# Sulfidogenic biotreatment of synthetic acid mine drainage and sulfide oxidation in anaerobic baffled reactor

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## ARTICLE INFO

## ABSTRACT

The treatment of synthetic acid mine drainage (AMD) water (pH 3.0–6.5) containing sulfate ($3.0–3.5\,\mathrm{g\,L^{-1}}$) and various metals (Co, Cu, Fe, Mn, Ni, and Zn) was studied in an ethanol-fed sulfate-reducing 4-compartment anaerobic baffled reactor (ABR) at 32 °C. The reactor was operated for 160 days at different chemical oxygen demand (COD)/sulfate ratios, hydraulic retention times (HRT), pH, and metal concentrations to study the robustness of the process. The last compartment of the reactor was aerated at different rates to study the bio-oxidation of sulfide to elemental sulfur. The highest sulfate reduction efficiency (88%) was obtained with a feed sulfate concentration of $3.5\,\mathrm{g\,L^{-1}}$, COD/sulfate mass ratio of 0.737, feed pH of 3.0 and HRT of 2 days without aeration in the 4th compartment. The corresponding COD removal efficiency was about 92%. The alkalinity produced in the sulfidogenic ethanol oxidation neutralized the acidic mine water from pH 3.0–4.5 to pH 7.0–8.0. Effluent soluble and total heavy metal concentrations were substantially reduced with removal efficiencies generally higher than 99%, except for Mn (25–77%). Limited aeration in the 4th compartment of ABR promoted incomplete oxidation of sulfide to elemental sulfur rather than complete oxidation to sulfate. Depending on the aeration rate and HRT, 32–74% of produced sulfide was oxidized to elemental sulfur. This study demonstrates that by optimizing operating conditions, sulfate reduction, metal removal, alkalinity generation, and excess sulfide oxidation can be achieved in a single ABR treating AMD.

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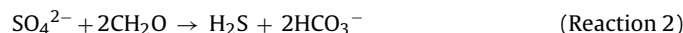
## 1. Introduction

Metal-containing acid mine drainage (AMD) is created by the interaction of air and water with metal sulfides, such as pyrite ($FeS_2$), commonly found in overburden and disused mine shafts consisting of low-grade minerals discarded from the mining operations. The production of AMD can be summarized by the oxidation of pyrite in the presence of air and water (Reaction (1)).

$$4FeS_2 + 14H_2O + 15O_2 \rightarrow 4Fe(OH)_3 + 8SO_4^{2-} + 16H^+ \quad \text{(Reaction 1)}$$

Other sulfide minerals are also oxidized in a similar way, releasing metals and acidic sulfate in solution. This oxidation process forms AMD which may also contain several metals and metalloids such as Cu, Fe, Zn, Al, Pb, As, Cd at $10–100\,\mathrm{mg\,L^{-1}}$ (Fe may be present at much higher concentrations) [1,2].

Sulfate reducing bioreactors have become an economically viable alternative to conventional chemical processes for the treatment of acidic and metal containing wastewaters [3]. Sulfate reducing bacteria (SRB) have an ability to reduce sulfate to hydro-

gen sulfide, which produce 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 [4,5].

$$SO_4^{2-} + 2CH_2O \rightarrow H_2S + 2HCO_3^- \quad \text{(Reaction 2)}$$

$$H_2S + M^{2+} \rightarrow MS(s) + 2H^+ \quad \text{(Reaction 3)}$$

Several types of anaerobic reactors have been employed in the biological removal of sulfate, including suspended growth [3,6] and attached growth [4,7–9] bioprocesses. The significant advantage of an anaerobic baffled reactor (ABR) is its compartmentalized structure, which limits the exposure of the biomass to adverse environmental conditions, such as low pH and high metal concentrations [10,11].

Sulfate reduction processes produce hydrogen sulfide, a toxic and corrosive compound. Although several studies have been conducted on sulfidogenic AMD treatment (see Ref. [12] for further information), the removal of sulfide from the effluent of the AMD treating bioprocesses has not been extensively studied. The oxidation of sulfide to elemental sulfur is of great importance as elemental sulfur can be separated by sedimentation from the efflu-
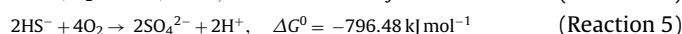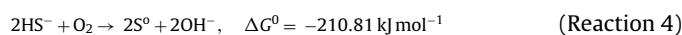
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**Table 1**
Operational conditions of anaerobic baffled reactor.

