



# Effects of Hydraulic Retention Time, COD/SO<sub>4</sub><sup>2-</sup> Ratio, Influent Iron Concentration, and Sulfate Kinetics on Treatment of a Synthetic Acid Mine Drainage

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## Abstract

The effects of hydraulic retention time (HRT), chemical oxygen demand (COD), and influent Fe<sup>2+</sup> concentrations were studied in an up-flow anaerobic sludge blanket reactor for acid mine drainage treatment using whey as a carbon source. The reactor was operated in four phases: phase I: HRT of 16 h, COD/SO<sub>4</sub><sup>2-</sup> ratio of 1.0, and an influent Fe<sup>2+</sup> of 100 mg/L; phase II: HRT of 16 h, COD/SO<sub>4</sub><sup>2-</sup> ratio of 0.8, and an influent Fe<sup>2+</sup> of 100 mg/L; phase III: HRT of 24 h, COD/SO<sub>4</sub><sup>2-</sup> ratio of 0.8, and an influent Fe<sup>2+</sup> of 100 mg/L; and phase IV: HRT of 24 h, COD/SO<sub>4</sub><sup>2-</sup> ratio of 0.8, and an influent Fe<sup>2+</sup> of 200 mg/L. The best performance was in phase I, with sulfate removal of 70 ± 8%, COD removal of 80.8 ± 7.3%, effluent pH of 6.7 ± 0.17, and sulfide generation of 91.5 ± 30.3 mg/l. The reactor reduced Cu concentrations by 92.4 ± 2.7%, Zn by 48.5 ± 4.0%, and Fe by 99.4 ± 0.4% for the entire study. Increasing the HRT and COD/SO<sub>4</sub><sup>2-</sup> ratio caused a decrease in COD and sulfate removal. The increase in influent Fe<sup>2+</sup> in phase IV improved the results, but not enough to reestablish the phase I results. Still, the reactor presented steady metal removal and sulfide generation during the entire study.

**Keywords** AMD · Whey · UASB · HRT · Bioreactor · Sulfate reducing bacteria

## Introduction

Acid mine drainage (AMD) is an acidic solution generated when sulfide minerals, mainly pyrite (Fe<sub>2</sub>S), in mining waste are naturally oxidized in the presence of water, atmospheric air and microbial activity (Naidu et al. 2019). AMD can also cause significant environmental pollution, serious impacts on the health of neighboring communities, and the loss of biodiversity if not managed appropriately (Anawar 2015; Nejeschlebová et al. 2015). This residue causes serious problems; in most of cases because of its low pH and high concentrations of toxic dissolved metals, metalloids, and sulfate (Han et al. 2017; Ouyang et al. 2015; Modabberi et al. 2013).

Conventional AMD treatment can be expensive, due to the costs of reagents, operation, and maintenance (Skousen

et al. 2017). Many studies have employed sulfate-reducing bacteria (SRB) as an alternative way to treat AMD, and different reactor configurations have been described (Bai et al. 2013; Castillo et al. 2012; Costa et al. 2020; Deng et al. 2016; Godoi et al. 2017; Kieu et al. 2011; Sahinkaya et al. 2015; Sampaio et al. 2019; Vieira et al. 2020; Zhang and Wang 2016). According to Sahinkaya et al. (2011), AMD is deficient in carbon compounds and can be treated in sulfidogenic anaerobic reactors if an electron donor is added. Reducing the sulfate to sulfide increases the pH by bicarbonate formation and removes most metals as sulfides. Many studies have been using different carbon sources to promote more efficient AMD treatment in different reactor configurations.

Liamleam and Annachatre (2007) pointed out compounds that can be used as electron donors in the reactors, such as organic waste, ethanol, lactate, whey, and others. The state of Minas Gerais, Brazil, is one of the main milk and derivatives producers in Brazil (Ferreira et al. 2008). This industry sector generates wastewater that requires proper treatment before returning to watercourses, since its high organic load and concentration of nutrients such as nitrogen

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and phosphorus may cause eutrophication of rivers and lakes (Carvalho et al. 2013).

