pH/control (◇—◇) and influent (■—■); and (b) Eh/SRB (●—●), Eh/control (○—○) with time for 10 mg l^{-1} experiments; (c) Cu (♦—♦), Fe (▲—▲), Zn (●—●), Ni (■—■) and As (×—×) with time for 50 mg l^{-1} SRB amended experiments; (d) Cu concentration for 5 mg l^{-1} (□—□), 10 mg l^{-1} (Δ—Δ), 20 mg l^{-1} (♦—♦) and 50 mg l^{-1} (▲—▲) SRB amended experiments; (e) sulfide (▲—▲) and sulfate (■—■) concentrations with time in SRB amended 10 mg l^{-1} experiments compared to sulfide (Δ—Δ) and sulfate (□—□) controls; and (f) the extent of sulfate reduced between 0.5 and 7 days in SRB amended systems at four different influent metal concentrations. All values are effluent concentrations (unless stated otherwise) and are from the average of duplicate experiments. The experimental time was 14 days.

column to the nitrogen box, resulting in lower sulfide values than was actually present. It is also plausible that some loss occurred via the formation of colloidal sulfides which were filtered off during the measurements. The observation that the insides of the PVC used to construct the reactor had turned dark grey implied that some of volatile H_2S was lost (or stripped) from the column or that diffusion of H_2S into the wall had occurred, consequently contributing to the further loss of dissolved sulfide in the effluent (S_{lost}). Mass-balance

Table 3
Typical water chemical parameters as measured in the effluent of the control compared to effluent concentrations in the water phase of SRB columns at the end of the experiment (day 14)

Sample[a]	pH	Eh (mV)	Concentration ($mg\,l^{-1}$)												
			SO_4^{2-}[b]	S_{in}[c]	S_{out}[c]	S_{metal}[c]	S_{diss}[c]	S_{lost}[c]	Al	As	Cu	Fe	Mg	Ni	Zn
Control															
Inf	4.34	+223.7	2248	750		—	<d.l.[d]	—	10.3	10.7	11.0	11.0	613	10.0	10.6
Eff	4.36	+220.2	2240		748	—	<d.l.[d]	—	10.3	10.6	10.9	10.9	612	9.6	10.4
SRB															
Inf	4.56	+221.2	2315	772		—	<d.l.[d]		5.2	5.1	5.1	5.1	633	5.1	5.1
Eff	7.31	−202.1	389		130	13	46	630	5.0	1.1	0.01	0.86	634	0.02	0.01
Inf	4.52	+225.3	2280	761		—	<d.l.[d]		10.1	10.6	10.8	11.6	614	9.5	10.3
Eff	7.29	−203.6	372		124	26	47	611	9.9	2.4	0.02	2.0	613	0.02	0.02
Inf	4.57	+224.4	2294	766		—	<d.l.[d]		20.0	20.1	20.1	20.1	615	20.1	20.1
Eff	7.28	−205.1	397		132	51	47	582	20.1	4.3	0.04	3.6	617	0.02	0.02
Inf	4.58	+222.9	2290	764		—	<d.l.[d]		50.1	50.6	50.3	50.8	595	49.9	50.4
Eff	7.35	−202.2	356		124	125	47	516	50.2	11.3	1.1	9.1	595	1.2	1.1

Values are from the average of duplicate experiments pertaining to reactors containing an initial metal influent concentration of 5,10, 20 or $50\,mg\,l^{-1}$. All concentrations are in $mg\,l^{-1}$ unless stated otherwise.

[a] Inf = influent, Eff = effluent.

[b] Calculated from total S analysis by ICP-AES.

[c] S_{in} = total sulfur (as elemental S) introduced into system, S_{out} = total sulfur measured in effluent, S_{metal} = sulfur used in precipitating metals from solution, S_{diss} = dissolved sulfide: $S^{2-} + HS^- + H_2S$, S_{lost} = sulfur lost via stripping and diffusion through reactor wall (i.e. $S_{lost} = S_{in} - S_{out} - S_{metal}$).