| Parameter | Periods | | | | | | |
|---|---|---|---|---|---|---|---|
| | I | I | III | IV | V | VI | VII |
| Days | 0–50 | 51–57 | 58–78 | 79–108 | 109–131 | 132–152 | 153–160 |
| HRT (days) | 1 | 1 | 2 | 2 | 2 | 2 | 1 |
| Feed sulfate concentration (mg L$^{-1}$) | 3000 | 3500 | 3500 | 3500 | 3500 | 3500 | 3500 |
| Feed ethanol concentration (mg COD L$^{-1}$) | 2010 | 2345 | 2345 | 2580 | 2380 | 2380 | 2380 |
| COD/SO$_4{}^{2-}$ mass ratio of feed | 0.67 | 0.67 | 0.67 | 0.737 | 0.68 | 0.68 | 0.68 |
| Total metal concentration (mg L$^{-1}$)[a] | Non | Full | Full | Full | Full | Full | Half |
| Feed pH | 6.0–6.5 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 4.5–5.0 |
| Aeration rate of 4th compartment (mL s$^{-1}$) | Non | Non | Non | Non | 15 | 7 | 7 |

[a] The concentrations are given in Table 2.

ent and can be re-used as fertilizer or as the raw material for sulfuric acid production [13,14]. Biological sulfide oxidation is also considered a clean alternative compared to physico-chemical alternatives due to less sludge production [13]. In the biological sulfide oxidation, various chemolithotrophic sulfide-oxidizing bacteria grow on reduced sulfur compounds, using the energy derived via the following reactions (Reactions (4) and (5)) [13–15]:

$$2HS^- + O_2 \rightarrow 2S^o + 2OH^-, \quad \Delta G^0 = -210.81 \text{ kJ mol}^{-1} \quad \text{(Reaction 4)}$$

$$2HS^- + 4O_2 \rightarrow 2SO_4{}^{2-} + 2H^+, \quad \Delta G^0 = -796.48 \text{ kJ mol}^{-1} \quad \text{(Reaction 5)}$$

The complete oxidation of sulfide to sulfate produces more energy; however, the elemental sulfur production is preferred as it can be separated by sedimentation and re-used. Elemental sulfur formation without producing sulfate can be achieved by limiting the oxygen supply [13–16].

Generally, two-step configurations are used for the treatment of AMD; sulfate reduction and sulfide oxidation. Using two separate reactors for these different processes may increase the cost of the process. Recently, Celis-García et al. [13] used a down-flow fluidized bed reactor to achieve sulfate reduction and sulfide oxidation in one reactor simultaneously. However, it may be difficult to optimize the conditions for both processes when two reactions are occurring simultaneously in one reactor compartment. In our study, both processes were achieved in one reactor, namely ABR, but in separate compartments.

This study aims at evaluating the efficiency of an ethanol supplemented sulfidogenic ABR for the treatment of simulated AMD containing high concentrations of sulfate, metals and acidity. For that purpose, the reactor was operated for 160 days at different chemical oxygen demand (COD)/sulfate ratios, hydraulic retention times (HRTs), pH, and metal concentrations. The last compartment of the reactor was aerated at different rates to study the bio-oxidation of sulfide to elemental sulfur. To the best of our knowledge, this is the first study on simultaneous AMD treatment and sulfide oxidation in ABR.

## 2. Materials and methods

### 2.1. Bioreactor

A laboratory scale ABR (Fig. 1) was used in the present study. The reactor was inoculated with sulfidogenic ABR sludge previously fed with lactate or ethanol and synthetic AMD for more than 550 days [17,18]. The reactor was divided into four equal compartments of 5.0 L by vertical baffles, each compartment having down-comer and riser regions created by further vertical baffle. Hence, HRT of each compartment was equal to a quarter of the reactors HRT. Sludge occupied a quarter of the total reactor volume and the HRT calculations were based on the sludge volume. The reactor was maintained at $32 \pm 3\,°C$ in a temperature controlled room.

The sulfate concentration in the synthetic wastewater containing micro and macro nutrients ($56 \text{ mg L}^{-1}$ KH$_2$PO$_4$; $110 \text{ mg L}^{-1}$
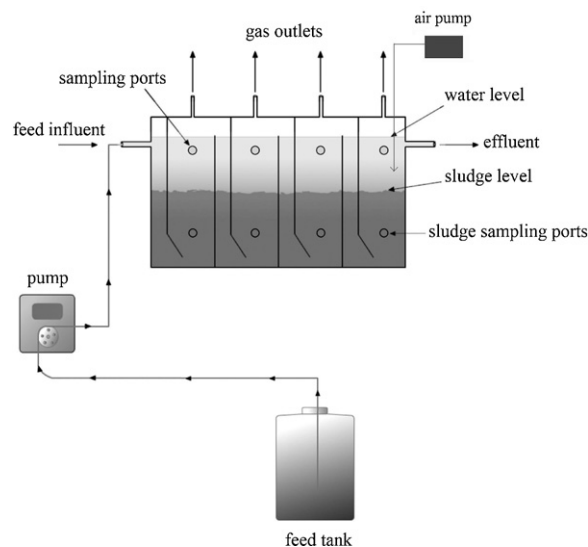