Rodríguez et al. (2012) observed that the optimum stoichiometric condition for sulfate reduction occurred at a ratio of the chemical oxygen demand (COD) and sulfate of 0.67. According to Shahinkaya et al. (2011), less organic matter in the system (lower COD/SO<sub>4</sub><sup>2-</sup> ratios) can favor fermentative bacteria and methanogenic archaea over sulfate-reducing bacteria (SRB). It is also important to note that sulfide can inhibit SRB and methanogenic archaea, depending on its free form. This occurs once sulfide passes through the cell membrane, causing protein denaturation, enzymatic inhibition, and interference of the sulfur consumption metabolism.

Rodríguez et al. (2012) used an UASB reactor to remove sulfate from actual AMD, using ethanol as a carbon source with a COD/SO<sub>4</sub><sup>2-</sup> ratio of 1.0, and achieved a sulfate removal of 85.6%. Poinapen et al. (2009) also evaluated the sulfate removal of synthetic AMD, with primary sewage sludge as the carbon source, a HRT of 13.5 h, and COD/SO<sub>4</sub><sup>2-</sup> ratio of 1.25 in an UASB reactor and obtained sulfate removal rates above 90%.

Jiménez-Rodríguez et al. (2010) obtained 68.1% sulfate removal and 55.4% COD removal for a low COD/SO<sub>4</sub><sup>2-</sup> ratio (0.5), using cheese whey as carbon source in a fixed bed anaerobic reactor treating AMD. Christensen et al. (1996) also studied AMD treatment in a fixed bed anaerobic reactor using whey as carbon source; sulfate removal ranged from 68 to 99%. The authors also monitored the removal of Fe, Cu, and Zn, and the presence of dissolved sulfide (1.8–4.1 mM) in the effluent, and found that the sulfate has been converted to sulfide and the precipitates that formed at the end of the reactor operation were metal sulfides (Jong and Parry 2003).

Oyekola et al. (2010) used an anaerobic reactor with lactate as carbon source for AMD treatment and varied the HRT between 5 and 1 days and 0.5 days, with a sulfate concentration of 1000 mg/L. They observed a sulfate removal efficiency of  $\approx 50\%$  for the lower HRT, while the higher HRTs resulted in  $\approx 86\%$  sulfate removal. Thus, using sulphidogenic UASB reactors is a viable alternative, although an external carbon source is required. As indicated by Christensen et al. (1996), using whey as carbon source can provide high sulfate and metal removal rates.

Despite increasing studies that employ a varied of organic compounds as electron donors in bioreactors, no data are available to assess the relative effectiveness of whey powder as carbon source in AMD treatment. In view of the literature cited above, there is an emergent need for AMD treatment that achieves high sulfate and COD removal, and the precipitation of metals as well as pH neutralization. Therefore, this study evaluated the performance of an up-flow anaerobic sludge blanket reactor (UASB) for the treatment of AMD,

using whey powder as carbon source and varying the COD/SO<sub>4</sub><sup>2-</sup> ratio, HRT, and influent Fe<sup>2+</sup> concentration.

## Materials and Methods

### Up-Flow Anaerobic Sludge Bed Reactor (UASB)

A UASB reactor made of acrylic with a total volume of 10.4 L (21 cm high, 20 cm diameter), 13 sampling points plus the outlet sampling point with 0.5 cm of distance between each was used in this study (Fig. 1). At the beginning of operation, the reactor was filled with 3 L of granular sludge from an UASB reactor previously used to treat poultry slaughterhouse wastewater of Poultry Dacar Co., in the city of Tietê, São Paulo, Brazil. This reactor used for the treatment of synthetic AMD wastewater (7.4 L) was operated for a year, using Alibra® commercial whey powder as a carbon source.

### Synthetic Acid Mine Drainage

For a sulfate concentration of 1500 mg/L, the reactor was fed with synthetic AMD with the following concentrations, in mg/L: Na<sub>2</sub>SO<sub>4</sub> (1572), MgSO<sub>4</sub>•7H<sub>2</sub>O (660), FeSO<sub>4</sub>•7H<sub>2</sub>O (496), NH<sub>4</sub>Cl (115.4), NaH<sub>2</sub>PO<sub>4</sub> (68.2), ZnCl<sub>2</sub> (42), and CuSO<sub>4</sub>•5H<sub>2</sub>O (20). The pH was then adjusted to 4 by the addition of 4 M HCl.

