Waste and Biomass Valorization (2019) 10:2907–2914
https://doi.org/10.1007/s12649-018-0329-3

ORIGINAL PAPER

Precipitation of Copper (II) in a Two-Stage Continuous Treatment System Using Sulfate Reducing Bacteria

Ayla Bilgin[1,2] · **Peter R. Jaffé**[2]

Received: 6 January 2018 / Accepted: 8 May 2018 / Published online: 11 May 2018

Abstract

Biologically driven precipitation of dissolved copper and other trace metals has been used to treat contaminated aqueous streams. However, high dissolved trace metal concentrations can lead to toxicity, and their bioremediation difficult. Furthermore sorption of trace metals onto biomass might result in large amounts of contaminated byproducts. The aim of this work was to develop and test a two-stage reactor to bypass the toxic effects on the bacteria and chemically precipitate copper without contaminating the bulk of the biomass. Hence, copper removal using a sulfate reducing bacteria culture was investigated in a two-stage continuous treatment system. The first reactor was a sand-filled biological reactor in which the sulfate is reduced, followed by a second reactor/clarifier where the chemical precipitation and sedimentation of a CuS phase occurs. The influent Cu^{2+} concentration was varied systematically between 15 and 600 mg/L, and the precipitation of Cu^{2+} metal as CuS was achieved in the second reactor, resulting in complete (within detection limits) Cu^{2+} removal. EDS analysis on the solid phase collected from the second reactor confirmed the presence of Cu and S in the precipitate. EDS analysis on the solid phase collected from the second reactor confirmed the presence of Cu and S in the precipitate, and a CuS phase with minimal biomass was obtained. This configuration avoids toxicity effects of heavy metals in the biological reactor, as well as the contamination of biomass with the trace metal. Furthermore, the biomass free CuS precipitates can be easily disposed or even used to recover the trace metal.

Keywords Heavy metal · Copper · Cooper sulfide · Anaerobic treatment · Sulfate reducing bacteria

Introduction

Heavy metal contamination arises from many activities, such as smelting and mining, and often causes contamination of the aquatic and soil systems [1, 2]. Copper, which this study focuses on, inhibits microbial metabolic processes (nitrification, denitrification, and glucose fermentation) at high concentrations, while it is a necessary micronutrient for many enzymes and co-enzymes at low concentrations [3, 4]. Contamination of soils and water by copper is a major problem, especially in soils with low pH where it sorbs less and is more bioavailable [5, 6]. Because they may enter the food chain, the presence of heavy metals in the soil has become an environmental problem of significant concern. Some of the in-situ remediation techniques for trace metals, such as chemical stabilization, acid leaching, excavation and transport have several environmental disadvantages such as high cost and low efficiency [7–9]. Conventional physicochemical methods such as chemical precipitation, membrane filtration, reverse osmosis, electrochemical treatments, adsorption, and evaporation are generally expensive and are insufficient in the treatment of highly diluted heavy metals [2, 10, 11].

Trace metals can be precipitated as hydroxides or oxyhydroxides. Hydroxide precipitation is one of the methods commonly used for the removal of heavy metals from industrial and/or polluted groundwater. However, it has disadvantages such as the re-solubilization of metals with changes in the pH and that precipitation of the trace metals is difficult in the presence of chelating agents. Alternatively, trace metals can also be precipitated as a sulfide [12–14]. For this purpose, sulfide can be either added directly or produced via biological reduction of sulfate. Compared to biologically produced sulfide, adding sulfide via a chemical reaction

✉ Ayla Bilgin
ayla.bilgin@gmail.com

[1] Department of Environmental Engineering, Artvin Coruh University, Seyitler Campus, 08100 Artvin, Turkey

[2] Department of Civil and Environmental Engineering, Princeton University, Princeton, NJ 08544, USA

has the advantage that the precipitate includes less sludge [15–17], which will be produced from the biomass growth. For in-situ treatment, the implementation of bioremediation technologies using sulfate reducing bacteria (SRB) is advantageous for the removal of metals such as copper from contaminated groundwater, such as acid rock drainage, being very effective in metal removal at low concentrations. The solubility of the sludge resulting from the precipitation of metal sulfides is less than that of the hydroxides compounds formed via chemical treatment and is much more stable [18–21] under anoxic conditions. In addition, biologically precipitated metal sulfides can be recovered and recycled [18].

Since many wastewaters contain both trace metals and sulfate, the biological sulfate reduction method to precipitate selected trace metals has gained interests in recent years. Applications have been carried out in full-scale bioreactors, as well as in in-situ remediation techniques [22, 23]. Hence, precipitation of heavy metals as metal sulfide, using SRB to generate the sulfide, is considered an effective alternative to physicochemical methods. This method consists of two steps that can happen either in the same reactor or in consecutive reactors: (1) SRB oxidize simple organic compounds (acetate, lactate, etc.) under anaerobic conditions using sulfate as the electron acceptor, while producing hydrogen sulfide and bicarbonate. (2) the biologically produced hydrogen sulfide then reacts with heavy metals, and precipitates as insoluble metal sulfide [17, 24, 25], as shown in the Eqs. (1) and (2) below.

