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ACID MINE DRAINAGE TREATMENT AND METAL REMOVAL BASED ON A BIOLOGICAL SULFATE-REDUCING PROCESS

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Abstract - The key purpose of this research was to explore the capacity of an anaerobic stirred batch reactor (ASBR) to deal with acid mine drainage (AMD) based on the activity of sulfate reducing bacteria (SRB). The tests showed that SRB produced hydrogen sulfide that precipitated the metals Fe^{2+}, Zn^{2+}, and Cu^{2+}. Ethanol was used as both the only source of carbon and electron donor. Throughout the experiment, the ratio of chemical oxygen demand (COD) to sulfate was constant at 1.0. The reactor was operated for 218 days using synthetic AMD at pH 4.0 containing 1000 and 1500 $mg{\cdot}L^{-1}$of sulfate,100 $mg{\cdot}L^{-1}$of Fe^{2+}, 20 $mg{\cdot}L^{-1}Zn^{2+}$, and 5 $mg{\cdot}L^{-1}Cu^{2+}$. The metal removal rates were greater than 99 %with effluent pH of 6.5 to 7.4. The sulfide concentration reached 56.6 $mg{\cdot}L^{-1}$ and sulfate removal was 43 to 65 %.

Keywords: acid mine drainage, metal precipitation, sulfate removal, stirred batch reactor.

INTRODUCTION

Acid mine drainage (AMD) is a dangerous form of pollution characterized by high acidity (pH near 2-3) and substantial quantities of sulfate and soluble metals (Fe, Zn, Cu, Ni, Pb, and Cd ions). The accumulation of sulfate in sediments and aquatic systems causes the release of toxic sulfides that can damage the environment (Utgikar et al., 2002; Ghigliazza et al., 2000).

The exploitation of sulfide minerals such as pyrite results in the aerobic oxidation of iron and sulfur when exposed to air and water (Kaksonen et al., 2003a). Atmospheric oxygen rapidly oxidizes pyrite, releasing large amounts of sulfuric acid and ferric iron, which precipitate as ferric hydroxide (called yellow boy) as shown in Eq. 1 (Robinson-Lora and Brennan, 2009).

$$\begin{aligned} &4FeS_{2(S)} + 150_{2(aq)} + 14H_2O_{(1)} \longrightarrow \\ &4Fe(OH)_{3(S)} \downarrow + 8SO_4^{2-}{}_{(aq)} + 16H^{+}{}_{(aq)} \end{aligned} \tag{1}$$

One important aspect is the formation of AMD, which is a problem not only in active mining operations, but also at abandoned mine sites. AMD into receiving water bodies has serious environmental impacts (Moosa et al., 2005).

A number of biological processes can remove metals from wastewater and generate alkalinity

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*To whom correspondence should be addressed. Email: gisellesancinetti@gmail.com (consume acidity), and therefore have potential uses in neutralizing AMD. However, due to the elevation of pH that typically occurs during the removal of metals and sulfate, sulfate-reduction appears to be the most promising bioprocess for AMD treatment and metal recovery. This process is based on the production of hydrogen sulfide and alkalinity by sulfate-reducing bacteria (SRB) (Kaksonen and Puhakka, 2007; Mizuno et al., 1998; Robinson-Lora and Brennan, 2009).

Although sulfate is a chemically inert, non-volatile, and non-toxic compound, high sulfate concentrations can cause imbalances in the natural sulfur cycle. In anaerobic environments that are rich in oxidized sulfur compounds, sulfate reduction occurs (along with methanogenesis) as an end step in the anaerobic mineralization process (Lens et al., 1998).

The production of sulfide is a major problem associated with the anaerobic treatment of sulfate-rich wastewaters. The sulfide produced in an anaerobic reactor is speciated as S^{2-}, HS^-, and H_2S in solution. Among the ionized species (HS^- and S^{2-}) and non-ionized species (H_2S), only the latter is able to pass through cellular membranes, and is therefore more toxic (Lens et al., 1998; Hirasawa, 2008).

Biological sulfate removal is a cost-effective alternative for removing sulfate. The process consists of dissimilatory sulfate reduction to sulfide. The SRB are enhanced by their ability to effectively compete with other anaerobic bacteria for available organic substrate, as well as the sensitivity of other bacteria to sulfide (Hulshoff Pol et al., 1998).

