

Sequential precipitation of Cu and Fe using a three-stage sulfidogenic fluidized-bed reactor system

Deniz Ucar^a, Ozan K. Bekmezci^a, Anna H. Kaksonen^b, Erkan Sahinkaya^{a,*}

^a *Harran University, Environmental Engineering Department, Osmanbey Campus, 63000 Sanliurfa, Turkey*

^b *CSIRO Land and Water, Underwood Avenue, Floreat, WA 6014, Australia*

*Corresponding author, e-mail: erkansahinkaya@yahoo.com

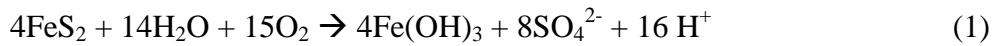
Abstract

The exposure of sulfides, such as pyrite (FeS_2) to water and air leads to the formation of acidic metal and sulfate containing waters, generally referred to as acid mine drainage (AMD). Under anaerobic conditions and in the presence of a suitable electron and carbon source, sulfate-reducing bacteria (SRB) can reduce sulfate to hydrogen sulfide which can precipitate metals as low-solubility sulfides. In the present study, a three-stage fluidized-bed reactor (FBR) system was operated at 35°C with ethanol as an electron and carbon source for SRB to sequentially precipitate Cu and Fe from synthetic AMD. The system consisted of two pre-settling tanks before a sulfidogenic FBR for the sequential precipitation of Cu and Fe with biogenic H_2S gas and HS^- containing effluent, respectively. Cu and Fe precipitation efficiencies were over 99% and sulfate and COD removals 60-90%. Biologically produced alkalinity increased the initial pH of the AMD from 3.0 to neutral values.

Keywords: Acid mine drainage, biotechnology, sulfide ores, reduction

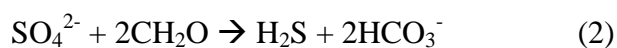
1. Introduction

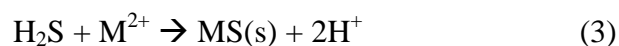
The commercial exploitation of sulfide minerals for valuable metals results in the oxidation of the exposed iron and sulfur with air and water (Reaction 1). This often leads to a production of acidic metal and sulfate containing wastewaters generally referred as acid mine drainages or AMD (Foucher et al. 2001; Garcia et al. 2001; Tsukamoto and Miller, 1999; Jong and Parry, 2003).



Other sulfide minerals are also oxidized in a similar way, releasing metals and sulfate in solution. This oxidation process forms AMD which may also contain several metals and metalloids such as Cu, Fe, Zn, Al, Pb, As, and Cd at high concentrations (Bechard et al., 1994 and Szczepanska et al., 1999).

Sulfate reducing bioreactors have become an economically viable alternative to conventional chemical processes for the treatment of acidic and metal containing wastewaters (Sahinkaya, 2009). Sulfate reducing bacteria (SRB) have an ability to reduce sulfate to hydrogen sulfide, which produces stable precipitates upon reaction with heavy metals. Moreover, bicarbonate produced in the sulfidogenic oxidation of provided electron donors increases the pH of the wastewater (Reactions 2 and 3). Hence, metals and sulfate can be concomitantly removed and pH increased from acidic to neutral or alkaline in a single reactor (Kaksonen et al., 2003 and Sahinkaya et al., 2009).





In one-stage biological reactors operated at neutral pHs, metals can be precipitated as metal-sulfides. However, this kind of application does not allow selective or separate metal precipitation, which is important to produce pure metal-sulfides that can directly be used in smelters to produce metals. In a recent study, Bijmans et al. (2009) showed selective recovery of nickel over iron in a gas lift bioreactor operated at pH 5, which allowed precipitation of nickel as NiS while the iron remained solubilized. Hence, controlling the reactor pH at low levels (<5) allowed the selective metal precipitation from mixed metal streams. One key factor constraining the design and application of selective metal recovery in one-stage bioreactor systems is the sensitivity of sulfate reducing bacteria (SRB) to even mild acidity (pH<5) (Johnson et al., 2006), which may be due to increased toxicity of sulfide and acetate at low pHs. Although sulfate reduction by SRB has been observed even at a very acidic condition of pH 3.8 (Kimura et al., 2006), pH >5 is still preferred to approach optimal rates in industrial-scale sulfate reduction processes (Bijmans et al., 2009).