$$2CH_2O + SO_4^{-2} \rightarrow H_2S + 2HCO_3^- \tag{1}$$

$$M^{2+} + H_2S \rightarrow MeS\downarrow + 2H^+ (Me = Metal) \tag{2}$$

Many anaerobic bacteria (i.e., *Desulfovibrio, Desulfomicrobium, Desulfobacter, Desulfosarcina, Desulfotomaculum, Thermodesulfobacteria, Archaeoglobus*) are capable of reducing sulfur to sulfide [26]. Precipitation of dissolved copper as copper sulfide using sulfide produced by SRB has been studied by many researchers. Copper removal studies have been carried out in batch reactors using SRB [18], *Desulfovibrio* A2 bacteria [27], as well as *Desulfovibrio* and *Desulfotomaculum* bacteria [28].

Metal precipitation using biological sulfate reduction in a single reactor is a low-cost solution for acid mine drainage (AMD) treatment. However, it may not perform satisfactorily if the waste water is very acidic or contains heavy metals at high concentrations [29]. Sulfate reduction and metal precipitation can also be carried out in a two-stage process consist of a biological stage followed by the precipitation stage. The advantage of a two-stage system is the elimination of toxic effects due to high acidity and/or metal concentrations in AMD streams [30, 31]. This technique has been applied to metals such as Cu and Zn [32–35]. Another approach is recycling some of the treated water to dilute the water that is toxic [36, 37]. However, in this system, recycling of water requires additional pumps and larger reactors to achieve a given hydraulic retention time, thus, increasing the investment and operating costs [29]. In an alternate system, metals can be pre-precipitated by recycling the sulfide-containing water or H_2S-containing gas, prior to the biological step [38]. If the sulfate and the metal are present in different water streams, the metal precipitation occurs with H_2S that was produced separately in the bioreactor [39, 40]. A study carried out by Alvarez et al., consisted of a three-stage system, including (1) the optimization of H_2S production by SRB utilizing volatile fatty acids, (2) formation of a biofilm reactor for sulfide production in a bench-scale upflow anaerobic packed bed reactor, and (3) the precipitation of metal sulfides by direct mixing of sulfide-rich supernatant from the sulfidogenic anaerobic bioreactor and metal leachate. The advantage of this system is the recirculation of the effluent after the precipitation step, in order to reduce the remaining sulfate. Removal yields of 100% for copper, over 94% for zinc and over 92% for lead were reported with an influent concentration of copper 0.018 mM, zinc 1.34 mM, and lead 0.0023 mM [41]. In addition to these purification systems, researchers have proposed three-stage systems, consisting of an alkaline reactor (limestone filter), a biological reactor, and a chemical reactor. This system is recommended for acid mine drainage waters having low pH and containing high metal concentrations [42, 43].

In this study the removal of Cu^{2+} from an aqueous stream was studied using a two-stage treatment system. In the two-stage system, the first reactor consists of the biological sand-column reactor (with a 2.53 h hydraulic retention time) and the second reactor consists of the chemical reactor (with a 7.33 h hydraulic retention time) where the CuS precipitates, and that also acts as an up-flow clarifier. In the literature, studies with a two-stage treatment system are few, and none has been reported with the configuration studied here. The configuration used here differs from other studies in that an up-flow packed bed column reactor filled with sand was used as the biological reactor. One of the most important advantages of this system is the sand bed also acts as a filter to remove the biomass, resulting in a low-solid effluent containing the HS^-. The effluent from the first reactor is then mixed with the stream containing the Cu^{+2}, and fed into the second up-flow reactor, where the CuS precipitate forms and settles, allowing the effluent to be free of the CuS phase and allowing for the collection of a CuS phase at the bottom of the reactor that is relatively pure and not contaminated with biomass. The advantages of this system are: (1) precipitation of metal sulfide absent of biomass, (2) treatment of metal concentrations way above their toxicity levels, (3) easy metal recovery, (4) easy adjustment of properties such as pH, hydraulic retention time (HRT) of either reactor, utilization of many possible organic waste streams (if needed augmented with sulfate) as the carbon source for the SRB.

Materials and Methods

Analytical Techniques

Copper analyses were carried out through the Neocuproine Method [44], using a Spectronic Genesys'2 spectrophotometer. Measurements were made measuring light attenuation at a wavelength of 457 nm. The calibration was obtained by preparing 0, 1, 10, 25 and 50 mg/L stock Cu^{2+} solutions in the bacterial medium using $CuCl_2$ as the Cu^{2+} source. All measurements were performed in triplicate. The upper limit for copper analysis of this method was determined to be 25 mg/L. Hence, concentrations in excess of 25 mg/L were diluted to yield concentrations below 25 mg/L prior to the analyses. The Cu^{2+} measurements were tested over a pH range between 4.0 and 9.0, and the optimum pH found as 5.0. Hence, copper analyses were carried out at pH 5.0 ± 0.1.

All liquid samples were collected after the second reactor with a 0.22 µm Millex-GP Syringe Filter, before SO_4 and TOC analyses were performed. SO_4 analyses were performed using a Thermo Scientific ion chromatograph. A Shimadzu-Total Organic Carbon Analyzer was used for the TOC measurements.