Measurement of the dissolved metal concentration can serve as an indicator of the SRB bioactivity, and heavy metal removal is a useful application of biological sulfate reduction. Sulfide generated by sulfate reduction is used to chemically precipitate metals as sulfides (Kaksonen et al., 2003b;Sahinkaya et al. 2011; Utgikar et al. 2002;Villa-Gomez et al., 2012; Xingyu et al., 2013), as shown in Eq.1 (Villa-Gomez et al., 2012):

$$H_2S + M^{2+} \rightarrow MS_{(S)} + 2H^+ \qquad (2)$$

where M^{2+}represents a divalent metal, such as Fe^{2+}, Zn^{2+}or Cu^{2+}.

The present study was performed to contribute to the development of a biological AMD treatment using an anaerobic sequential batch reactor (ASBR). A synthetic AMD solution was used instead of actual AMD because of the difficulty in acquiring sufficient quantities and also because of restrictions due to its composition. In addition, with the synthetic AMD, it was possible to control the influent solution to the reactor. This allowed us to investigate the precipitation of iron, zinc, and copper with sulfide produced by dissimilatory digestion by the SRB.

Other aspects that have been previously addressed include the use of granular biomass, mechanical agitation with a draft-tube system, geometric reactor configuration, and feeding strategy. These factors all affect the reactor performance and sludge granulation, which is desirable in batch systems because granulated sludge allows high cellular retention times (Mockaitis et al. 2010; Zaiat et al. 2001).

For wastewater that has no or insufficient electron donors and carbon sources for complete sulfate reduction, addition of an appropriate electron donor is required. The selection of the electron donor depends on the cost of the added electron donor per unit of reduced sulfate, as well as the resulting pollution in the waste stream, which should be low or easily removable. Therefore, the choice of carbon source for SRB activity can be the key to ensuring high performance, long-term efficiency, and economic viability of the treatment. SRB can use the sulfate present in AMD as the terminal electron acceptor during metabolism of organic matter.H_2S, which acts as a metal precipitating agent, is produced during this process (Costa et al., 2009; Hulshoff Pol et al., 1998; Kaksonen et al., 2003a; Kousi et al., 2011; Sarti et al., 2010)

The main purpose of the present study was to evaluate sulfate and metal removal by an ASBR with varying metal and sulfate loads in a synthetic AMD.

MATERIALS AND METHODS

ASBR reactor

The ASBR reactor (total volume of 7.0 L and operational volume of 5.5 L) was equipped with a Fiberglass jacket and water circulation system to maintain a process temperature of 30 ºC. Mixing was provided by a three-blade propeller system operating at 50 rpm. Internally, a perforated steel basket was used to provide better mechanical protection from the stirrer for the biomass and to minimize biomass loss during liquid withdrawals. The reactor was operated for 24-48 hours, depending on the experimental step.

Figure 1 provides photographs of the ASBR used in this experiment. In the left image, the system is opened, while in the right image the system is shown during operation. The reactor was wrapped with

Figure 1. Two aspects of the reactor: Left – before operation; right – during operation.

aluminum foil during operation to prevent light from passing through the reactor walls.

Inoculum

The inoculum was a granular sludge biomass generated in an upflow anaerobic sludge blanket reactor (UASB) used to treat poultry slaughterhouse wastewater in Tietê, São Paulo, Brazil. A sludge volume of 1.0 Lwas added to the batch reactor at the beginning of the experiment, with no further sludge addition throughout the experiment.

Synthetic wastewater and operational conditions

The composition of synthetic AMD using $ZnCl_2$ was in agreement with other research developed previously using the same reactor type (Vieira et al., 2016; Kousi et al., 2011; Kaksonen et al., 2003a; Villa-Gomes et al., 2012).

Synthetic wastewater was prepared to simulate acid mine drainage and added to the reactor at the beginning of each operational cycle. Cycle means the period, in hours, elapsed between loading and unloading the reactor. In addition, Step means the period of days that the reactor operated under a set of conditions. Both are shown in Table 2. Feeding and drainage were performed manually and lasted around 1.5 minutes. The initial pH of the synthetic AMD was set to 4.0 at the beginning of each cycle using 4M HCl. The reactor operated during 218 days.