### Experimental Procedure

Commercial whey powder was used as the carbon source, with varied concentrations of COD and Fe in the distinct reactor operational phases (Table 1). Whey powder was then added and the system was agitated to dissolve the powder in the synthetic AMD. The temperature of the feeding medium was maintained at 10 °C. The reactor was kept in a thermostatic chamber at room temperature, around 20 ± 5 °C. The reactor operation was divided in four phases (Table 1), in which the HRT, COD/SO<sub>4</sub><sup>2-</sup> ratio, or the influent Fe<sup>2+</sup> concentration was varied. A HRT of 16 h was maintained for the first two phases of the reactor operation. After that, the HRT was extended to 48 h due to good results obtained by Sampaio et al. (2019).

Sulfate, COD, pH, and metals were analyzed for both the influent and effluent of the reactor, while sulfide analysis were performed for the effluent, using Standard Methods for the Examination of Water and Wastewater (Rice et al. 2012). The parameters were monitored three times a week for the first three operational phases, and two times a week during the fourth operational phase. The pH, sulfate, and total dissolved sulfide (S<sup>2-</sup>) were measured using the electrometric method (4500-H<sup>+</sup> B), turbidimetric method (4500-SO<sub>4</sub><sup>2-</sup> E), and the methylene blue method (4500-S<sup>2-</sup> D), respectively. The COD

was determined using closed colorimetric reflux (5220 D), while  $\text{Fe}^{2+}$  was measured using 3500-Fe B.  $\text{Zn}^{2+}$  and  $\text{Cu}^{2+}$  were determined using an atomic absorption flame spectrometer (*ContrAA 300 Analytic Jena*). The alkalinity was evaluated using the method of Ripley et al. (1986), and volatile acids were evaluated by the Dilallo and Albertson method (1961), for both the influent and effluent.

At the end of each operational phase, a kinetic profile analysis was conducted in which eight equidistant sampling points were evaluated. Regarding the kinetic adjustment, the microbial growth was considered constant during the kinetic adjustment periods, since they transpired over a short period of time. The kinetic adjustment used by Cubas et al. (2004) is presented in Eq. 1, for a modified first order kinetic model:

$$S(t) = S_f + (S_i - S_f)e^{-k_{ap}t} \quad (1)$$

in which:  $t$ : hydraulic retention time (h);  $S_i$ : substrate influent concentration (mg/L);  $S_f$ : substrate residual concentration; and  $k_{ap}$ : apparent kinetic constant ( $\text{h}^{-1}$ ).

## Results and Discussion

The reactor was operated for 554 days. The parameters evaluated during each operational phase are presented in Table 2. It is important to note that it wasn't possible to analyze Cu and Zn during the first operational phase, even though these metals were added to the system since phase I. Therefore, copper and zinc removal data is presented for phases II to IV. The pH, and removal of sulfate, COD, Fe, Cu, Zn, and sulfide can be observed in Fig. 2.

The alterations made during the operational phases did not affect the effluent pH (Fig. 2a), which remained between 6.46 and 6.70 during the first three phases. In phase IV, the pH increased from 6.46 to 8.00. For this operational phase, the Fe concentration was increased from 100 mg/L (Phase III) to 200 mg/L (Phase IV). The increase in pH was due to bicarbonate generation (Eq. 2; Sahinkaya et al. 2011). The  $\text{H}_2\text{S}$  produced reacts with the metallic ions, precipitating them as sulfides and generating acidity. The excess sulfate is reduced and bicarbonate ions and hydroxyl are generated, neutralizing the pH (Cao et al. 2009; Sheoran et al. 2010). For a HRT of 16 h, the highest sulfate removal was 70%, in the first operational phase (Fig. 2b).