Bacterial Culture

A sulfate reducing bacteria medium was prepared, containing $MgSO_4$ (2 g/L), $CaCl_2.2H_2O$ (0.1 g/L), NH_4Cl (1 g/L), Na_2SO_4 (3.7 g/L), K_2HPO_4 (0.5 g/L), $FeSO_4.7H_2O$ (0.005 g/L), yeast extract (1 g/L) and $NaC_2H_3O_2$ (3.5 g/L or 42.7 mM). The pH of the medium was 7.5 ± 0.1.

To obtain a SRB seed, a soil sample was taken from a dried lake sediment, 10–15 cm depth below the surface, placed in a glass bottle, and brought to the laboratory. One gram of the soil sample was then added to 1000 mL of the medium and the slurry was incubated for 15 days in an anaerobic chamber at room temperature.

Reactor Study

The study was carried out using a two-stage continuous up-flow reactor system. Test sand ASTM 20–30 (specially graded natural silica sand to pass a No. 20 (850 µ) sieve) was used as the first reactor's porous medium. The effluent of the first reactor was feed into the second reactor in addition to the flow containing dissolved Cu^{2+}. The columns used for both reactors were made of glass and had a length of 30 cm with the inner diameter of 3 cm.

The first reactor was inoculated with 1/2 pore volume of the bacterial medium using the acclimated bacterial culture. Pumping was then discontinued for 48 h to allow the bacteria to adhere to the sand grains. After this, pumping was resumed and the bacterial medium was pumped into the first reactor at a flowrate of 25.2 mL/h. The effluent from the first reactor was fed to the second reactor plus 3.7 mL/h of a Cu^{+2} solution, resulting in a flow of 0.008 mL/s. A peristaltic pump (ISMATEC High Precision Multichannel Pump) was used to supply the flow to both reactors. Hence, the HRT of the first reactor was 2.53 hand the HRT of the second reactor was 7.33 h (assuming a porosity of 0.3). Copper was chemically precipitated in the second reactor with the sulfide produced in the first reactor. The CuS precipitate settled at the bottom of the second reactor, yielding a Cu-free effluent. Nitrogen gas was bubbled trough the influent medium to provide anaerobic conditions in the reactor. A schematic of the column reactor is shown in Fig. 1.

Preparation of Cu^{2+} Stock Solution

The experiments were conducted with solutions containing Cu^{2+}, which were prepared by dissolving analytical grade $CuCl_2$ in distilled water. Cu^{2+} concentrations ranged from 15 to 600 mg/L.

Biomass Concentration Measurements

Biomass concentrations in the sludge of the second reactor were measured as volatile suspended solid (VSS). The VSS concentration was measured according to method 2540D in Standard Methods for Water and Wastewater [44]. Briefly, the sludge was dried at 103–105 °C for 2 h and weighted. The sample was then ignited at 550 °C for 2 h and weighted again [45, 46]. The mass lost after incineration represents the volatile suspended solid (VSS).

Energy Dispersive Spectroscopy Analysis

Precipitates that accumulated at the bottom of the second reactor were collected and analyzed via energy dispersive spectroscopy (EDS) using a PHILIPS XL30 SEM. A drop of copper sulfide was placed on an aluminum SEM stub and dried for ~ 36 h in the fume hood. The sample was then coated with ~ 9 nm of iridium using the Leica sputter coater at zero tilt. The sample was analyzed in the XL30 SEM at primarily 5 and 8 kV due to charging observed at 10–12 kV.