There was no recirculation and the solution inside the reactor was completely drained and replaced at the end of each cycle. Ethanol was used as both the electron donor and organic carbon source for simplicity and because of its low cost. Detailed descriptions of the wastewater composition and operational steps are included in Table 1.

Six steps with varying AMD compositions were used, while the COD/SO_4^{2-} ratio was held constant at 1.0. The concentrations of Fe, Zn, and Cu were increased consecutively during Steps II, III, and IV, respectively, so that in Step IV all three metals were at their maximum concentrations. The COD and sulfate concentrations were increased from 1000 $mg \cdot L^{-1}$ to 1500 $mg \cdot L^{-1}$ during Step V, and maintained at the higher concentration for Step VI. The cycle times for Steps I through V were all 24 hours. In Step VI, the cycle time was increased from 24 to 48 hours.

Table 1. Operational steps, step duration time, AMD composition and operation cycle.

Steps	Ethanol	$MgSO_4^{2-}.7H_2O$	Na_2SO_4	$FeSO_4.7H_2O$	$ZnCl_2$	$CuSO_4.5H_2O$	COD	SO_4^{2-}	Cycle Hours	Time (day)
	$mL.L^{-1}$	$mg.L^{-1}$	$mg.L^{-1}$	$mg.L^{-1}$	$mg.L^{-1}$	$mg.L^{-1}$	$mg.L^{-1}$	$mg.L^{-1}$		
I	0.607	88	1420	17	15		1000	1000	24	64
II	0.607	88	1174	498	15		1000	1000	24	43
III	0.607	88	1174	498	42		1000	1000	24	13
1V	0.607	88	1163	498	42	19	1000	1000	24	23
V	0.910	88	1903	498	42	19	1500	1500	24	40
VI	0.910	88	1903	498	42	19	1500	1500	48	30

Table 2. Main results achieved in each operational step.

Step (S)	I	II	III	IV	V	VI
pH$_{Affluent}$	4.04 ± 0.10	3.96 ± 0.09	4.01 ± 0.04	3.95 ± 0.07	3.97 ± 0.10	4.05 ± 0.09
pH$_{Effluent}$	6.70 ± 0.29	6.64 ± 0.21	6.55 ± 0.17	6.59 ± 0.12	6.71 ± 0.20	7.39 ± 0.17
SO_4^{2-}Removal (%)	43 ± 13	44 ± 6	51 ± 4	48 ± 4	56 ± 7	65 ± 3
COD Removal (%)	90 ± 9	88 ± 8	82 ± 5	83 ± 3	66 ± 9	90 ± 4
SO_4^{2-}Feed (mg.L^{-1})	1034 ± 132	1032 ± 80	1009 ± 47	1027 ± 68	1459 ± 187	1267 ± 74
SO_4^{2-}Effluent (mg.L^{-1})	575 ± 121	579 ± 70	490 ± 30	531 ± 53	648 ± 128	441 ± 121
COD Feed (mg.L^{-1})	1096 ± 337	1052 ± 61	1074 ± 41	1084 ± 38	1491 ± 145	1434 ± 52
COD Effluent (mg.L^{-1})	127 ± 124	130 ± 83	192 ± 51	181 ± 26	502 ± 140	141 ± 61
COD/ SO_4^{2-}	1.1 ± 0.34	1.0 ± 0.11	1.1 ± 0.06	1.1 ± 0.08	1.0 ± 0.13	1.1 ± 0.08
Sulfide Effluent (mg.L^{-1})	27.8 ± 23.0	3.7 ± 7.6	6.9 ± 2.4	12.7 ± 4.3	42.1 ± 13.3	56.6 ± 24.9
Fe^{2+}Removal - %		97.4 ± 2.05	99.8 ± 0.21	99.9 ± 0.03	99.9 ± 0.14	99.5 ± 0.27
Zn^{2+}Removal - %			99.5 ± 0.39	99.2 ± 0.36	99.4 ± 0.06	
Cu^{2+}Removal - %				99.2 ± 0.31	99.3 ± 0.08	
Cycle time (hour)	24	24	24	24	24	48
Duration (day)	64	44	14	24	41	31

Analytical methods

Every cycle, the pH, sulfate, COD, sulfide, Fe^{2+}, Zn^{2+}, and Cu^{2+} concentrations were monitored.