In the literature, although several studies has been conducted with one-stage reactor, limited information is available with two- and three-stage metal-recovering sulfidogenic bioreactor (see Kaksonen et al. (2007) for review). This study aimed to evaluate the performance of two- and three-stage sulfidogenic bioreactors for separate precipitation of Cu and Fe from acidic and sulfate containing wastewater. The system consisted of one (for two-stage process) or two (for three stage process) pre-settling tanks before a sulfidogenic fluidized-bed reactor (FBR) was used for the sequential precipitation of Cu and Fe with biogenic H₂S gas and HS⁻ containing effluent, respectively.

2. Materials and Methods

2.1. Bioreactor

A laboratory scale reactor that could be operated as a two- and a three-stage FBR was used in the present study (Fig. 1). The bioreactor was inoculated with sulfidogenic anaerobic baffled reactor (ABR) sludge previously fed with lactate or ethanol and synthetic AMD for over 550 days (Bayrakdar et al., 2009; Sahinkaya and Yucesoy, 2010).

When operated as two-stage system, the system consisted of a Cu pre-settling tank and a sulfate reducing bioreactor (Fig. 1A). It was operated for 228 days with an influent feed consisted of sulfate, ethanol and Cu (Table 1).

In the second stage of the study (day 229 to 254), the two-stage reactor was retrofitted as a three stage-reactor system consisted of two instead of one pre-settling tanks before the sulfidogenic FBR (Fig. 1b). The aim was to separately precipitate Cu and Fe in the 1st and 2nd settling tank, respectively. This would enable the sequential precipitation of Cu and Fe with concomitant generation of biogenic H₂S gas and HS⁻ containing effluent, respectively. The empty non-fluidized bed volume of the FBR and the volume of each settling tanks were 600 and 250 mL, respectively. The main sulfidogenic FBR was continuously purged with nitrogen gas (flow rate 0.8 L/min) to strip off any H₂S. The stripped H₂S was introduced into the first settling tank. Alkaline liquor was not recycled to the first settling tank such that the pH therein was always maintained at <5. This would facilitate effective Cu recovery from this tank, Alkaline and dissolved sulfide (HS⁻) containing reactor liquid was recycled to the second settling tank with a flow rate of 400 mL/day to precipitate iron as FeS.