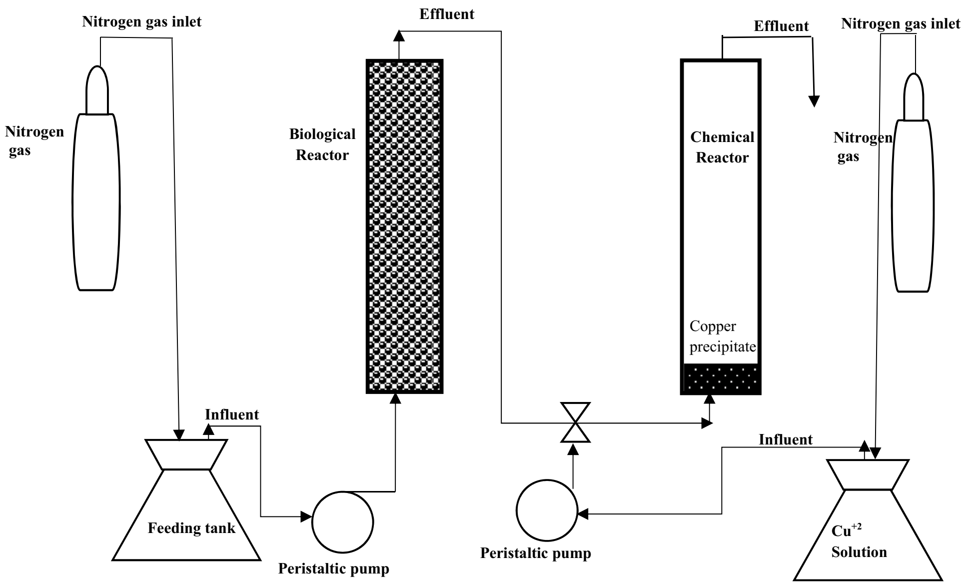

Fig. 1 Schematic diagram of the reactor system

Result and Discussion

The reactor was operated as described above for a 6-month period. The Cu^{2+} input concentrations into the second reactor were 15, 20, 50, 75, 100, 200, 300, 400, 500, and 600 mg/L. Constant inflow was maintained in the reactors at each concentration over a 4 to 5-day period, and then increased to the next concentration level. Results show that concentrations of SO_4^{2-} and TOC decreased with time, indicating that over the time the system was operated the biomass in reactor 1 kept increasing and reducing more SO_4^{2-}. After 6 months sulfate in the two-stage continuous treatment system reached a minimum concentration of 790 mg/L or a 78% reduction (Table 1) corresponding to a sulfate reduction rate of approximately 163.2 mmol SO_4/L/day. Complete (within detection limits) Cu^{2+} removal was achieved for all Cu^{2+} concentrations in the influent. As the Cu^{2+} concentration was increased, presumably more CuS precipitated since the HS^-, estimated by the amount of SO_4^{2-} removed, was in excess of the stoichiometric requirements (Table 1). This also indicates that the CuS precipitation was not kinetically limited within the HRT of reactor 2.

During the last six stages of this experiment (Cu^{2+} inputs between 100 and 600 mg/L) the average SO_4^{2-} removed was 3910 mg/L or 40.7 mM. Hence, theoretically one could have removed up to 45.75 mM or 2907 mg/L of Cu^{2+}. In this study, the reactor was operated up to a concentration of

Table 1 Effects of sulfate and TOC concentrations on copper removal

Cu^{2+} (mg/L) (In)[a]	Cu^{2+} (mmol/L) (In)[a]	TOC (mg/L) (In)	SO_4^{2-} (mg/L) (In)	SO_4^{2-} (mmol/L) (In)	Cu^{2+} (mg/L) (Out)	TOC (mg/L) (Out)[b]	SO_4^{2-} (mg/L) (Out)[b]	SO_4^{2-} (mmol/L) (Out)[b]	ΔSO_4^{2-} (mmol/L) (In-Out)[b]
15	0.24	1100	4103	42.74	0	765	3610	37.60	5.14
20	0.31	1100	4103	42.74	0	575	3070	31.98	10.76
50	0.79	1100	4103	42.74	0	589	2888	30.08	12.66
75	1.18	1100	4103	42.74	0	675	2540	26.46	16.28
100	1.57	1100	4103	42.74	0	430	1580	16.46	26.28
200	3.15	1100	4103	42.74	0	698	560	5.83	36.91
300	4.72	1100	4103	42.74	0	535	930	9.69	33.05
400	6.30	1100	4103	42.74	0	600	840	8.75	33.99
500	7.87	1100	4103	42.74	0	549	800	8.33	34.41
600	9.45	1100	4103	42.74	0	490	790	8.23	34.51

[a] Cu^{2+} was injected into the stream between columns 1 and 2. After mixing concentrations entering column 2 decreased to ~ 1/8th of these values

[b] Measured in the outflow of column 2. Decrease is due to reaction plus dilution by a factor of ~ 7/8th (3.7/28.9)

600 mg/L Cu^{2+}, and the Cu^{2+} concentration in the effluent was zero (below detection) achieving 100% Cu^{2+} removal.

At the end of the experiment, the pH increased from 7.5 in the inflow to 8.0 in the effluent. Equation 1 shows that alkalinity is being produced during sulfate reduction, while Eq. 2 shows that alkalinity is being consumed. Since much more sulfate was being reduced than sulfide precipitated, the net effect is an increase in pH.

A HRT of several hours is desirable to reduce a significant amount of sulfate by SRB. In this study, the HRT was 2.53 h for the biological reactor, which was more than sufficient for the purposes reducing sufficient sulfate for treating the Cu^{2+} stream. HRTs much higher than that might not provide much gain since it has been shown that an increase in HRT from 12 h to 10 days increased sulfate reduction only by an additional 8% [47].

Characterization of the CuS Phase

EDS analyses were carried out and SEM photographs were taken to characterize the precipitate formed by the removal of the Cu^{2+} metal in the second reactor (Figs. 2, 3).

This precipitate was taken from the bottom of the second reactor at the end of approximately 6 months of operation when the Cu^{2+} input concentration was 200 mg/L. Operation continued with increasing Cu^{2+} concentrations in the influent to reactor 2 up to a Cu^{2+} concentration of 600 mgL. EDS analyses confirmed the presence of the Cu and S in the