[d] d.l. = detection limit ($3 \times$ SD (blank) = $0.31\,mg\,l^{-1}$).

calculations in Table 3 suggest that this phenomenon represented 80% of the total sulfur budget.

3.6. Heavy metal removal

The aqueous phase of the effluent was analysed for metal ion concentrations and is also presented in Table 3. The results pertaining to a system inoculated with SRB and containing an initial influent metal concentration of $10\,mg\,l^{-1}$ are shown in Fig. 3(a). The concentrations of Zn, Cu and Ni were dramatically lowered to less than $0.05\,mg\,l^{-1}$ after 4, 6 and 7 days, respectively. This represents >99.5% removal of the initial concentrations of Cu, Zn and Ni in the influent. The removal of Fe was incomplete (Fig. 3(b)) and only 82.3% of the initial Fe was removed corresponding to the lowest level of Fe detected at $1.95\,mg\,l^{-1}$ at day 14. These results are comparable to those reported by Dvorak et al. [11], who obtained greater than 95% removal of Cu, Ni, and Zn using anaerobic reactors to treat metal contaminated water in an underground coal mine.

The metal removal was attributed to the precipitation of insoluble metal sulfides as a result of the sulfides produced by the biological activities of SRB. Among the heavy metals it was noted that copper was the first metal to be removed, then zinc followed closely by nickel, and lastly iron (Figs. 3(a) and (b)). This removal pattern is reflected in the trend in solubility products of the respective metal sulfides; with log K_s of CuS, ZnS and NiS equivalent to –40.94, −28.39 and −27.98 [19], which are much lower than FeS at −22.39. CuS is particularly insoluble over a broad range of pH [1] and sulfide precipitation of copper is thus a rapid and efficient process. Machemer and Wildeman [10] investigated metal removal processes in an experimental constructed wetland receiving acid mine drainage, and found that the removal of Cu, Zn and Fe closely followed the trend in K_s values. Figs. 3(a) and (b) also illustrate that metal ions were removed faster in the reactor inoculated with SRB than in the control (Fig. 3(c)). The very small decrease in metal concentrations in the effluent of the control indicated that adsorption processes are likely to be responsible for the slight loss in metal concentration over time. When compared to systems containing SRB these results indicate that pre-conditioned SRB growing on lactate can neutralise relatively low pH influent and decrease Cu, Zn, Ni and Fe concentrations simultaneously.

Conversely, magnesium and aluminium remained relatively constant throughout the study (Fig. 3(b)).