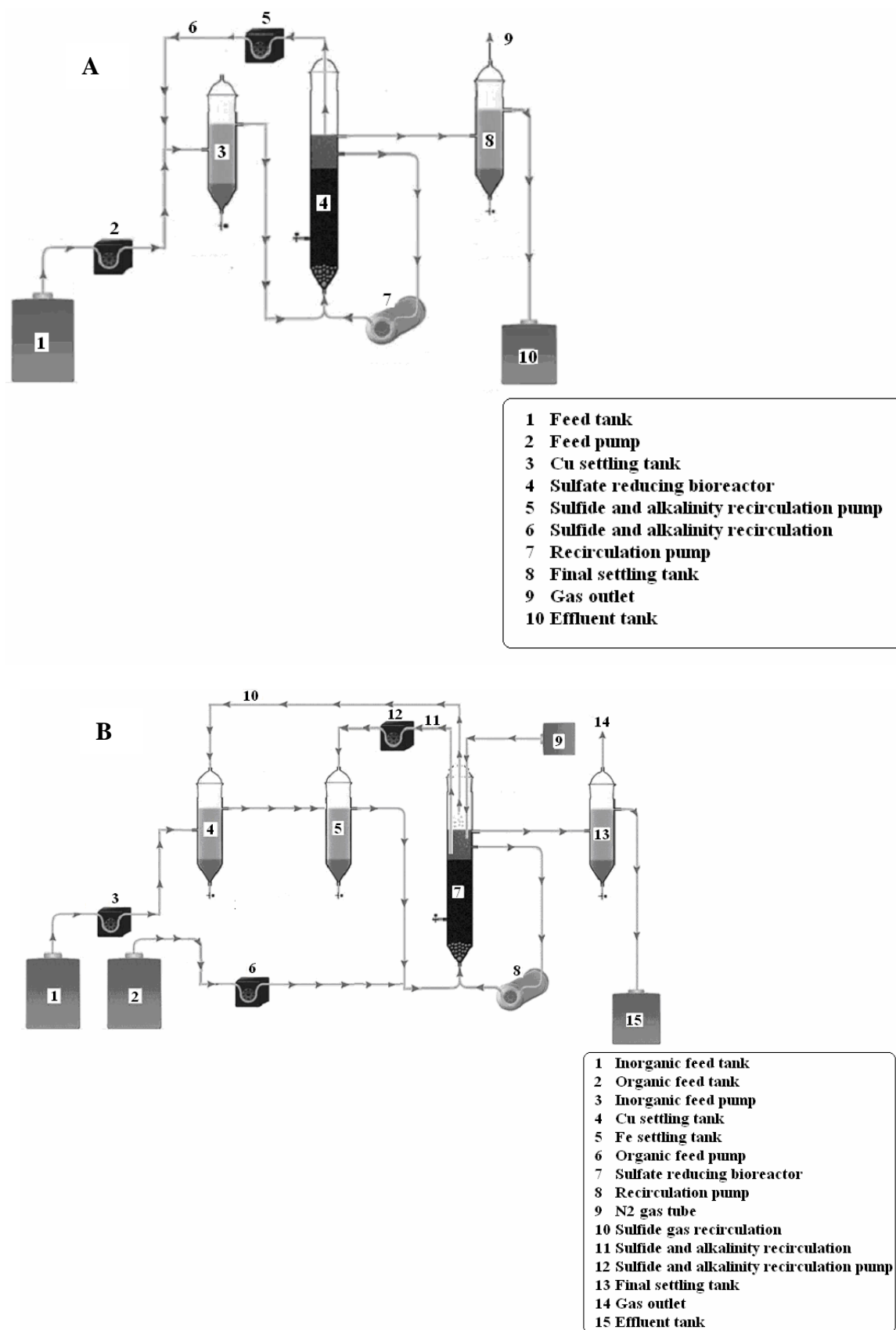


Figure 1. Two-stage (A) and three-stage (B) sulfidogenic fluidized-bed reactor systems

Table 1. Operational conditions of FBR

Parameter	Periods*				
	1	2	3	4	5
Days	0-85	86-112	113-145	146-228	229-254
Sulfate (mg/L)	2000	2000	2000	2000	2000
COD (mg/L)	1340	1340	1340	1700	1700
COD/sulfate	0.67	0.67	0.67	0.85	0.85
pH	7.0	4.0	4.0	4.0	4.0
HRT (day)	1	1	1	1	1
Cu (mg/L)	0	50	100	100	300
Fe (mg/L)	0	0	0	0	150

*Between periods 1 and 4, the reactor was operated as a two-stage process. In period 5, the reactor was operated as a three-stage process.

2.2. Experimental procedure

For the first 85 days, the two-stage reactor was fed with an alkaline (pH close to 7.0) and metal-deficient water to enrich ethanol oxidizing sulfate reducing bacteria. Then the reactor performance was evaluated at different pHs (7.0-4.0), Cu concentrations and chemical oxygen demand (COD)/sulfate ratios (Table 1). The sulfate concentration in the synthetic wastewater containing micro and macro nutrients (56 mg/L KH_2PO_4 ; 110 mg/L NH_4Cl ; 11 mg/L ascorbic acid and 50 mg/L yeast extract) was 2000 mg/L for the duration of the study. Ethanol was used as a carbon and electron source to maintain a $\text{COD}/\text{SO}_4^{2-}$ ratio of 0.67 or 0.85 (Tables 1).

The three stage-reactor system was fed with synthetic wastewater containing sulfate, ethanol, Fe and Cu (Table 1). Unlike the two-stage process where ethanol and inorganic nutrients mixture were combined and fed to the system, in the three-stage process organic and inorganic feed was separately fed to the bioreactor and 1st stage settling tank, respectively. This was to avoid sulfate reduction in the 1st stage settling tank, which may cause alkalinity production and Fe precipitation. The feed solution was kept refrigerated at 4°C to ensure that

COD removal, metal precipitation, and sulfate reduction did not occur in the feed container over the duration of the study. The reactor influent, and the effluent were sampled three times per week for the measurement of pH, alkalinity, COD, sulfate, dissolved sulfide. The effluent of settling tanks was also sampled for the measurement of sulfide, soluble and total metals.