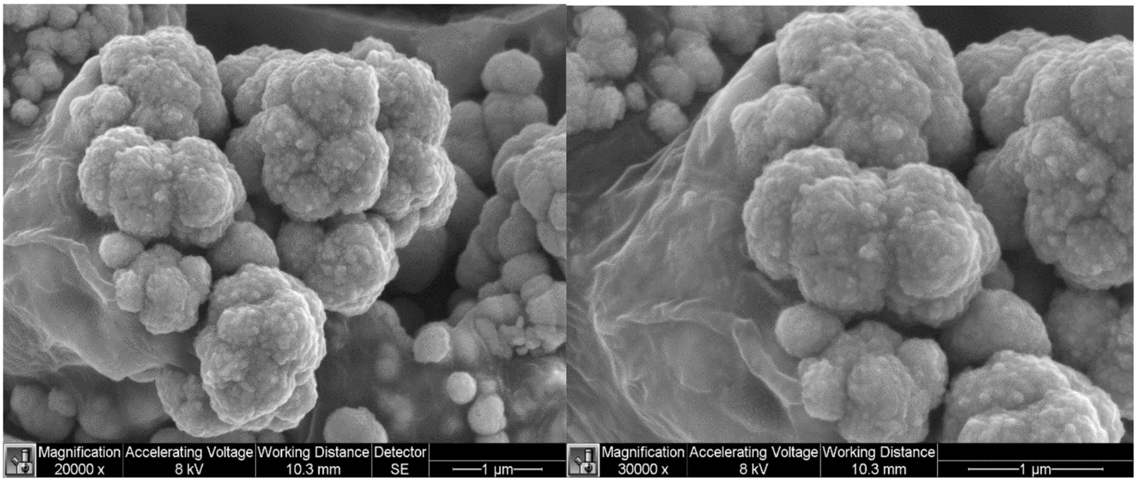

Fig. 2 Scanning electron microscopy (SEM) photographs of the precipitate

Fig. 3 EDS spectrum of the precipitate

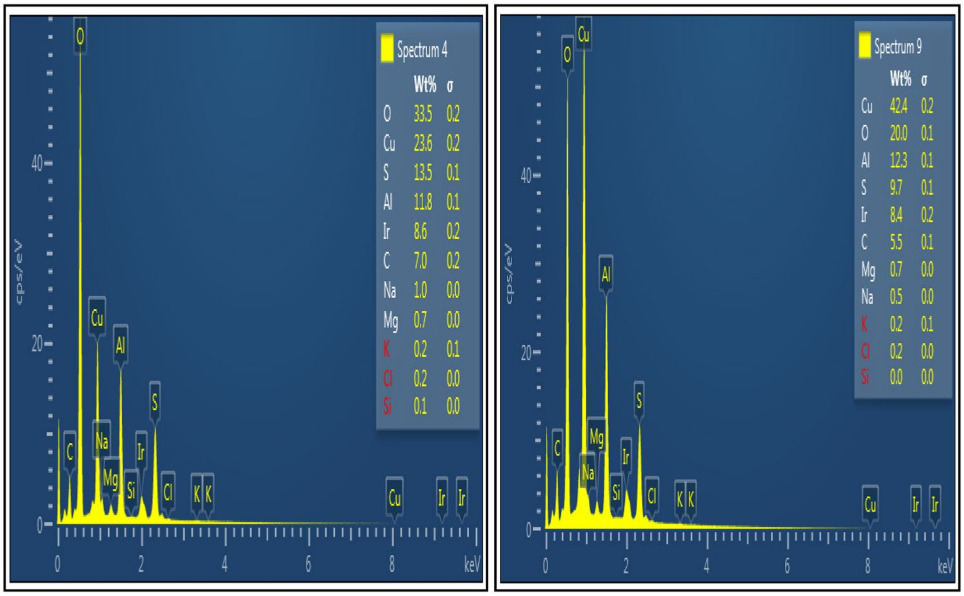

Table 2 EDS analysis of Cu-sulfide precipitates

Samples name	Cu (wt%)	S (wt%)	Atomic ratio (Cu/S)
1	44.92	28.72	0.78
2	67.76	16.91	1.99

precipitate and the results are consistent with what similar studies have reported [18].

Copper sulfides exists as chemical compounds ranging from copper-rich chalcocite (Cu_2S) to copper-deficient villamaninite (CuS_2) with other intermediate compounds, in-between, such as covellite (CuS), djurleite ($Cu_{1.95}$ S), and anilite ($Cu_{1.75}S$) [48, 49], digenite ($Cu_{1.8}S$), geerite ($Cu_{1.6}S$), spionkopite ($Cu_{1.4}S$), yarrovite ($Cu_{1.12}S$), among others [50]. In studies reported in the literature, covellite (CuS) and chalcocite (Cu_2S) have been reported as being most common in the solid phase of biogenic Cu-sulfite precipitates [51, 52].

EDS analysis of the Cu-sulfides collected in this study showed that they contained 44.92–67.76 wt% Cu and 14.11–28.72 wt% S (Table 2). Covellite (CuS) contains 66.5 wt% Cu and 33.5 wt% S with a Cu/S = 1. Chalcocite (Cu_2S) contains 79.9 wt% Cu and 20.1 wt% S with a Cu/S = 2 [34]. The partial elemental composition of the precipitates showed that Cu, S and O were the dominant elements in precipitates (Fig. 3) and the Cu/S atomic ratio of samples ranged from 0.78 to 1.99 (Table 2). Hence based on these analyses the precipitates appear to be predominantly covellite (CuS) but also contain chalcocite (Cu_2S). Similar results have been found in the other studies [27, 34]. Gramp et al. studied biogenic copper sulfide formation and found Cu/S atomic ratios between 0.83 and 0.99 [34].