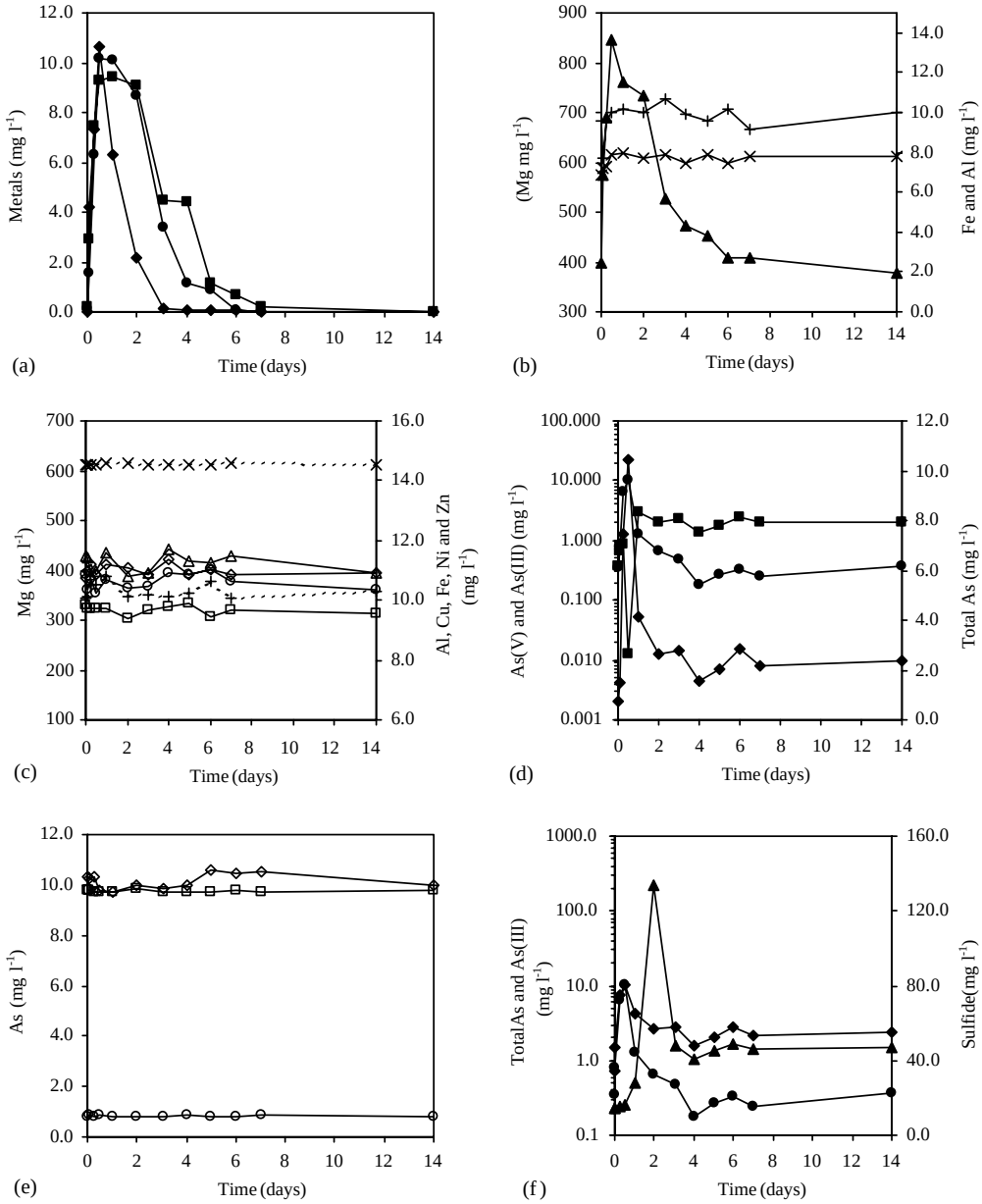

Fig. 3. Variations of effluent (a) Cu (♦—♦), Zn (●—●) and Ni (■—■); (b) Mg (×—×), Fe (▲—▲) and Al (+—+) with time in SRB amended 10 mg l^{-1} experiments; (c) Mg (× --- ×), Fe (Δ—Δ), Al (+ --- +), Zn (○—○), Ni (□—□) and Cu (◇—◇) with time in control 10 mg l^{-1} experiments; and (d) total As (♦—♦), As(III) (●—●) and As(V) (■—■) with time in SRB amended 10 mg l^{-1} experiments (note the log scale for As(III) and As(V)); (e) total As(◇—◇), As(III) (○—○) and As(V) (□—□) with time in control 10 mg l^{-1} experiment; and (f) total As (♦—♦) and As(III) (●—●) compared to dissolved sulfide (▲—▲) concentrations in SRB amended 10 mg l^{-1} experiments (note the log scale for total arsenic and As(III)). All values are from the average of duplicate experiments. Experimental time was 14 days.