The pH was measured using a pHmeter (PG 1800, GEHAKA).

Samples were prepared for photometric analysis using zinc acetate and the two specific reagents indicated in the method.The most important aspect of the photometry calculation is the measurement of the absorbance at each wavelength by the sample,as described by the Beer-Lambert law.

Absorbances of the prepared samples were measured using a spectrophotometer (HACH model DR 3900) that allowed several adjustments in a range of wavelengths. Specific program were then used to determine sulfide concentration.

Using a spectrophotometric method, COD can be quickly quantified based on there action with potassium dichromate ($K_2Cr_2O_7$) in a warm acidic environment. Samples were prepared using a solution of silver sulfate (Ag_2SO_4) dissolved in concentrated sulfuric acid (H_2SO_4). After digestion, the sample was analyzed using the spectrophotometer at a wavelength of 620 nm to determine the COD concentration.

The sulfate content was determined using a spectrophotometer (HACH model DR 3900) at a wavelength of 420 nm. Because the sulfate ions associated with acetic acid react with barium chloride ($BaCl_2$) and precipitate as uniform barium sulfate crystals, the light absorption measurements were compared to a standard calibration curve.

The reaction between Fe^{2+} and 1,10-phenanthroline produces a red-colored complex that that can be used to determine iron content. The produced color intensity is independent of pH in the range of 2.0 to 9.0, and the complex is optically stable for a long time. The iron must be present in the ferrous form (Fe^{2+}), so the reducing agent hydroxylamine was added before the development of the colored reaction.

All analyses were performed in accordance with the Standard Methods for the Examination of Water and Wastewater (APHA, 2012).

The determinations of Cu^{2+} and Zn^{2+}concentrations were performed at the Escola de Engenharia de São Carlos (EESC, USP, Campus I, Sanitation Laboratory) following the standard method SM 3111 Busing atomic absorption equipment.

RESULTS AND DISCUSSION

Table 2 shows an overview of the parameter values during all steps. Based on this information, some comparisons can be made between the parameter values at different steps.

pH Monitoring

The average influent pH was 4.00, while the average effluent pH was consistently higher than 6.5 as a result of the SRB dissimilatory metabolism, as indicated by Eq.3. This pH increase was related to the culture acclimation for ethanol oxidation and also indicates that the SRB metabolic process was not inhibited by the initially low pH (Sahinkaya, 2009; Kousi et al., 2011).

$$2CH_3CH_2OH + SO_4^{2-} \rightarrow 2CH_3COO^- + HS^- + H^+ + 2H_2O \quad (3)$$

The pH increase indicates that the culture was well adapted to the experimental conditions, even in the presence of varying concentrations of heavy metals.

Figure 2 presents the pH results for each step using the Boxplot statistical tool. The pH during Steps I to V was between 6.5 and 7.0, while in Step VI the pH was between 7.0 and 7.5. The higher pH value during Step VI was a result of the largest observed sulfate removal, which could have been caused by the increased operational time.

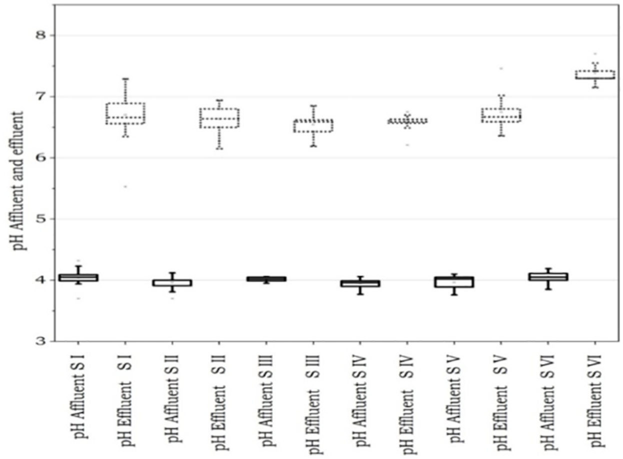


Figure 2. pH results achieved in each operational step (S).