2.3. Analytical techniques

Samples were centrifuged using Hettich Rotofix 32 centrifuge 3000 g for 10 min, before the measurement of sulfate, dissolved sulfide, and COD from the supernatant. Total dissolved sulfide was analyzed spectrometrically using a Shimadzu UV-1601 Spectrophotometer following the method described by Cord-Ruwisch (1985). A turbidimetric method was used to measure sulfate concentrations (APHA, 1999). Both COD and alkalinity were also measured according to APHA standard methods (APHA, 1999). Before COD measurements, samples were acidified with concentrated H_2SO_4 to $\text{pH} \leq 2$ and purged with N_2 gas for approximately 5 minutes to remove H_2S . For the alkalinity measurements, unfiltered samples were titrated with 0.1 N HCl to a pH 4.5 endpoint. For soluble metal measurements, the samples were first filtered through 0.45 μm polyethersulfone membrane syringe filters and then acidified with concentrated H_2SO_4 to $\text{pH} \leq 2$. For total metal concentration measurements, samples were first acidified to approximately pH 1.0 with concentrated H_2SO_4 to solubilise metal precipitates. Then, samples were filtered through 0.45 μm filters to remove biomass and other particles. Metal concentrations were measured Inductively Coupled Plasma (ICP) combined with Atomic Emission Spectroscopy (AES, Perkin Elmer Optima 5300). The data presented represent the mean values and standard deviations of all measurements ($n \geq 2$).

3. Results and Discussion

3.1. COD and sulfate removal

In the period 1, the reactor was fed with metal deficient water at pH close 7.0 to enrich ethanol oxidizing sulfate reducers. The reactor started with approximately 40% sulfate reduction which progressively increased to approximately 75% within 30 days (Fig. 2). At the end of period 1, the sulfate reduction averaged 80%. The reactor started with high COD removal efficiency without any lag period and averaged 98%. The rapid start-up of the reactor with high sulfate and COD removal efficiency suggests that the selected inoculum (enriched biomass from an ABR operated with synthetic AMD for more than 550 days) was a suitable bacterial source for the present study.

In the periods 2 and 3, sulfate removal efficiency remained stable with average efficiencies of 76% and 74%, respectively. In period 4, the feed COD concentration was increased from 1340 to 1700 mg/L and COD/sulfate ration from 0.67 to 0.85%. As a result, the sulfate removal efficiency increased to $91\pm4\%$ between days 155 and 182 (Fig. 2). Subsequently, sulfate removal performance decreased and averaged $60\pm10\%$ between days 194 and 228. The average sulfate reduction efficiency during whole period 4 was $76\pm18\%$. Unlike the sulfate reduction, COD removal was quite stable and it averaged $92\pm6\%$ (Fig. 2 and Table 2). The remaining COD in the effluent of the reactor was due to remaining volatile fatty acids (Fig. 3C), mainly acetate.