This is presumably due to the fact that MgS has a very high solubility product and Al does not form stable sulfides in the presence of water. The results for Mg and Al agree with studies conducted by Maree and Strydom [20], but contradict the results of Christensen et al. [1], who reported that aluminium in contaminated mine water was reduced from 17.7 mg l^{-1} to $<5\,\mu g\,l^{-1}$ in a batch treatment system using mixed SRB culture grown on lactose. However, it must be noted that the treatment system also received 5.7% whey as an additional source

of carbon and was studied over a longer duration (>150 days). Dvorak et al. [11] found that Al was removed to less than 0.2 mg l^{-1} in a pilot-scale anaerobic reactor packed with mushroom compost and limestone. He suggested that the removal of Al resulted from its hydrolysis to insoluble $Al(OH)_3$, since the effluent was found to be saturated with $Al(OH)_3$. Stability studies on the influent substrate used in this study indicated that no significant amounts of Mg and Al hydrolysed until around pH 10.5 (data not shown). In view of this, and given that the highest pH value attained in this study was approximately 7.4, it seems reasonable to conclude that any minor loss of either metal during the experiment was likely due to adsorption processes either onto metal sulfide precipitates and/or the reactor walls.

3.7. Arsenic removal

The removal rate of As in the SRB column (Fig. 3(d)) was relatively rapid when compared to the control columns (Fig. 3(e)), which showed no appreciable As removal. Fig. 3(d) illustrates that at the end of the sampling period (day 14), total As in the aqueous phase of the effluent had decreased to 2.4 mg l^{-1} from an initial concentration of 10.6 mg l^{-1}, a >77.5% removal. This compares well to Simonton et al. [21], who reported consistent removal for As and Cr (>60–80%) from solution using SRB (*Desulfovibrio desulfuricans*) in columns containing silica sand. Uhrie et al. [22] found that after 6 days of incubation, 96% of the initial 10 mg l^{-1} As was removed from solution in serum bottles containing sulfidogenically active SRB biomass. The results for arsenic was characterised by an initial low total As concentration followed by a rapid increase in total As concentration between 1 and 12 h, with As(III) ≫ As(V). Since the SRB column was initially subjected to an incubation period, the observed rapid increase in As levels was probably a result of the proton dissolution of arsenic precipitates or release of adsorbed arsenic already present, as influent at a lower pH was introduced into the column.

Total dissolved As then decreased between 12 and 48 h as a result of dropping As(III) concentration. As(V) increased during this period; with As(V) consistently higher than As(III). After this period, the concentration of total As, As(III) and As(V) remained constant. When anoxic conditions were stabilised (~day 3) arsenic concentrations fell in the presence of sulfide. Dissolved total arsenic concentrations were then observed to increase then decrease with corresponding sulfide levels (Fig. 3(f)). This is consistent with either the precipitation of arsenic sulfides such as As_2S_3 or concomitant removal of arsenic with Cu-, Zn-, Ni- and Fe-sulfides, followed by re-dissolution or desorption of the As to form soluble thioarsenite (As(III)) complexes. This behaviour was also reported by Castro et al. [23] using SRB fed with organic wastes to treat contaminated water in an open-pit mine. Webster [24] found that in highly sulfidic microcosms, As(III) solubility was increased by sulfide through the formation of thioarsenite complexes.