The amount of biomass in the sludge was 2% based on the VSS analysis. This shows the effectiveness of the two-stage system with the biological reaction being in a sand medium in terms of maintaining the biomass and metal precipitate separate and yielding a relatively organic free copper sulfide phase.

Conclusion

The interaction of SRB with heavy metals can be complex since heavy metals have toxic and inhibitory effects on SRB. Biological methods to precipitate trace metals may also result in a large amount of biomass that is contaminated with the trace metals and/or mixed with the metal precipitate. Here a simple system to overcome these limitations was set up and its performance was demonstrated. The toxic effect of the heavy metal on SRB was mitigated by having two separate reactors. Furthermore, a sandy medium was used for the SRB reactor to filter out biomass so as to minimize the contamination of biomass by the trace metal. In the first reactor, SRB reduced sulfate in a sandy medium resulting in an effluent with HS^- and minimal biomass. The effluent from this reactor was fed into a second up-flow reactor to which a stream containing Cu^{2+} was also fed. Copper precipitated with the sulfide in the second reactor and settled to its bottom. Analyzing the precipitate via EDS showed that the copper precipitated as cooper sulfides.

The HRT of the biological reactor was 2.53 h. and was sufficient to reduce an excess of sulfate required for the precipitation of the CuS phase in the second reactor. The HRT of the second reactor was 7.3 h. and was sufficient to allow for the CuS precipitate to form, resulting in a complete Cu^{2+} removal over the range of Cu concentrations studied. This HRT resulted in an up-flow velocity of 4.1 cm/h., which allowed for the settling of the CuS phase and no CuS particles were observed in the effluent. The minimum HRT for the biological reactor depends on the amount of HS^- that needs to be produced for a specific application, and is a function of the influent source and strengths (e.g. concentration of TOC and SO_4^{2-}), surface area of the medium, and as shown here length of operation. The reactor was operated here for a total of 6 months, it is possible that much longer operation times might result in more accumulation of biomass and a higher-pressure loss, which should be evaluated in long-term trials.

The two-reactor system utilized here has the advantage that it can be used for metal removal at high concentration since the metal is not fed to the biological reactor, which has the added advantage that the CuS precipitates separately from biomass and is relatively pure. This has the advantage of reducing disposal costs or that the precipitate can be used easily in metal recovery. The system can also take advantage of existing organic waste streams and result in their partial treatment. Depending on the waste stream composition, the waste stream might have to be augmented with SO_4^{2-}.

Acknowledgements This study has been supported by 2219 Post-doctoral program and the authors present their thanks to the institution of The Scientific and Technological Research Council of Turkey (TUBITAK). At the same time, the authors are thankful for the support and assistance during the process of working with Environmental Biogeochemistry Research Group of Princeton University: Melany Ruiz Urigüen, Shan Huang, Arianna Sherman, Weitao Shuai, Il Han.

References

1. Vimala, R., Das, N.: Biosorption of cadmium (II) and lead (II) from aqueous solution using mushrooms: a comparative study. J. Hazard. Mater. **168**, 376–382 (2009)
2. Li, Y., Wu, Y., Wang, Q., Wang, C., Wang, P.: Biosorption of copper, manganese, cadmium, and zinc by Pseudomonas putida

isolated from contaminated sediments. Desalination Water Treat. **52**(37–39), 7218–7224 (2014)