**Fig. 1.** A schematic diagram of the anaerobic baffled reactor.

NH$_4$Cl; $11 \text{ mg L}^{-1}$ ascorbic acid and $50 \text{ mg L}^{-1}$ yeast extract) was $3000 \text{ mg L}^{-1}$ for the period I and $3500 \text{ mg L}^{-1}$ for the rest of the study. Ethanol (963, 1124, 1141 or $1236 \text{ mg L}^{-1}$) was used as a carbon and electron source to maintain a COD/SO$_4{}^{2-}$ ratio of between 0.67 and 0.74 (Table 1). 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.

### 2.2. Experimental procedures

The performance of the ethanol-fed ABR was evaluated for 160 days, which was divided into 7 periods (Table 1). Firstly, the reactor was fed with a moderately acidic (pH 6.0–6.5) solution containing $3000 \text{ mg SO}_4{}^{2-} \text{ L}^{-1}$ without metal supplementation (period I, days 0–50) to enrich ethanol oxidizing sulfate reducing bacteria. Then, the reactor performance was evaluated at different pHs, metal concentrations (Tables 1 and 2), sulfate, and organic carbon loadings and at two aeration rates in the 4th compartment (Table 1).

**Table 2**
Feed metal concentrations for the sulfidogenic anaerobic baffled reactor.

| Metal | Full (periods I–IV) mg L$^{-1}$ | Half (periods V–VII) mg L$^{-1}$ |
|---|---|---|
| Fe | 400 | 200 |
| Cu | 50 | 25 |
| Co | 10 | 5 |
| Mn | 10 | 5 |
| Zn | 10 | 5 |
| Ni | 5 | 2.5 |

The metal concentrations for periods 2–7 (Table 2) were selected according to highest measured values in a local AMD water in a copper mine area near Elazığ, Turkey.

The reactor influent, each ABR compartment, and the effluent were sampled 3 times per week for the measurement of pH, alkalinity, COD, sulfate, and dissolved sulfide. The effluent was also sampled weekly and biweekly for soluble and total metals, respectively.

Sulfide oxidation and sulfate production rates in the last compartment were calculated using the Eqs. (1) and (2), respectively;

$$R_s \, (\text{mmol L}^{-1} \, \text{d}^{-1}) = \frac{So - S}{HRT} \qquad (1)$$

$$R_{su} \, (\text{mmol L}^{-1} \, \text{d}^{-1}) = \frac{Su - Su, o}{HRT} \qquad (2)$$

where $R_s$ and $R_{su}$ are sulfide oxidation and sulfate production rates, respectively. So and S, Su,o and Su are the 4th compartment influent and effluent sulfide and sulfate concentrations as mmol L$^{-1}$ in respective order. HRT is the hydraulic retention time in the 4th compartment of the ABR as days.

Elemental sulfur generation could not be measured from effluent samples, because much of the elemental sulfur remained in the ABR attached to the biofilm or settled. Therefore, elemental sulfur production was calculated by subtracting the concentration of effluent sulfate from the concentration of oxidized sulfide. This approach is valid because thiosulfate ($S_2O_3^{2-}$) was not observed by ion-chromatography (Schimadzu, Prominence HIC-NS) at any time during the study. Hence, sulfur production efficiency was calculated from

$$E_{S^{\circ}} = \frac{R_s - R_{su}}{R_s} \times 100 \qquad (3)$$

where $E_{S^{\circ}}$ is the percentage sulfur production efficiency, and $R_s$ and $R_{su}$ are sulfide oxidation and sulfate production rates, respectively.

### 2.3. Analytical techniques

Samples were centrifuged using Hettich Rotofix 32 centrifuge $3000 \times g$ for 10 min, before the measurement of sulfate, dissolved sulfide, and COD from the supernatant. Total sulfide was analyzed spectrometrically using a Shimadzu UV-1601 Spectrophotometer following the method described by Cord-Ruwisch [19]. A turbidimetric method was used to measure sulfate concentrations [20]. Both COD and alkalinity were also measured according to APHA standard methods [20]. Before COD measurements, samples were acidified with concentrated $H_2SO_4$ to below pH 2 and purged with $N_2$ gas for approximately 5 min to remove $H_2S$. For the alkalinity measurements, unfiltered samples were titrated by 0.1 N HCl to a pH 4.5 endpoint. For soluble metal measurements, the sample was first filtered through 0.45 μm polyethersulfone membrane syringe filters and then acidified with concentrated $H_2SO_4$ to below pH 2.0. For total metal concentration measurements, samples were first acidified approximately to pH 1.0 with concentrated $H_2SO_4$ to solubilise metal precipitates. Then, samples were filtered through 0.45 μm to remove biomass and other particles. Metal concentrations were measured ICP-OM (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. Sulfate reduction and COD oxidation