The removal pattern for total arsenic, however, appeared to coincide with decreasing Cu, Zn, Ni and Fe, and did not follow the trend in solubility products of the respective metal sulfides, if it is assumed that amorphous As_2S_3 was formed. The formation of As_2S_3 was expected to follow after FeS since the log K_s for amorphous As_2S_3 is −11.9 [25], which is significantly higher than that for FeS (log $K_s = -27.39$). It was also noted that total arsenic in the effluent took only 3 days to decrease to a level of 2.81 mg l^{-1}, while levels of Cu, Zn, Ni and Fe dropped to comparable levels in 2, 3.5, 4.5 and 7 days, respectively. In view of this, the initial removal of arsenic is perhaps best explained by the adsorption or concomitant co-precipitation with Zn-, Cu-, Fe- and Ni-sulfides. Arsenic and other metals are known to co-precipitate with iron sulfides [6] or be adsorbed by metal sulfides [5]. The formation of insoluble arsenic sulfides may have occurred later when reducing conditions (Eh < −180 mV) were more established due to the increase in sulfate reduction by SRB activity. Rittle et al. [4] found that some of the arsenic was precipitated as an Fe–As–S solid phase by actively sulfidogenic microcosms containing As. This contradicts studies by Dowdle et al. [26] who reported that As(III) itself was not immobilised as As-sulfides in anoxic salt marsh sediments. However, their studies involved significantly higher As concentration of ~750 mg l^{-1} as apposed to 100 mg l^{-1} employed by Rittle et al. [4] and ⩽50 mg l^{-1} used in this study. In view of this, it appears that the concentration of As species is a critical factor, and that the rate of arsenic reduction is variable in differing environments. Although the exact processes responsible for arsenic removal are not clear, it is evident that when compared to controls containing no SRB the action of bacterial sulfate reduction in this particular system greatly enhanced the removal rate. It is also recognised that this work has highlighted variables that affect the rates of sulfate reduction, sulfide precipitation and the eventual formation of mineral phases in this system. Work is continuing to determine which mechanism is in fact responsible for As removal, including secondary ion mass spectrometry (SIMS) studies.

4. Conclusion

This investigation demonstrated microbial sulfate reduction and subsequent precipitation of Cu, Zn, Ni, Fe and As by a mixed population of SRB in an UAPB reactor containing silica sand. After an initial lag phase or adaptation period, sulfate reduction began and pH increased, redox potentials dropped and dissolved

concentrations of Cu, Zn, As, Ni and Fe were significantly reduced in SRB inoculated systems supplied with lactate. These results were consistent with those found by other investigators. In continuous-flow column experiments containing SRB, effluent pH above pH 7.2 and greater than 80% sulfate removal efficiencies were attained due to the activity of SRB. Conversely, pH levels remained low (pH $\sim$4.5) with no sulfate reduction detected in systems containing no SRB. Metal removal efficiencies of more than 97.5% for Cu, Zn and Ni, and $>$82% for Fe were achieved in the column experiments. The treatment process also removed $>$77.5% of the initial concentration of As, but was ineffective in removing Mg and Al. The results presented here have relevance to SRB found in natural systems and also to efforts to use similar systems to remediate water quality in mildly acidic metal and sulfate contaminated water.

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References

[1] Christensen B, Laake M, Lien T. Treatment of acid mine water by sulfate-reducing bacteria: results from a bench scale experiment. Water Res 1996;30(7):1617–24.

[2] Chang IS, Shin PK, Kim BH. Biological treatment of acid mine drainage under sulphate-reducing conditions with solid waste materials as substrate. Water Res 2000;34(4): 1269–77.

[3] Turpeinen R, Pantsar-Kallio M, Höggblom M, Kairesalo T. Influence of microbes on the mobilization, toxicity and biomethylation of arsenic in soils. Sci Total Environ 1999;236:173–80.

[4] Rittle KA, Drever JI, Colberg P JS. Precipitation of arsenic during bacterial sulfate reduction. Geomicrobiology 1995;13:1–11.

[5] Davis JA. Complexation of trace metals by adsorbed natural organic matter. Geochim Cosmochim Acta 1984;48:679–91.

[6] Moore JN, Brook EJ, Johns C. Grain size partitioning of metals in contaminated coarse-grained river floodplain sediment: Clark Fork River, Montana. Environ Geol Water Sci 1989;14:107–15.

[7] Masscheleyn PH, Delaune RD, Patrick Jr. WH. Effect of redox potential and pH on arsenic speciation and solubility in a contaminated soil. Environ Sci Technol 1991;25:1414–9.

[8] Elliott P, Ragusa S, Catcheside D. Growth of sulfate-reducing bacteria under acidic conditions in an anaerobic bioreactor as a treatment system for acid mine drainage. Water Res 1998;32(12):3724–30.