The increase in pH levels confirms that the system consumed acidity, which is important because it indicates that the environmental conditions were beneficial to metallic sulfide precipitation. H_2S gas acts as a weak acid and, when the pH is around 6.5, it releases protons to form HS^-. In an acidic solution, sulfide volatilization is expected to increase, consequently producing a decrease in the metallic sulfide formation potential. At pH around 6.5, 50% of the sulfide species are present in the form of HS- (Kaksonen and Puhakka, 2007). This pH value is therefore compatible with the environmental conditions necessary for sulfide or hydroxide metal precipitation, especially Fe^{2+}, Zn^{2+}, and Cu^{2+}.

The microorganisms had good activity inside the reactor because of their fitness to the environment.

Metal sulfide precipitation is an important process in the hydrometallurgical treatment of ores and effluents (Lewis, 2010). Although hydroxide precipitation is widely used in industry for metal removal, there are some advantages to sulfide precipitation, including lower solubility of the metal sulfide precipitates, the potential for selective metal removal, faster reaction rates, better settling properties, and the potential for re-use of the sulfide precipitates by smelting. The concentration of sulfur species is a strong function of pH, as shown by the equilibrium among $H_2S/HS^-/S^{2-}$ in Figure 3. The presence of S^{2-} is only relevant at a pH above 16, while small changes in pH between 6.0 and 8.0 change the H_2S concentration sharply.

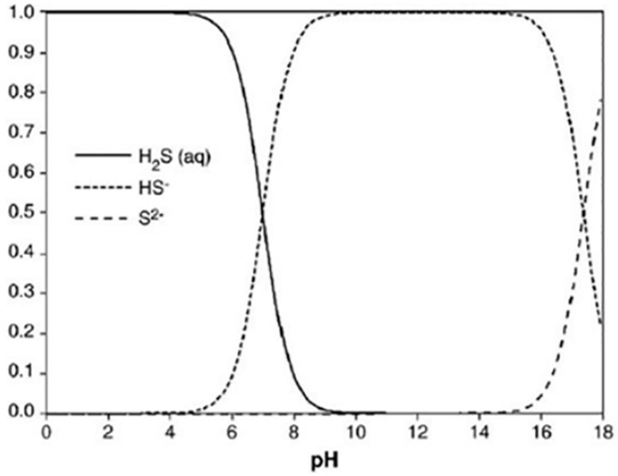


Figure 3. pH dependence of sulfide speciation (Source: Lewis, 2010).

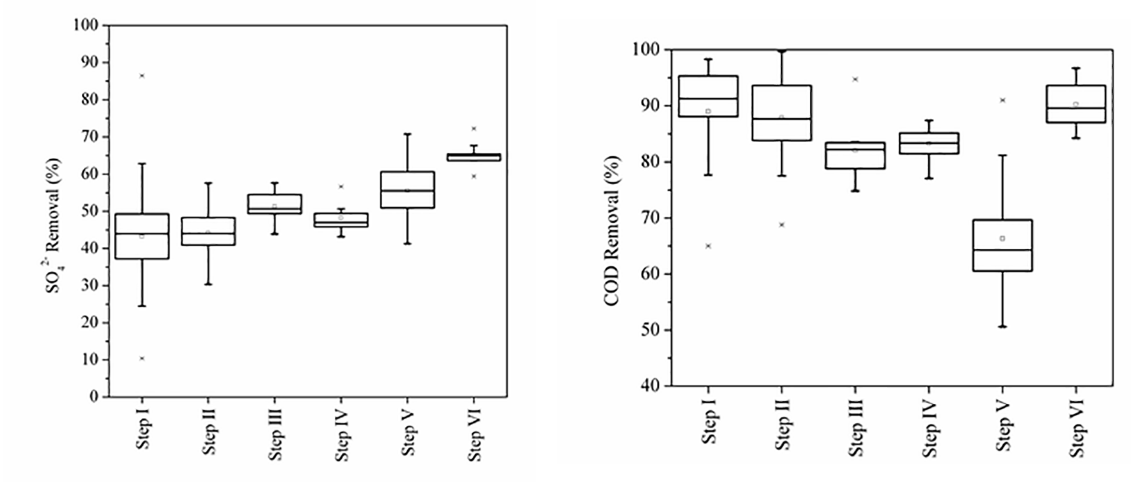
The main mechanism for metal removal in bioreactors is precipitation in the form of oxyhydroxides, carbonates, or sulfide minerals. Sorption mechanisms (e.g., adsorption and surface precipitation) and co-precipitation with (or adsorption onto) Fe and Mn oxides can also occur (Neculita, 2008).