In phase II, the HRT was kept at 16 h and the COD/ $\text{SO}_4^{2-}$  ratio was decrease to 0.8. In this phase, the sulfate removal efficiency was less than in the previous phase (55.2%) and kept decreasing in the third operational phase (43.1%), with a higher HRT (24 h) and a COD/ $\text{SO}_4^{2-}$  ratio of 0.8. This phase also showed wider variations in the data. For these two operational phases, the sulfate removal values were much less than those found by other authors, but

kept the same trend indicated by Oyekola (2010), who used lactate as a carbon source for AMD treatment, with sulfate removal values of  $\approx 50\%$  for a HRT of 12 h and 86% for a HRT of 24 h.

The HRT change generated decreased sulfate removal over time, which was probably exacerbated by the COD/ $\text{SO}_4^{2-}$  decrease from 1.0 to 0.8 from phase I–II, generating an even more unfavorable situation for the SRB. This aligns with Poinapen et al. (2009), who with an even lower HRT (13.5 h), achieved sulfate removals above 90% using a COD/ $\text{SO}_4^{2-}$  ratio of 1.25, greater than both COD/ $\text{SO}_4^{2-}$  ratios used in the present study, to treat a synthetic AMD with an influent sulfate concentration of 1500 mg/L using primary sewage sludge as the carbon source in an UASB reactor.

Shahinkaya et al. (2011), who treated actual AMD with ethanol as the carbon source in a sulphidogenic anaerobic reactor, found that increasing the COD/ $\text{SO}_4^{2-}$  ratio from 0.67 to 0.85 significantly improved sulfate removal, from 70 to 90%. The authors emphasized that this was related to the competition between methanogenic archaea, fermentative bacteria, and SRB for the carbon source. Still, regarding the HRT, COD removal was not negatively affected when the HRT was reduced from 24 to 16 h, maintaining removal rates of  $\approx 95\%$ . However, reducing HRT from 16 to 12 h decreased COD removal to 80% (Fig. 2c).

This adverse situation due to decreased HRT and COD/ $\text{SO}_4^{2-}$  ratios was not observed in the acidities from the three first reactor's operational phases, which showed similar average values, as well as alkalinity concentrations as bicarbonate. This, as well as the stability of pH, indicate that there was no formation or accumulation of intermediate acids in the system, and also indicated that the generated alkalinity was sufficient to neutralize the acids present in the medium and raise the effluent pH.

In phase III, even with the increased HRT (24 h), less sulfate removal was observed compared to phases I and II.

**Table 1** Up-flow anaerobic sludge blanket (UASB) reactor's operational phases

Phase	I	II	III	IV
HRT (h)	16	16	24	24
COD/sulfate ratio	1.0	0.8	0.8	0.8
$\text{SO}_4^{2-}$ (mg/L)	1500	1500	1500	1500
$\text{SO}_4^{2-}$ (mg/L·d <sup>-1</sup> )	2152	2123	2299	2256
COD (mg/L)	1500	1200	1200	1200
COD (mg/L·d <sup>-1</sup> )	2201	1773	1761	1840
Iron (mg/L)	100	100	100	200
Zinc (mg/L)	20	20	20	20
Copper (mg/L)	5	5	5	5
Operating Time (d)	122	121	186	127

This could be related to operational conditions of the previous phases.

In phase IV (a HRT of 24 h, a COD/SO<sub>4</sub><sup>2-</sup> ratio of 0.8, and an influent Fe<sup>2+</sup> concentration of 200 mg/L), sulfate removal increased. A better recovery and adaptation of the system were observed in this phase due to the increased Fe concentrations, which improved sulfate removal efficiency, as the metals, such as iron, precipitated as metal sulfides (Bekmezci et al. 2011).

The increased metal concentrations shifted the sulfate reduction reaction towards sulfide generation, increasing sulfate removal and lowering sulfide concentrations, since the sulfide reacts with the metals. In the present study, sulfate removal increased by 8% from Phase III to Phase IV, as the influent Fe<sup>2+</sup> concentration increased from 100 to 200 mg/L.