The performance of ABR in terms of sulfate reduction and COD removal is presented in Fig. 2. The reactor started with 45% sulfate reduction and 60% COD removal without any lag period due

the inoculation with active biomass from an ABR fed with a lactate or ethanol containing synthetic wastewaters for more than 550 days [17,18]. In the first period of the reactor operation, sulfate reduction and COD oxidation averaged 65 and 72%, respectively (Table 3). The sulfate removal performance increased to 88% by period IV (Table 3). The sulfate reduction rates in the whole ABR were between 1.3 and 2.0 g L$^{-1}$ day$^{-1}$ throughout the reactor operation (data not shown). Previously reported maximum sulfate reduction rates in an ethanol-fed fluidized bed reactor and ABR were approximately 4 g L$^{-1}$ day$^{-1}$ [4] and 3 g L$^{-1}$ day$^{-1}$ [18], respectively. When excluding the aerated compartment 4 and calculating the sulfate reduction rate per the first 3 compartments which had a total HRT of 0.75 day during the period VII, the sulfate reduction rate was approximately 3.5 g L$^{-1}$ day$^{-1}$, which is similar to the values observed previously [4,18].

Aeration in ABR compartment 4 during periods V–VII resulted in production of sulfate from the complete oxidation of sulfide (Fig. 2A and Table 3). During period V, the sulfate concentrations in the 3rd and 4th compartments were approximately 875 and 1800 mg L$^{-1}$, respectively, corresponding to approximately 75 and 48% sulfate reduction efficiencies (Table 3). With a decrease in the aeration rate from 15 to 7 mL s$^{-1}$ in period VI (Table 1), the sulfate concentration at the effluent of 4th compartment decreased from approximately 1800 to 1500 mg L$^{-1}$ (Fig. 2A), corresponding to 48 and 56% sulfate removal efficiencies (Table 3), respectively. In the last period, the metal concentration in the feed was decreased by half and HRT was decreased from 2 days to 1 day (Tables 1 and 2), resulting in a decrease of sulfate in the last compartment to approximately 1100 mg L$^{-1}$ (Fig. 2A), corresponding to 68% sulfate reduction efficiency (Table 3). This is in agreement with the literature findings as the decreased sulfate production and increased sulfur formation at higher sulfide loadings have been reported by others [15,16].

COD removal continued to increase to 98% until period VII at which reduced HRT decreased COD removal to 93% (Table 3). After start-up period, COD concentration in the 4th compartment remained between 200 and 700 mg L$^{-1}$ until period V, after which aeration of the last compartment decreased COD concentration to <100 mg L$^{-1}$ (Fig. 2B and Table 3). Hence, decreasing pH and adding metal to the feed did not adversely affect the reactor performance. Aeration of the last compartment increased the COD removal efficiency from approximately 92 to 97% (Fig. 2B and Table 3). This was probably due to aerobic degradation of the remaining COD.

The treatment efficiency of the reactor showed an increasing trend over time in terms of sulfate reduction and COD oxidation (Fig. 2 and Table 3). There may be several possible reasons for this increase. Sulfide is toxic to SRB and addition of metal to the feed solution caused removal of $H_2S$ by metal sulfide precipitation and increasing sulfate reduction performance. Moreover, the SRB biomass concentration may have increased over time and the bacteria may have been adapted to the operating conditions [7,17,18].

Although pH in the first compartment decreased to 4.5–5.0 after the first period, significant COD oxidation and sulfate reduction was still present. This finding is in agreement with the findings of Bijmans et al. [21]. They selectively recovered nickel from a nickel–iron-containing solution at pH 5.0 using a single stage sulfate reducing bioreactor.