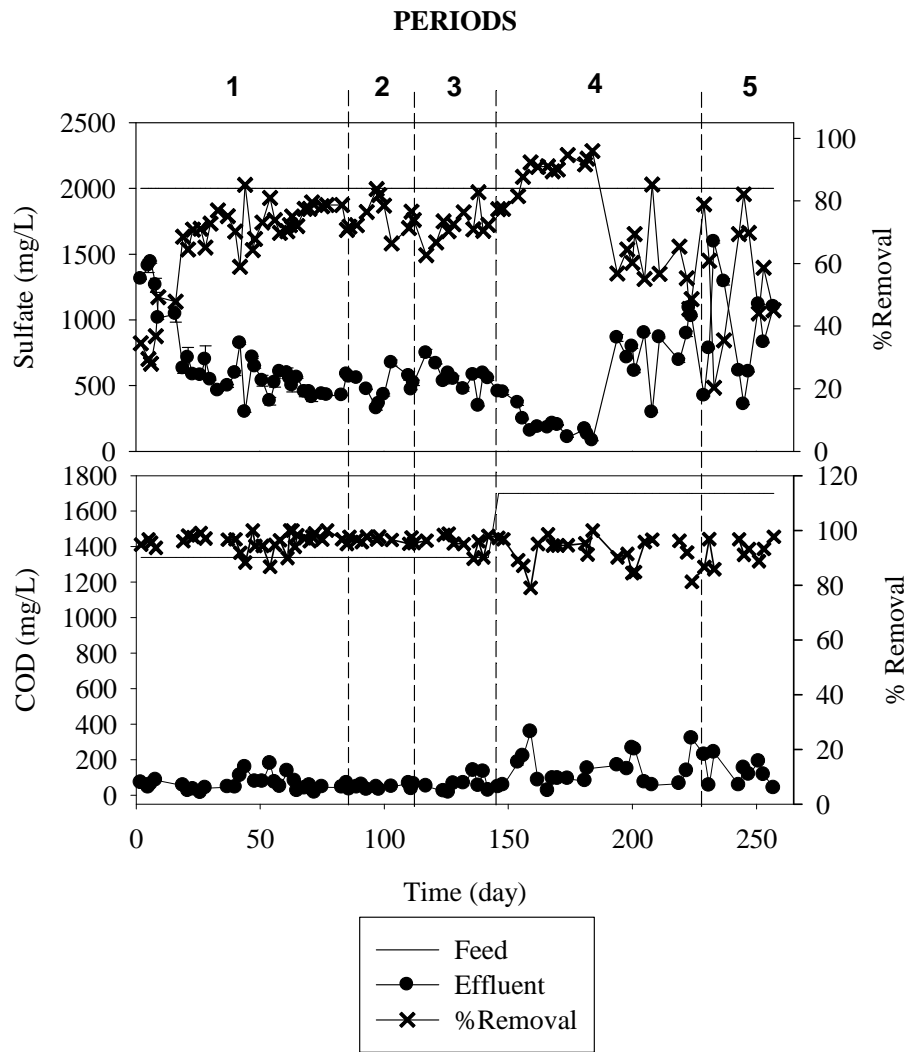


Fig. 2. Sulfate and COD removal performance over five periods in the sulfidogenic fluidized-bed reactor system

Table 2. Average Sulfate and COD removal performance in the sulfidogenic fluidized-bed reactor system

Periods	Sulfate removal (%)	COD removal (%)
1	75±5	96±4
2	76±6	96±1
3	74±4	95±4
4	76±18	92±6
5	57±20	92±4

In period 5, the reactor was operated as a three-stage process to precipitate Cu and Fe separately. The feed Cu and Fe concentrations were 300 and 150 mg/L, respectively. During this period, sulfate reduction efficiency was unstable, ranged from 20 to 65% with an average of $57 \pm 20\%$. The decrease in sulfate reduction efficiency was possibly due to the increase of Cu and addition of iron to the feed of the reactor. Also, operating the reactor as a three-stage process may have had an adverse effect on reactor performance.

3.2. Alkalinity and sulfide production

Sulfidogenic oxidation of ethanol to acetate does not produce alkalinity and acetate oxidation is necessary to produce sufficient alkalinity to increase the pH of AMD to neutral values. Alkalinity and pH variations for the feed and effluent of the FBR are illustrated in Fig. 3A and B. The effluent pH of the reactor was close to 8.0 throughout the reactor operation although the feed pH was decreased to about 4.0 after the first period. The effluent alkalinity increased in the period 1 as a result of increasing sulfate reduction efficiency. After period 2, the effluent alkalinity decreased steadily due to a decrease in the feed pH to 4 and an increase feed metal concentration (Fig. 3B). The increase in the feed metal concentration caused consumption of the alkalinity according to reaction 3.

For the first 5 days of the experiment, dissolved sulfide was not detected in the FBR although sulfate reduction occurred at approximately 30% efficiency (Figs. 2 and 3D). A possible reason was the adsorption of sulfide by activated carbon used as carrier material (Xiao et al., 2008). After that, sulfide concentration increased to approximately 400 mg/L due to increasing sulfate reduction performance. In period 2, sulfide concentration in the FBR decreased to approximately 250 mg/L with the introduction of Cu. In period 2, the reactor was

operated as a two-stage process. Changes in the dissolved sulfide concentration in the effluent of settling are presented in Fig. 3D. The sulfide concentration in the bioreactor and settling tank decreased due to increasing the feed Cu concentration from 50 mg/L (period 2) to 100 mg/L (period 3).