[9] Kim SD, Kilbane JJ, Cha DK. Prevention of acid mine drainage by sulfate reducing bacteria: organic substrate addition to mine waste piles. Environ Eng Sci 1999;16(2): 139–45.

[10] Machemer SD, Wildeman TR. Adsorption compared with sulfide precipitation as metal removal processes from acid mine drainage in a constructed wetland. J Contam Hydrol 1992;9:115–31.

[11] Dvorak DH, Hedin RS, Edenborn HM, McIntire PE. Treatment of metal-contaminated water using bacterial sulfate reduction: results from pilot scale reactors. Biotechnol Bioeng 1992;40:609–16.

[12] Postgate JR. The sulphate-reducing bacteria, 2nd ed. Cambridge: Cambridge University Press; 1984.

[13] Stroh A, Völlkopf U. Optimization and use of flow injection vapour generation inductively coupled plasma mass spectrometry for the determination of arsenic, antimony and mercury in water and sea-water at ultratrace levels. J Anal Atom Spectrom 1993;8:35–40.

[14] Samanta G, Chowdhury TR, Mandal B K, Biswas BK, Chowdhury UK, Basu GK, Chanda CR, Lodh D, Chakraborti D. Flow injection hydride generation atomic absorption spectrometry for determination of arsenic in water and biological samples from arsenic-affected districts of West Bengal, India, and Bangladesh. Microchem J 1999;62:174–91.

[15] Greenberg AE, Clesceri LS, Eaton AD, editors. Standard methods for the examination of water and wastewater, 18th ed. Washington, DC: American Public Health Association, 1992.

[16] Meyer-Reil LA. Microbial life in sedimentary biofilms—the challenge to microbial ecologists. Mar Ecol Prog Ser 1994;112:303–11.

[17] Sani RK, Peyton BM, Brown LT. Copper-induced inhibition of growth of Desulfovibrio desulfuricans G20: assessment of its toxicity and correlation with those of zinc and lead. Appl Environ Microbiol 2001;67(10):4765–72.

[18] Song YC, Piak BC, Shin HS, La SJ. Influence of electron donor and toxic materials on the activity of sulfate-reducing bacteria for the treatment of electroplating wastewater. Water Sci Technol 1998;38:187–94.

[19] Di Toro DM, Mahony JD, Hansen DJ, Scott KJ, Hicks MB, Mayr SM, Redmond MS. Toxicity of cadmium in sediments: the role of acid volatile sulfide. Environ Toxicol Chem 1990;9:1487–502.

[20] Maree JP, Strydom WF. Biological sulphate removal from industrial effluent in an upflow packed bed reactor. Water Res 1987;21(2):141–6.

[21] Simonton S, Dimsha M, Thomson B, Barton LL, Cathey G. Long-term stability of metals immobilized by microbial reduction. Proceedings of the 2000 Conference on Hazardous Waste Research: Environmental Challenges and Solutions to Resource Development, Production and Use, Southeast Denver, CO, 2000. p. 394–403.

[22] Uhrie JL, Drever JI, Colberg PJS, Nesbitt CC. In situ immobilization of heavy metals associated with uranium leach mines by bacterial sulfate reduction. Hydrometallurgy 1996;43:231–9.

[23] Castro JM, Wielinga BW, Gannon JE, Moore J N. Stimulation of sulfate-reducing bacteria in lake water from a former open-pit mine through addition of organic wastes. Water Environ Res 1999;71(2):218–23.

[24] Webster J. The solubility of As_2S_3 and speciation of As in dilute and sulfide-bearing fluids at 25 and 90°C. Geochim Cosmochim Acta 1990;54:1009–17.

[25] Eary LE. The solubility of amorphous As_2S_3 from 25 to 90°C. Geochim Cosmochim Acta 1992;56:2267–80.

[26] Dowdle PR, Laverman AM, Oremland RS. Bacterial dissimilatory reduction of arsenic(V) to arsenic(III) in anoxic sediments. Appl Environ Microbiol 1996;62(5):1664–9.