Many previous studies have focused on the formation of metal bisulfide complexes, partly because they are often intermediates in metal sulfide precipitation, but also because they can account for the high concentrations of metals sometimes found in the environment (Lewis, 2010).

Sulfate and COD removal

An increasing trend in the percentage of sulfate removal was observed during all steps of the experiment. The increase in sulfate removal occurred even with extra metal addition, as shown in Table 2 and Figure 4A. The increased removal was likely caused by an increase in the quantity of available electrons at the higher concentrations of COD and sulfate used for Steps V and VI, as well as the time cycle increase during Step VI (Kaksonen and Puhakka, 2007; Sahinkaya, 2009; Vieira et al., 2016).

In a study by Kaksonen and Puhakka (2007), the biological reduction of sulfate was inhibited by low pH, hydrogen sulfite, high metal concentrations, and some ions, all of which indicated that the initial metal concentrations can inhibit the process. This was not observed during the present experiment.



(A) (B)

Figure 4. (A) SO_4^{2-} removal and (B) COD removal in each ASBR operational step.

The Pearson coefficient of variation is a relative dispersion measurement and represents the standard deviation expressed as a percentage of the average. The coefficients of variation calculated for Steps I to VI were 30.2%, 13.6%, 7.8%, 8.3%, 12.5%, and 4.6%, respectively, indicating that the data range became gradually narrower. This reinforces the conclusion that increased cycle time was the most important factor to increase sulfate removal.

The sulfate removal increased smoothly from Step II to IV, and increased sharply during Steps V and VI (Table 2 and in Figure 4A).

One possibility for the low decrease during Step IV is that the microorganisms were not completely adapted to the environment after the addition of copper. However, during Steps V and VI, the system showed good adaptation and the sulfate removal rate increased sharply, even with a 50 % increase in the sulfate and COD loads. During Step VI, the longer cycle time allowed for the highest removal rate.

The importance of competition between SRB and methanogenic archea increases with a decrease in the COD/SO_4^{2-} ratio in the wastewater. The outcome of this competition determines to what extent sulfide and methane, the end products of the anaerobic mineralization process, are produced (Lens and Kuenen, 2001).

Using the stoichiometric COD/SO_4^{2-} ratio of 0.67 as a reference, this work was developed using a ratio of 1.0 from the start and, as a consequence, the microbial competition favored sulfide production.

During Steps II and III, there was a decrease in the COD removal as a consequence of the Fe^{2+} and Zn^{2+} additions, respectively (Figure 4B).The lower removal rate was maintained during Step IV when Zn^{2+} was added. However, the lowest value was reached during Step V when the COD concentration in the influent was increased from 1000 $mg \cdot L^{-1}$ to 1500 $mg \cdot L^{-1}$. This negative impact was overcome during Step VI, with the cycle time increase from 24 to 48 hours. This further confirms that the microorganisms were well adapted to the reactor environment.

Effect of metal addition

The relationship between heavy metals and SRB is complex, with the metals potentially causing toxicity or metabolism inhibition. Beginning in Step II, Fe^{2+}, Zn^{2+}, and Cu^{2+} were added step-wise to the feeding solution to evaluate their settling characteristics with the sulfide generated by sulfate reduction. Even with these metals present in the solution, the sulfate reduction produced alkalinity, maintaining the pH above 6.5

Metal removal capacity is related to the amount of sulfide generated. The sulfide concentrations throughout the experiment are illustrated in Figure 5.

The metal removals were 97.4 to 99.9 % for Fe^{2+}, 99.4 to 99.5 % for Zn^{2+}, and 99.2 to 99.3 % for Cu^{2+}.