Phase IV also saw lower intermediate acid concentrations than Phase III, from 0.3 to 0.1 mg/L, which was reflected in the effluent pH, which increased from 6.46 to 7.99. This can also be associated with the increased iron concentrations, since the alkalinity (as bicarbonate) remained almost constant.

It is important to note that the average sulfide concentration remained within the same range throughout all of the operational phases, but this value was high from the beginning, so it doesn't negate the apparent shift of the sulfate reduction reaction towards sulfide generation, followed by subsequent reaction and precipitation with metals as metal sulfides. The higher availability of metals could have also improved sulfide removal, thereby reducing its inhibitory effect on SRB and methanogenic archaea, and resulting in better sulfate removal rates.

In phase I, COD removal was 80%. The least COD removal was obtained in phase II (72%), with a HRT of 16 h and COD/SO<sub>4</sub><sup>2-</sup> ratio of 0.8. Phase III was the most severe for the system, with 71% COD removal initially, but this recovered to 77% COD removal. As was observed for

sulfate, this phase helped the reactor adapt to the last operational phase, when COD removal reached its highest value in this study (83.5 ± 3.4%).

It is important to consider that, when the COD/SO<sub>4</sub><sup>2-</sup> ratio decreased from 1.0 (phase I) to 0.8 (phase II), COD removal decreased less than sulfate removal. The same trend was observed when the HRT was increased from 16 (phase II) to 24 h (phase III) and the influent Fe<sup>2+</sup> concentration was increased from 100 (phase III) to 200 mg/L (phase IV), COD removal recovered more quickly than sulfate removal.

The higher availability of metals in phase IV could have led to better sulfide removal, which would have negatively affected the SBR more than the methanogenic archaea, since, as already discussed, the predominant form of sulfide between a pH of 8 and 12 inhibits SBR activity more than methanogenic archaea activity. This may explain why the variations imposed throughout the operational phases and their effects on competition between the methanogenic archaea, fermentative bacteria, and SRB, greatly affected sulfate removal, while COD removal efficiency recovered more quickly.

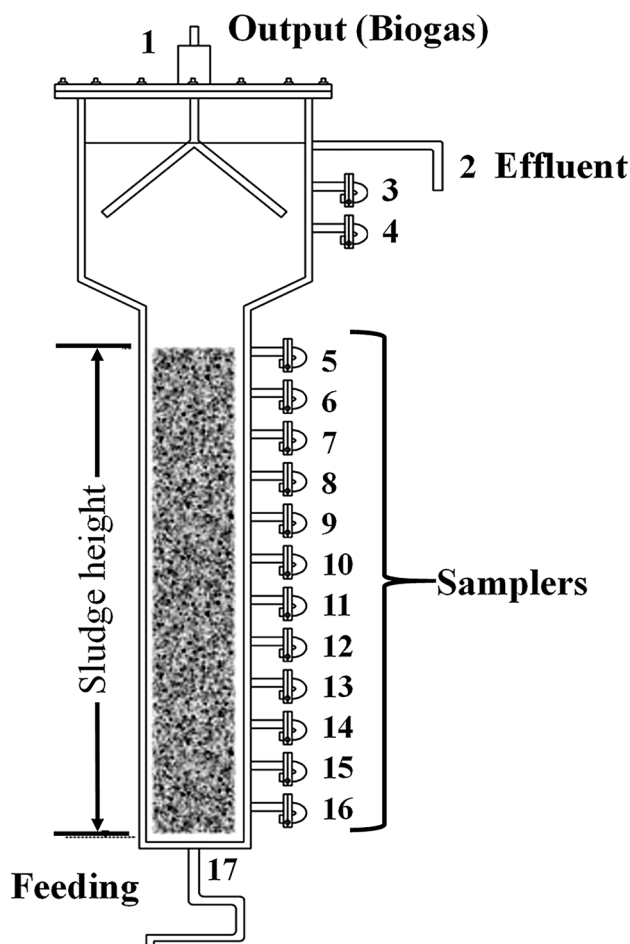
Less COD availability in the medium generates greater competition between microorganisms, which affects COD removal rates, as was observed by Jiménez-Rodríguez et al. (2010) using cheese whey as carbon source to treat AMD. They saw COD removal for a COD/SO<sub>4</sub><sup>2-</sup> ratio of 0.5, which was even less than all of the removal rates obtained in the four operational phases in this study.