### 3.2. Alkalinity production and metal removal

Although the feed pH was decreased to 3.0, the effluent pH was close to neutral due to alkalinity production during sulfidogenic electron donor oxidation (Reaction (2)) (Fig. 3A and B). With the addition of metals and the reduction of feed pH to 3.0, the pH and alkalinity in the 1st compartment decreased to approximately 4.5 and 0 mg L$^{-1}$, respectively (Fig. 3A and B). The formation sulfide–metal precipitate causes acidity generation according to
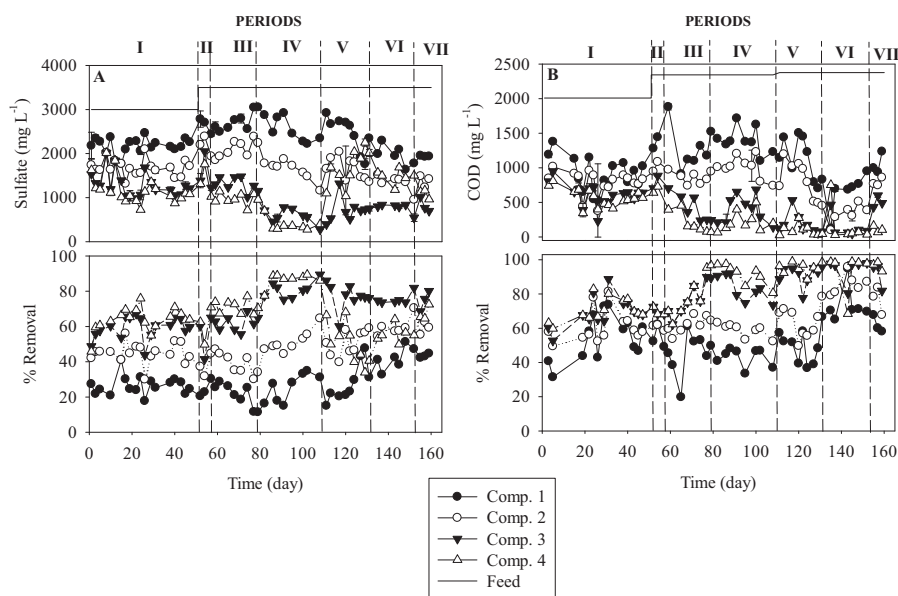
**Fig. 2.** Sulfate (A) and COD (B) removals in the anaerobic baffled reactor.

Reaction (3). As most of the metals were precipitated in the first compartment, the acidity generation in the first compartment should be balanced with the alkalinity production by sulfate reduction (Reaction (2)). The pH and alkalinity increased in the other compartments and effluent alkalinity was approximately 2500 mg L$^{-1}$ CaCO$_3$ in the 4th compartment at the end of period IV (Fig. 3A and B). The alkalinity decreased in 4th compartment when aeration was started, possibly be due to complete oxidation of sulfide to sulfate. As shown in Reactions (4) and (5), the partial oxidation of sulfide to elemental sulfur produces alkalinity, whereas, the complete oxidation of sulfide to sulfate consumes alkalinity [13,15]. After period V, alkalinity in 4th compartment increased as less sulfide was oxidized to sulfate (Fig. 2A).

In the first period (days 0–50) the reactor was fed with metal deficient AMD (Table 1) and sulfide concentration increased (Fig. 3C) with time supporting sulfate removal data (Fig. 2A). With the addition of metal to the feed, the sulfide concentration decreased sharply due to formation of metal–sulfide precipitates (Fig. 3C). In the period II (days 51–57), the sulfide concentration in the first compartment decreased from around 200 mg/L to around 15 mg/L. Similar decrease was observed in the other compartments. Therefore, metals were precipitated with the sulfide produced by sulfate reduction process.

The concentrations of the major ions Ca, K, Mg and Na did not change appreciably in the influent and effluent throughout the reactor operation (data not shown). At least 99% of Co, Cu, Fe, Ni and Zn precipitated within the first two compartments of the ABR, and the precipitation efficiency reached 100% in the ABR effluent (data not shown). The precipitation of Mn was less efficient and the

average removal efficiency in the whole ABR varied between 25% and 77% depending on the period. The soluble metal concentrations in the effluent were 0.00–0.01 mg L$^{-1}$ Co, 0.00–0.03 mg L$^{-1}$ Cu, 0.06–0.66 mg L$^{-1}$ Fe, 0.6–7.5 mg L$^{-1}$ Mn, 0.00–0.03 mg L$^{-1}$ Ni, and 0.00–0.14 mg L$^{-1}$ Zn. The total and soluble effluent concentrations of Co, Cu, Fe, Ni and Zn were similar, which indicates good removal and settling of the metal–sulfide precipitates.

The average effluent concentration and the removal efficiency of Mn were $3.8 \pm 2.6$ mg L$^{-1}$ and $50 \pm 20$%, respectively. The effluent concentrations of Mn were significantly higher than those of other metals. This is likely due to higher solubility product ($K_{sp}$) of MnS compared to those of Co, Cu, Ni, and Zn sulfides [22]. The solubility products of Mn sulfides ($2.5 \times 10^{-13}$ to $2.5 \times 10^{-10}$) and Mn hydroxide ($1.9 \times 10^{-13}$) are relatively high. It is possible that Mn was removed as Mn(OH)$_2$ rather than MnS due to lower $K_{sp}$ value of Mn(OH)$_2$ compared to MnS. Of the many metals and metalloids, Mn is one of the most difficult to remove due to the complexity of the interactions governing Mn solubility. Mn precipitation may be inhibited if the Fe/Mn ratio is high [23] as the case in our study. Mn precipitates may dissolve in the case of high Fe$^{2+}$ concentrations. Also, other metals used in our study may react with sulfide before Mn due to high $K_{sp}$ of MnS.