3. Zhang, Z.Z., Deng, R., Cheng, Y.F., Zhou, Y.H., Buayi, X., Zhang, X., Wang, H.Z., Jin, R.C.: Behavior and fate of copper ions in an anammox granular sludge reactor and strategies for remediation. J. Hazard. Mater. **30**(300), 838–846 (2015)
4. Ochoa-Herrera, V., Leon, G., Banihani, Q., Field, J.A., Sierra-Alvarez, R.: Toxicity of copper(II) ions to microorganisms in biological wastewater treatment systems. Sci. Total Environ. **412–413**, 380–385 (2011)
5. Radziemska, M., Jeznach, J., Mazur, Z., Fronczyk, J., Bilgin, A.: Assessment of the effect of reactive materials on the content of selected elements in Indian mustard grown in Cu-contaminated soils. J. Water Land Dev. **28**, 53–60 (2016)
6. Fronczyk, J., Radziemska, M., Mazur, Z.: Copper removal from contaminated groundwater using natural and engineered limestone sand in permeable reactive barriers. Fresenius Environ. Bull. **24**(1a), 228–234 (2015)
7. Lasat, M.M.: Phytoextraction of toxic metals: a review of biological mechanisms. J. Environ. Qual. **31**, 109–120 (2002)
8. Kumar, R.N., Nagendran, R.: Changes in nutrient profile of soil subjected to bioleaching for removal of heavy metals using *Acidithiobacillus thiooxidans*. J. Hazard. Mater. **156**, 102–107 (2008)
9. Das, C., Bhowal, A., Datta, S.: Bioremediation of copper-contaminated soil by co-application of bioaugmentation and biostimulation with organic nutrient. Bioremediat. J. **15**(2), 90–98 (2011)
10. Mata, Y.N., Blazquez, M.L., Ballester, A., Gonzalez, F., Munoz, J.A.: Sugar-beet pulp pectin gels as biosorbent for heavy metals: preparation and determination of biosorption and desorption characteristics. Chem. Eng. J. **150**, 289–301 (2009)
11. Perez-Marin, A.B., Ballester, A., Gonzalez, F., Blazquez, M.L., Munoz, J.A., Saez, J., Meseguer Zapata, V.: Study of cadmium, zinc, and lead biosorption by orange wastes using the subsequent addition method. Bioresour. Technol. **99**, 8101–8106 (2008)
12. Peters, R.W., Ku, Y., Batthacharyya, D.: Evaluation of recent treatment techniques for removal of heavy metals from industrial wastewaters. Paper Presented at AIChE Meeting, Philadelphia, PA, 19–22: (1984)
13. Patterson, J.W.: Metals control technology; past, present and future. Separation and recovery. In: Patterson, J.W., Passino, R., (eds.) Metals Speciation, Separation, and Recovery. Proceedings of the Second International Symposium on Metals Speciation, Separation, and Recovery, Rome, Italy, May 14–19, 1989. Lewis Publishers, Chelsea (1990)
14. Veeken, A.H.M., de Vries, S., Van der Mark, A., Rulkens, W.H.: Selective precipitation of heavy metals as controlled by a sulfide-selective electrode. Sep. Sci. Technol. **38**(1), 1–19 (2003)
15. Gallegos-Garcia, M., Celis, L.B., Rangel-Mendez, R., Razo-Flores, E.: Precipitation and recovery of metal sulfides from metal containing acidic wastewater in a sulfidogenic down-flow fluidized bed reactor. Biotechnol. Bioeng. **102**, 91–99 (2009)
16. Tsukamoto, T.K., Killion, H.A., Miller, G.C.: Column experiments for microbiological treatment of acid mine drainage: low-temperature, low-pH and matrix investigations. Water Res. **38**, 1405–1418 (2004)
17. Kieu, H.T., Müller, E., Horn, H.: Heavy metal removal in anaerobic semi-continuous stirred tank reactors by a consortium of sulfate-reducing bacteria. Water Res. **45**(13), 3863–3870 (2011)
18. Jalali, K., Baldwin, S.A.: The role of sulphate reducing bacteria in copper removal from aqueous sulphate solutions. Water Res. **34**(3), 797–806 (2000)
19. Rossi, G.: Environmental applications. Biohydrometallurgy, ch. 6. McGraw Hill, Hamburg (2000)
20. Villa Gómez, D.K.: Simultaneous sulfate reduction and metal precipitation in an inverse fluidized bed reactor. PhD thesis, UNESCO-IHE Institute for Water Education, Delft, The Netherlands (2013)
21. Remoudaki, E., Hatzikioseyian, A., Kousi, P., Tsezos, M.: The mechanism of metals precipitation by biologically generated alkalinity in biofilm reactors. Water Res. **37**, 3843–3854 (2003)
22. Picavet, M., Dijkman, H., Buisman, C.: Development of a novel efficient bioreactor for sulphate reduction. Electron. J. Environ. Agric. Food Chem. **2**(2), 297–302 (2003)
23. Sampaio, R.M.M., Timmers, R.A., Xu, Y., Keesman, K.J., Lens, P.N.L.: Selective precipitation of Cu from Zn in a pS controlled continuously stirred tank reactor. J. Hazard. Mater. **165**, 256–265 (2009)
24. Hulshof, A.H.M., Blowes, D.W., Gould, W.D.: Evaluation of in situ layers for treatment of acid min drainage: a field comparison. Water Res. **40**, 1816–1826 (2006)
25. Neculita, C.M., Zagury, G.J., Bussiere, B.: Passive treatment of acid mine drainage in bioreactors using sulfate-reducing bacteria: critical review and research needs. J. Environ. Qual. **36**, 1–16 (2007)
26. Odom, J.M., Singleton, R.: The Sulfate-Reducing Bacteria: Contemporary Perspectives. Springer, New York (1993)
27. Karnachuk, O.V., Sasaki, K., Gerasimchuk, A.L., Sukhanova, O., Ivasenko, D.A., Kaksonen, A.H., Puhakka, J.A., Tuovinen, O.H.: Precipitation of Cu-sulfides by copper-tolerant *Desulfovibrio* isolates. Geomicrobiol. J. **25**(5), 219–227 (2008)
28. Luptakova, A., Kusnierova, M.: Bioremediation of acid mine drainage contaminated by SRB. Hydrometallurgy, **77**, 97–102 (2005)
29. Hao, O.J.: Metal effects on sulfur cycle bacteria and metal removal by sulfate reducing bacteria. In: Lens, P.N.L., Hulshoff Pol, L. (eds.) Environmental Technologies to Treat Sulfur Pollution: Principles and Engineering, pp. 393–414. IWA Publishing, London (2000)
30. Johnson, D.B., Hallberg, K.B.: Acid mine drainage: remediation options. Sci. Total Environ. **338**, 3–14 (2005)
31. Tabak, H.H., Scharp, R., Burckle, J., Kawahara, F.K., Govind, R.: Advances in biotreatment of acid mine drainage and biorecovery of metals: 1. Metal precipitation for recovery and recycle. Biodegradation **14**(6), 423–436 (2003)
32. Al-Tarazi, M., Heesink, A.B.M., Versteeg, G.F., Azzam, M.O.J., Azzam, K.: Precipitation of CuS and ZnS in a bubble column reactor. AIChE J. **51**(1), 235–246 (2005)
33. Foucher, S., Battaglia-Brunet, F., Ignatiadis, I., Morin, D.: Treatment by sulfate-reducing bacteria of Chessy acid-mine drainage and metals recovery. Chem. Eng. Sci. **56**(4), 1639–1645 (2001)
34. Gramp, P.J., Sasaki, K., Binghman, J.M., Karnachuk, O.V., Tuovinen, O.H.: Formation of covellite (CuS) under biological sulfate-reducing conditions. Geomicrobiol. J. **23**(8), 613–619 (2006)
35. Esposito, G., Veeken, A., Weijma, J., Lens, P.N.L.: Use of biogenic sulfide for ZnS precipitation. Sep. Purif. Technol. **51**(1), 31–39 (2006)
36. Ma, X., Hua, Y.: Cd^{2+} removal from wastewater by sulfate reducing bacteria with an anaerobic fluidized bed reactor. J. Environ. Sci. **9**, 366–371 (1997)
37. Steed, V.S., Suidan, M.T., Gupta, M., Miyahara, T., Acheson, C.M., Sayles, G.D.: Development of a sulfate-reducing biological process to remove heavy metals from acid mine drainage. Water Environ. Res. **72**, 530–535 (2000)
38. Kaksonen, A., Puhakka, J.: Sulfate reduction based bioprocesses for the treatment of acid mine drainage and the recovery of metals. Eng. Life **6**, 541–564 (2007)
39. Dijkman, H., Buisman, C.J.N., Bayer, H.G.: Biotechnology in the mining and metallurgical industries: cost savings through selective precipitation of metal sulfides. In: Proceedings of the Copper 99—Cobre 99 International Conference, Phoenix, Arizona, USA,