Regarding iron removal (Fig. 2d), the reactor kept removal ranges above 99% in all phases, even in phase IV when the influent Fe<sup>2+</sup> concentration was doubled. The same pattern was observed by Xingyu et al. (2013), who observed 97.83% Fe removal for an influent Fe<sup>2+</sup> concentration of 200 mg/L using a sulfidogenic reactor for AMD treatment

**Table 2** Average values of the up-flow anaerobic sludge blanket reactor operation parameters

Phase	I	II	III	IV
Effluent pH	6.70 ± 0.17	6.58 ± 0.20	6.70 ± 0.43	8.00 ± 0.19
Sulfate removal (%)	70.0 ± 8.0	55.2 ± 16.9	43.1 ± 14.3	51.3 ± 7.0
Sulfate removal rate (mg/L d <sup>-1</sup> )	1505.9 ± 194.6	1179.4 ± 424.8	1047.4 ± 400.5	1152.9 ± 155.6
COD removal (%)	80.8 ± 7.3	72.0 ± 7.5	77.4 ± 10.0	83.5 ± 3.4
COD removal rate (mg/L d <sup>-1</sup> )	1795.8 ± 447.9	1275.9 ± 136.3	1362.8 ± 181.6	1539 ± 160.8
Iron removal (%)	99.7 ± 0.4	99.2 ± 0.5	99.3 ± 0.5	99.5 ± 0.3
Copper removal (%)	—	93.1 ± 3.5	92.3 ± 3.7	91.7 ± 1.0
Zinc removal (%)	—	50.8 ± 2.7	47.5 ± 4.4	47.3 ± 4.9
Effluent sulfide (mg/L)	91.5 ± 30.3	112.7 ± 46.9	137.3 ± 34.8	136.8 ± 41.5
HS <sup>-</sup> (mg/L)	35.4 ± 11.7	36.5 ± 15.2	53.1 ± 13.5	126.7 ± 38.4
COD <sub>HS<sup>-</sup></sub> (mg/L)	68.7 ± 22.8	70.8 ± 29.5	103.0 ± 26.1	245.7 ± 74.5
Effluent acids (mg/L)	240 ± 120	272.4 ± 91	333.8 ± 107	95.9 ± 39
Effluent alkalinity (mg/L as CaCO <sub>3</sub> )	108.8 ± 23	61.9 ± 19	98.4 ± 47	99.4 ± 23





**Fig. 1** Schematic representation of the upflow anaerobic sludge blanket (UASB) reactor (Cunha et al. 2018)

with activated sludge from a local wastewater treatment station as the carbon source.

It is also important to note that Jiménez-Rodríguez et al. (2009), who evaluated the effect of pH variation (3.5–5.5) on Fe, Cu, Zn, and Al removal from AMD using an anaerobic reactor and cheese whey as the carbon source. They found that the best metal removal was obtained at a pH of 5.5, resulting in 91.3% Fe removal. That indicates that the effluent pHs during phases I–IV of this study (all above 6.4) may have aided Fe removal, resulting in values above 99% for all operating phases.

Sulfate removal decreased throughout the operational phases, but the doubled iron concentration increased sulfate removal from 43 to 51%, from phases III–IV, respectively. In addition, the acids, which had been stable throughout the first three phases only showed a downward trend in phase IV, with enough alkalinity to raise the average pH from 6.5 (phase III) to 8.0 (phase IV).