### 3.3. Oxidation of sulfide to elemental sulfur and sulfate

Sulfide oxidation efficiency, elemental sulfur production efficiency and the sulfur balance after the commencement of aeration are shown in Fig. 4. During aeration, oxygen concentration in the 4th compartment was consistently lower than 0.15 mg L$^{-1}$.

**Table 3**
Steady-state chemical oxygen demand (COD) and percent sulfate removal efficiencies (mean ± standard deviation) of the anaerobic baffled reactor.

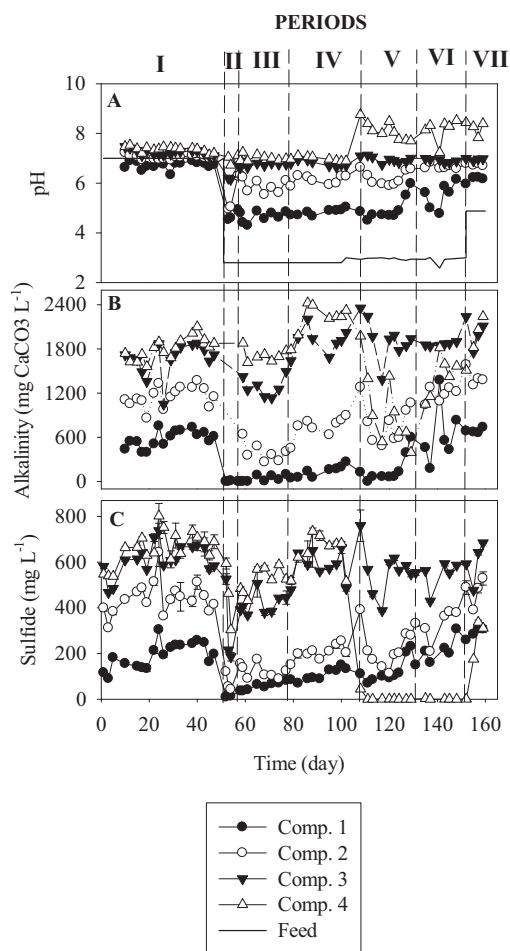| Periods | Compartment 1 | | Compartment 2 | | Compartment 3 | | Compartment 4 | |
|---------|---------------|---------|---------------|---------|---------------|---------|---------------|---------|
| | Sulfate (%) | COD (%) | Sulfate (%) | COD (%) | Sulfate (%) | COD (%) | Sulfate (%) | COD (%) |
| I | 27 ± 5 | 55 ± 12 | 45 ± 6 | 61 ± 9 | 59 ± 6 | 69 ± 8 | 64 ± 7 | 72 ± 7 |
| II | 25 ± 5 | 42 ± 5 | 38 ± 7 | 57 ± 4 | 56 ± 13 | 66 ± 6 | 61 ± 11 | 71 ± 3 |
| III | 24 ± 4 | 67 ± 6 | 41 ± 5 | 64 ± 3 | 62 ± 5 | 81 ± 7 | 72 ± 4 | 91 ± 6 |
| IV | 27 ± 8 | 46 ± 8 | 52 ± 7 | 61 ± 7 | 81 ± 5 | 85 ± 8 | 88 ± 2 | 92 ± 5 |
| V | 33 ± 11 | 53 ± 13 | 51 ± 6 | 66 ± 12 | 75 ± 3 | 92 ± 6 | 48 ± 13 | 96 ± 3 |
| VI | 42 ± 7 | 72 ± 5 | 60 ± 6 | 86 ± 4 | 76 ± 3 | 94 ± 1 | 56 ± 5 | 98 ± 1 |
| VII | 43 ± 1 | 56 ± 7 | 59 ± 4 | 67 ± 2 | 75 ± 6 | 79 ± 4 | 68 ± 5 | 95 ± 2 |

**PERIODS**



**Fig. 3.** Solution pH, alkalinity and dissolved sulfide concentration in feed and each compartment of the anaerobic baffled reactor.
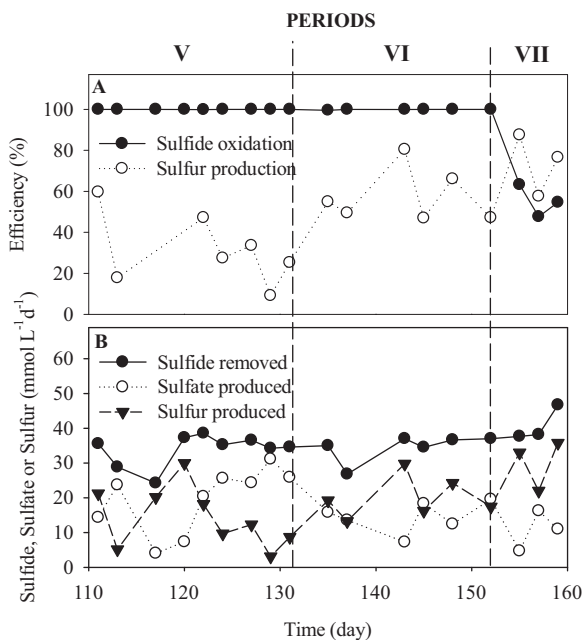
**PERIODS**