October 10–13, 1999, Vol. IV: Hydrometallurgy of Copper (Eds: S. K. Young, D. B. Dreisinger, R. P. Hackl, D. G. Dixon), The Minerals, Metals & Materials Society, Warrandale, PA (USA), 113–126 (1999)

40. Vegt, A.L., Krol, J., Buisman, C.J.N.: Biological sulfate removal and metal recovery from mine waters. In: Proceedings of the International Biohydrometallurgy Symposium IBS97, BIOMINE 97, Sydney, Australia, August 4–6, 1997, Australian Mineral Foundation, Glenside, South Australia, 1–10 (1997)
41. Alvarez, M.T., Crespo, C., Mattiasson, B.: Precipitation of Zn(II), Cu(II) and Pb(II) at bench-scale using biogenic hydrogen sulfide from the utilization of volatile fatty acids. Chemosphere **66**, 1677–1683 (2007)
42. Prasad, D., Wai, M., Berube, P., Henry, J.G.: Evaluating substrates in the biological treatment of acid mine drainage. Environ. Technol. **20**(5), 449–458 (1999)
43. Prasad, D., Henry, J.G.: Removal of sulphates acidity and iron from acid mine drainage in a bench scale biochemical treatment system. Environ. Technol. **30**(2), 151–160 (2009)
44. APHA; AWWA. Standard Methods for Water and Wastewater Examinations, 21st edn.; American Public Health Association (APHA); American Water Works Association (AWWA): Washington, DC (2005)
45. Metcalf and Eddy. Wastewater Engineering: Treatment, Disposal, and Reuse, 3rd edn.; McGraw-Hill, Inc.: New York, NY (1991)
46. Grady, C.P.L., Daigger, G.T., Lim, H.C.: Biological Wastewater Treatment, 2nd edn.; Marcel Dekker Inc.: New York, NY (1999)
47. Isa, Z., Grusenmeyer, S., Verstraete, W.: Sulfate reduction relative to methane production in high-rate anaerobic digestion: microbiological aspects. Appl. Environ. Microbiol. **51**(3), 580–587 (1986)
48. Goble, R.J.: The leaching of copper from anilite and the production of a metastable copper sulfide structure. Can. Miner. **19**, 583–591 (1981)
49. Tezuka, K., Sheets, W.C., Kurihara, R., Shan, Y.J., Imoto, H., Marks, T.J., Poeppelmeier, K.R.: Synthesis of covellite (CuS) from the elements. Solid State Sci. **9**(1), 95–99 (2007)
50. Cao, J., Zhang, G., Mao, Z., Fang, Z., Yang, C.: Precipitation of valuable metals from bioleaching solution by biogenic sulfides. Miner. Eng. **22**, 289–295 (2009)
51. McNeil, M.B., Jones, J.M., Little, B.J.: Mineralogical fingerprints for corrosion processes induced by sulfate reducing bacteria. NACE Annual Conference, Paper **580**:1–16: (1991)
52. Baas-Becking, L.G.M., Moore, D.: Biogenic sulfides. Econ. Geol. **56**, 259–272 (1961)