Several alternative mechanisms could have contributed to the metal removal rates, including sorption onto biofilms or complexation and precipitation with other compounds that compete with the metal sulfide precipitation, altering the fate of metals in the system. Metal sulfide precipitation can only be achieved when the sulfide concentration

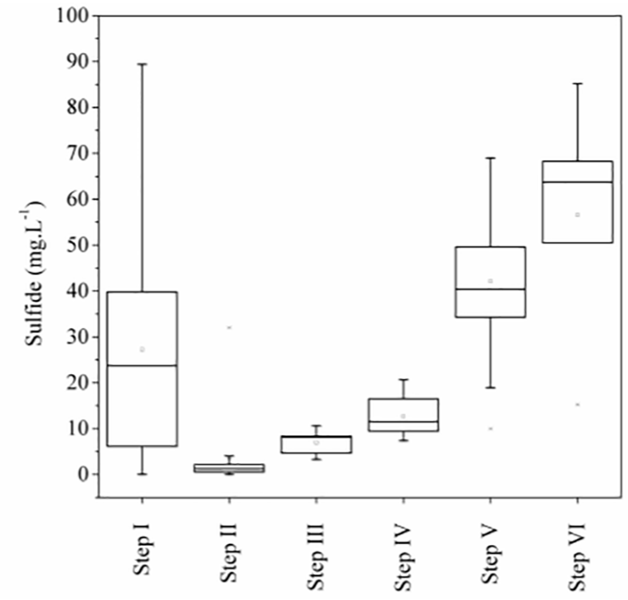


Figure 5. Sulfide concentration in each operational step.

is maintained above the stoichiometric value, as indicated by Eq. 2 (Villa-Gomez et al., 2012).

The system became gradually more adapted at each step and stronger at generating sulfide above the stoichiometric ratio, ensuring high metal removal rates (Figure 5). This was reinforced in Steps V and VI, in which the sulfate concentration and cycle time were respectively increased.

This increased sulfide concentration is an indication that the system had the capacity to remove more metal than was used. This also suggests that the metal concentrations were not close to the reported toxicity limits. The removal mechanisms in a reactor dealing with sulfate reduction depend strongly on the sulfide concentration, while precipitate growth and settlement are more associated with agglomeration. The metal removal rates followed the same order as the expected precipitation rates based on the metallic sulfide solubility (that is, the less soluble sulfides settled first) (Villa-Gomez et al., 2012).

Considering the sulfide concentration changes together with the metal removal results, the ASBR used in the experiment could have the capacity to remove larger metal quantities than those used in this experiment.

CONCLUSION

This paper developed important conclusions regarding pH, sulfate, and metal removal efficiencies during ASBR treatment of AMD.

As a key finding, the sulfate removal ranged from 43 % to 65 %, with the highest value achieved when both the sulfate input and cycle time were increased.

The sulfate removal trend increased with metal addition.

The Fe^{2+}, Zn^{2+}, and Cu^{2+} removal efficiencies were high from the moment they were added, reflecting operational stability.

The process had sufficient capacity to receive large metal loads, and absorbed the sulfate concentration increase from 1000 mg·L^{-1} to 1500 mg·L^{-1} without any negative impacts. The ASBR is therefore promising for use in treating AMD because it generates alkalinity, is stable, and has high sulfate, Fe^{2+}, Zn^{2+} and Cu^{2+} removal efficiencies.

Additional aspects of AMD treatment using an ASBR still need to be considered in future research. For instance, gas could be bubbled through a zinc acetate solution to trap hydrogen sulfide in the off-gas (Utgikar et al., 2002). The COD/SO$_4^{2-}$ ratio for biological sulfate reduction needs to be optimized, and the most suitable inert support for the biomass needs to be identified. The microbial community present in the inoculum needs to be assessed and optimized. Changes in the experimental conditions, such as to the influent sulfate concentration or COD/SO$_4^{2-}$ ratio, may lead to a better understanding of batch reactor operation for the treatment of sulfate-rich wastewater from industrial processes (Sarti et al., 2010; Sarti and Zaiat, 2011).

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NOMENCLATURES

BR - anaerobic stirred batch reactor
AMD - acid mine drainage
SRB - sulfate reducing bacteria
COD - chemical oxygen demand (mg.L^{-1})
UAS - Bupflow anaerobic sludge blanket

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