**Fig. 4.** Sulfide oxidation and elemental sulfur production efficiencies (A) and rates (B) in the last compartment of the anaerobic baffled reactor.

It should be noted that in addition to sulfide oxidation, some of the sulfide was used for metal precipitation and some was possibly stripped into gas phase due to aeration. In periods V and VI, the sulfide concentrations in the influent of the 4th compartment were $564 \pm 97$ and $545 \pm 67$ mg L$^{-1}$, respectively. The aeration rates in periods V and VI were 15 and 7 mL s$^{-1}$, respectively (Table 1). The sulfide removal during periods V and VI was complete (Fig. 4A). The elemental sulfur production from oxidized sulfide was $32 \pm 17\%$ and $60 \pm 14\%$, respectively, as calculated based on sulfate and sulfide results (Fig. 4B). Hence, decreasing aeration rate from 15 mL s$^{-1}$ to 7 mL s$^{-1}$ resulted in increased elemental sulfur production without decreasing sulfide removal efficiency. The produced elemental sulfur accumulated in the last compartment, which was evidenced with the settling of whitish-yellow precipitate. Although the biomass source was from another ABR, and not previously exposed to oxygen, a rapid start-up of sulfide oxidation was observed. In period VI, the elemental sulfur production from sulfide oxidation increased, and the oxidation yielded less sulfate, which increased the overall sulfate removal efficiency of the ABR (Table 3). The average sulfate reduction efficiency of the ABR decreased appreciably after starting aeration in period V due to the formation of sulfate from sulfide oxidation (Table 3). Although, sulfate removal efficiency was approximately 75% in the 3rd compartment, it decreased to approximately 48% after aeration of the 4th compartment in period V (Table 3). After decreasing aeration rate in period VI, the sulfate reduction efficiency increased to 56% although the sulfide oxidation efficiency was still approximately 100%. Hence, the optimization of aeration rate is very important to increase the formation of elemental sulfur from sulfide oxidation [13–16]. In period VII, the feed metal concentrations and the HRT were decreased by half (Table 1). The average sulfide concentration in the influent of 4th compartment was approximately 600 mg L$^{-1}$ (Fig. 3C). Decreasing HRT of 4th compartment from 0.5 to 0.25 day decreased the sulfide oxidation efficiency from 100% to 55% (Fig. 4A). This may be due to limitation in biological activity due to excessive sulfide loading [15]. Although sulfide oxidation efficiency decreased appreciably, the elemental sulfur production from sulfide oxidation was approximately 74% (Fig. 4A). Although sulfide removal in period VII was incomplete, some production of sulfate from elemental sulfur oxidation was observed. In the ABR, the elemental sulfur particles were retained in the 4th compartment, which increased the oxidation potential from elemental sulfur to sulfate. In period VII, the effluent turned a yellow-greenish color, as observed in other studies [13,16,24], indicating the formation of polysulfides, which can be toxic to sulfide oxidizing bacteria [13]. The presence of sulfide oxidizing bacteria in the ABR was not checked. Due to the relatively high aeration rates it is possible that the sulfide oxidation has been chemical. In chemical sulfide oxidation intermediates, such as polysulfides, sulfite and thiosulfate, may have formed in addition to sulfur. Therefore the presented sulfur yields represent maximum estimates, which do not consider the formation of intermediates.

Sulfide oxidation rates in the periods V and VI were similar; $35 \pm 3$ mmol S L$^{-1}$ day$^{-1}$ (or 1 g S L$^{-1}$ day$^{-1}$) and $34 \pm 4$ mmol S L$^{-1}$ day$^{-1}$ (or 1.1 g S L$^{-1}$ day$^{-1}$), respectively (Fig. 4B). With a decrease of the HRT in 4th compartment from 0.5 to 0.25 day in the period VII, the sulfide oxidation rate increased slightly to $41 \pm 5$ mmol S L$^{-1}$ day$^{-1}$ (or 1.3 g S L$^{-1}$ day$^{-1}$) (Fig. 4B) although sulfide oxidation efficiency decreased appreciably (Fig. 4A). Elemental sulfur production rates averaged $11 \pm 7$, $21 \pm 7$ and $30 \pm 7$ mmol S L$^{-1}$ day$^{-1}$ in periods V, VI and VI, respectively (Fig. 4B). The maximum elemental sulfur production rate was approximately 36 mmol S L$^{-1}$ day$^{-1}$ (or 1.15 g S L$^{-1}$ day$^{-1}$) as observed in period VII (Fig. 4B). Similarly, Celis-García et al. [13] reported the maximum elemental sulfur production rate as 38.9 mmol L$^{-1}$ day$^{-1}$ in a down-flow fluidized bed reactor