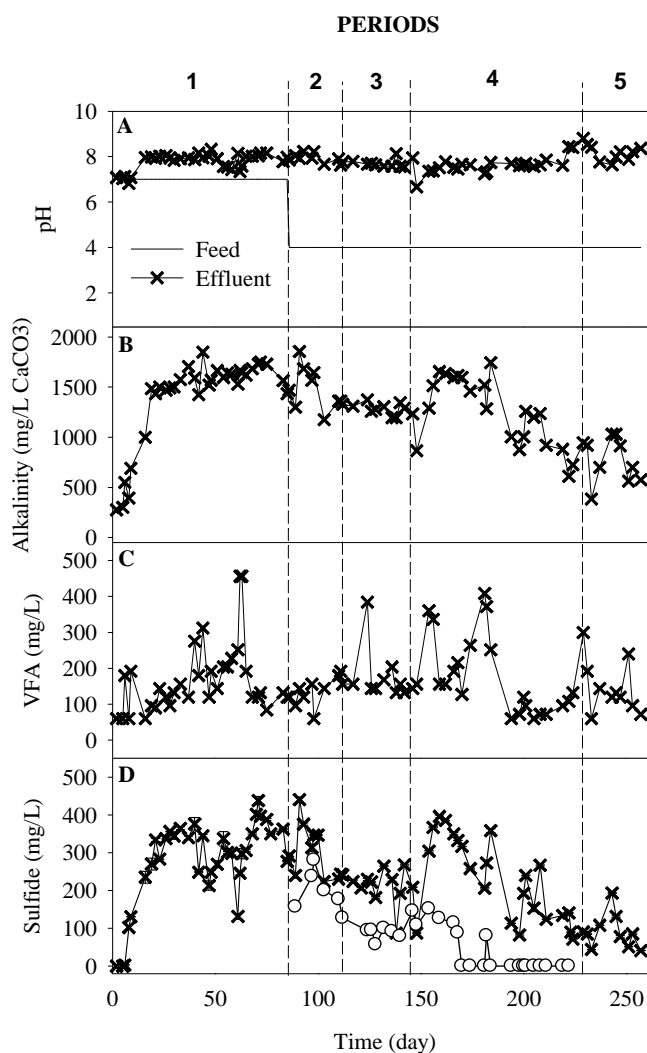


Figure 3. Variations of feed and effluent pH (A), effluent alkalinity (B), effluent VFA (C), and dissolved sulfide at the effluent of bioreactor (x) and first settling tank (o) (D)

In period 4, the COD/sulfate ratio was increased (from 0.67 to 0.85) to test if the sulfidogenic process was COD limiting. This change had caused a significant increase in the dissolved sulfide concentration from about 100 to 400 mg/L, signifying an increased sulfate reduction rate (Fig. 2). Thereafter, the sulfide concentration decreased steadily to 120 mg/L at

the end of period 4 as a result of decreasing sulfate reduction performance. The sulfide concentration in the settling tank also decreased due to a low sulfide concentration in the recycled effluent. In the last period, when the reactor was operated as a three-stage process, the dissolved sulfide concentration in the FBR was approximately 100 mg/L (Fig. 3D).

3.3. Electron flow to sulfate reduction

The reactor was inoculated with a consortium sourced from a sulfidogenic ABR previously operated for more than 550 days. Due to the inoculation of bioreactor with a sulfidogenic consortium, sulfate reduction commenced rapidly with approximately 40% sulfate reduction efficiency which subsequently increased to approximately 75% within 30 days. Methane production was not observed. Hence, most of the electrons produced in ethanol oxidation were used for sulfate reduction (Fig. 4) and the percent electron flow to sulfate reduction remained at approximately 75% until period 4. After day 195, sulfate reduction (Fig. 2) and percent electron flow to sulfate reduction decreased to approximately 55% (Fig. 4).

Throughout the reactor operation the electron flow to sulfate reduction averaged $70 \pm 13\%$ (Fig. 4). The remainder of the electrons was most probably used for fermentative reactions and biomass growth. This result corroborates well with many other published studies. For examples, previous studies have shown that 0.05-0.15 mg volatile suspended solids (VSS) was produced per mg sulfate reduced depending on reactor configuration and operational conditions (Sahinkaya, 2009; Kaksonen et al., 2004). Similarly, Bayrakdar et al. (2009) and Sahinkaya and Yucesoy (2010) reported that the percent electron flow to sulfate reduction was slightly higher than 85% in sulfidogenic ABR. Sahinkaya (2009) reported this value as 83% in a sulfidogenic continuously stirred tank reactor (CSTR) treating acidic and Zn-

containing wastewater. Kaksonen et al. (2004) reported the average electron donor utilized for sulfate reduction as $76 \pm 10\%$ in a mesophilic ethanol-fed FBR.