performing simultaneous sulfate reduction and sulfide oxidation. Celis-García et al. [13] concluded that under low aeration rates (1.0 L air L$^{-1}$ of reactor volume per day) 50% of the sulfide was transformed to elemental sulfur. When the aeration rate increased to 2.34 L air L$^{-1}$ of reactor volume per day, elemental sulfur recovery was only 30% and sulfide oxidation efficiency was approximately 73%. In our study, the high sulfide oxidation efficiency may be due to higher aeration rates (7–15 mL s$^{-1}$ or 242–518 L air L$^{-1}$ of reactor volume per day) and the separation of sulfate reduction and sulfide oxidation processes to different reactor compartments separated by baffles. The amount of dissolved oxygen present in the reactor is associated not only with aeration rate, but with the possible retention and use in biochemical reactions. In the study conducted by Celis-García et al. [13] both processes were occurring in the one reactor compartment simultaneously. Although achieving both processes simultaneously in one reactor offers some advantages, there remains a possibility of inhibiting sulfate reducing bacteria at high aeration rates. In another study, Lohwacharin and Annachhatre [15] used air lift reactor for sulfide oxidation with the aeration rates of 12–30 L air L$^{-1}$ of reactor volume per day. The use of much higher aeration rates in our study is due to inefficient air supply mechanisms associated with ABR compared to a down-flow FBR [13] and air-lift reactor [15], in which much higher oxygen diffusion rates can be achieved. Hence, aeration rate should be carefully adjusted to maintain appropriate dissolved oxygen concentrations in the reactor liquor, to prevent low or not complete sulfide oxidation. In our study, although sulfate reduction and sulfide oxidation was achieved in one reactor, both processes were separated due to the compartmentalization of the ABR. With the separation of both processes, addition of aeration to the last compartment did not adversely affect the sulfate reduction efficiency. Hence, with the use of ABR, sulfate reduction, metal removal, alkalinity generation, and excess sulfide oxidation can be simultaneously achieved in one reactor.

### 3.4. Practical implications

The study showed the high potential of ABR in the treatment of acidic (pH 3.0) mine drainage containing various metals (Co, Cu, Fe, Mn, Ni, and Zn) and sulfate (3.0–3.5 g/L). High sulfate reduction (as high as 90%) and COD oxidation (90–97%) efficiencies were obtained. Except for Mn, very high (close to 99%) metal removal efficiencies were obtained. Most of the metals were precipitated in the first compartment and this has great practical implication. The precipitated metals can be removed from the first compartment without giving serious harm to the reactor operation as the first compartment can be reseeded with sludge of other's compartment. By this way, valuable metals can be recovered without interrupting reactor operation, unlike other commonly used reactor configurations, such as up-flow sludge blanket reactor (UASB) and FBR. The study has shown, for the first time, that sulfide produced in ABR can be partially oxidized to elemental sulfur for sulfur recovery and avoiding discharge of hazardous sulfide. By this way, sulfate removal, alkalinity production, metal removal and sulfide oxidation to elemental sulfur were achieved in one reactor, which has great practical implication. Sulfide oxidation in the last compartment was studied under different operational conditions. Although sulfate production could not be completely avoided, the elemental sulfur formation efficiency was as high as 80% simultaneous with complete sulfide oxidation (period VI, Fig. 4A). The key parameters to obtain high elemental sulfur formation efficiency simultaneous with high sulfide oxidation efficiency are the aeration rate and sulfide loading.

## 4. Conclusions

This work demonstrates that ethanol-fed sulfidogenic 4-compartment ABR efficiently removed sulfate, metals and acidity from a synthetic acid mine drainage water. Moreover, aeration of the last compartment of the ABR allowed partial oxidation of the excess sulfide to elemental sulfur. The highest sulfate removal efficiency was approximately 88% and metal removal efficiencies were generally greater than 99%, except for Mn. The alkalinity produced in sulfidogenic ethanol oxidation neutralized the synthetic acidic mine water (pH 3.0–4.5) to a final pH of 7.0–8.0. Depending on the aeration rate and HRT, 32–74% of produced sulfide was oxidized to elemental sulfur. The compartmentalized structure of the ABR enables the simultaneous optimization of both sulfate reduction and sulfide oxidation in a single reactor.

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