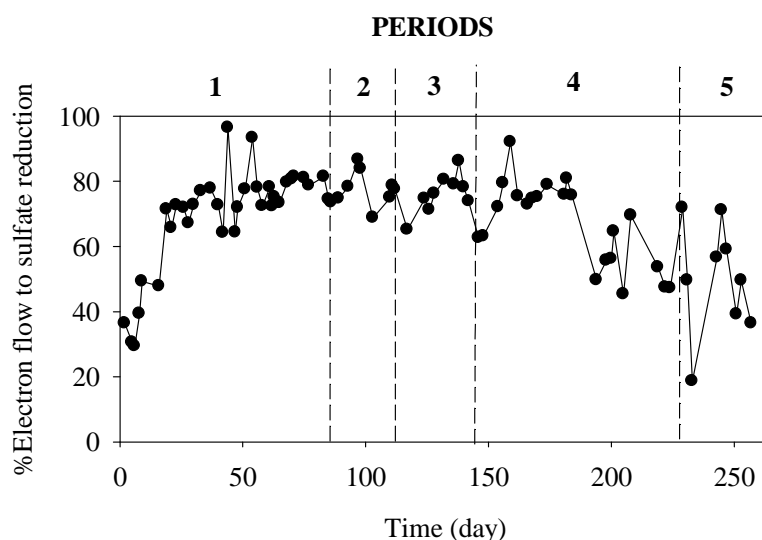


Fig. 4. Electron flow to sulfate reduction throughout the reactor operation

3.4. Metal Removal and recovery

Between periods 2 and 4, the reactor was operated as a two-stage process to recover Cu in a two-stage process (Fig. 5).

Copper was predominantly precipitated in the settling tank and it can be recovered without interrupting the bioreactor operation. Although by doubling the influent Cu concentration to 100 mg/L, the total effluent Cu concentration could still maintain at a low level of 0.1 mg/L. In the effluent of the pre-settling tank, the soluble and total metal concentrations were similar (data not shown), indicating that the Cu-sulfide precipitates settled out in the settling tank. Hence, the two-stage process is feasible for selective Cu recovery.

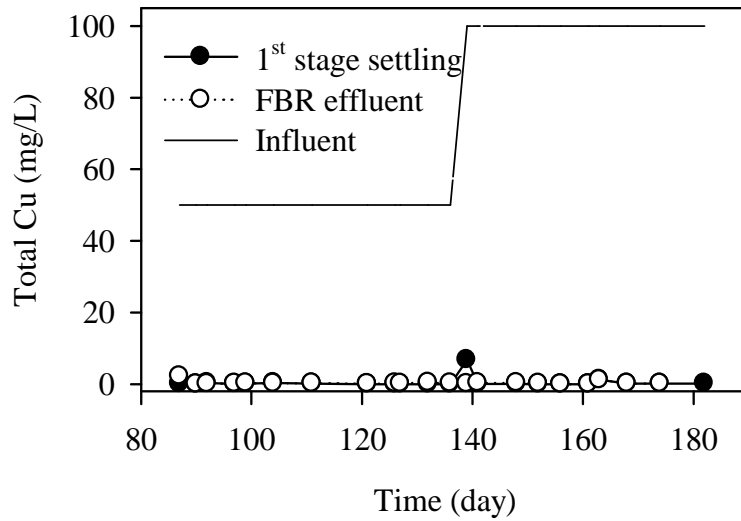


Figure 5. Total Cu concentrations in the influent, settling tank effluent and bioreactor effluent of the two-stage FBR system

Although, two-stage processes are applicable for wastewater containing a single metal, three or more stages are required for poly-metallic wastewater if selective precipitation is required. In order to study separate removal of Fe and Cu, a three stage process was initiated after day 230. Sulfide (H_2S) containing gas (Fig. 1) was recycled to the first settling tank in which Cu was precipitated separate from Fe at a $\text{pH} \leq 3$. Sulfide (HS^-)-containing liquid was recycled to the second settling tank to precipitate iron at $\text{pH} \geq 6.0$. Changes in the soluble and total Fe and Cu concentrations in the three-stage process are illustrated in Fig. 6.

Similar to the two-stage process, in the three-stage process both total and soluble Cu concentrations were similar as CuS precipitates efficiently settled (Sahinkaya et al., 2009). Although Cu separation was almost complete between days 230-243, the Cu removal efficiency decreased to approximately 50% due to decrease in the reactor performance and sulfide concentration after days 250 (Figs. 2 and 3).

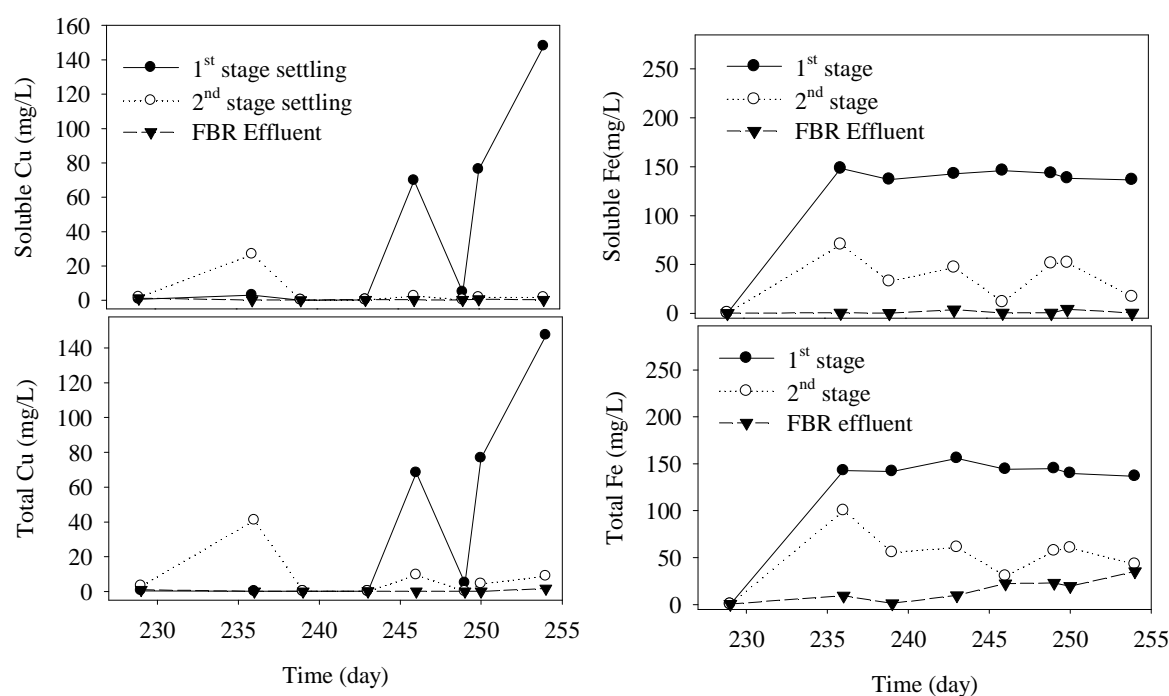


Figure 6. Cu and Fe concentrations in the three-stage sulfidogenic fluidized-bed reactor system

In the three-stage reactor system, the influent Fe concentration was 150 mg/L. Fe was not precipitated in the first tank (Fig. 6) although measurable sulfide was present due to the low pH in the settling tank. Although feed pH was maintained at 4.0, the pH in the first settling tank was approximately 3.0 due to acid production during the precipitation of metals according to reaction 3. In the second settling tank, Fe precipitated efficiently and the average effluent concentration decreased to approximately 50 mg/L, corresponding to approximately 65% removal efficiency. The remaining Fe was removed in the bioreactor. The total Fe concentration was slightly higher than the soluble Fe concentration especially in the effluent of the bioreactor. These results suggest that the three-stage bioreactor is a suitable alternative for the treatment of AMD and for removing Cu and Fe separately.

4. Conclusions

In this study, sequential precipitation of Cu and Fe from acidic (pH 4) sulfate containing wastewater was achieved using an ethanol-fed sulfidogenic fluidized-bed reactor (FBR) system at 35°C consisting of a bioreactor and two pre-settling tanks. In this three-stage system with feed influent Cu and Fe concentrations of 300 and 150 mg/L, respectively, almost all Cu (over 99%) was initially precipitated in the first pre-settling tank which received the biogenic H₂S gas stripped-off from the FBR. Decreased sulfate reduction rates in the FBR significantly reduced Cu precipitation efficiency. As pH remained <3, no Fe was precipitated in the first pre-settling tank, whereas about 65% of Fe was precipitated in the second settling tank with recycled bioreactor liquid and the remainder precipitated in the FBR resulting in over 99% Fe removal. Thus the three-stage FBR system allows selective precipitation and recovery of Cu and Fe from acidic sulfate containing wastewaters. Biologically produced alkalinity increased the initial pH of the water from 3.0 to neutral values.

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