It wasn't possible to analyze Cu and Zn removal for the first phase of the reactor's operation, so the results are

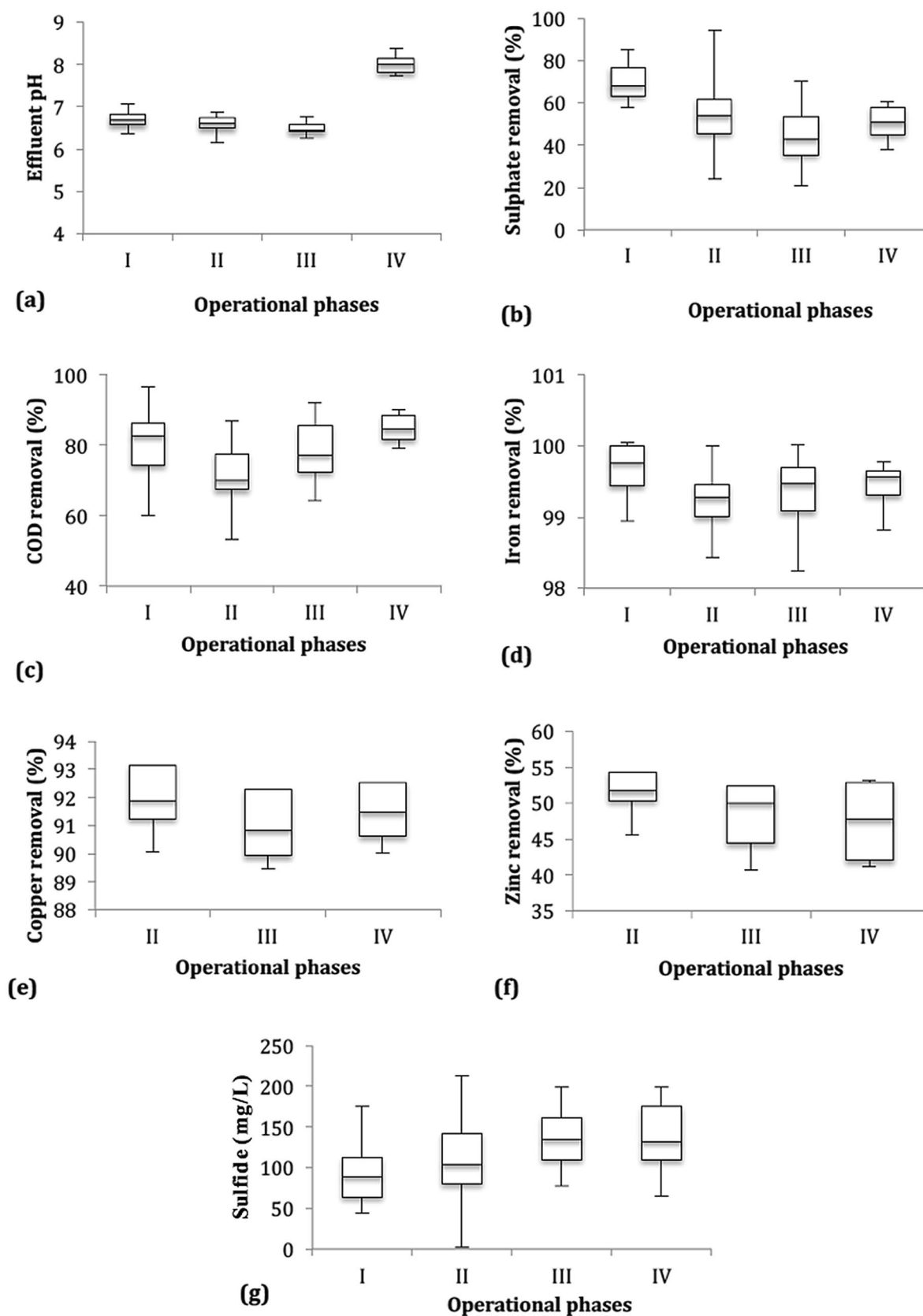
shown for phases II, III, and IV. For Cu removal (Fig. 2e), the values ranged between 91.7 and 93.1%, while for zinc (Fig. 2f), removal rates ranged between 47.3 and 50.8%. In addition, Cu and Zn have great binding affinity for the extracellular polymeric substances (EPS) produced by SRB which is a potential advantage of using this microbial group to remove heavy metals from AMD or other metal-rich wastewater. (Yue et al. 2015).

Choudhary and Sheoran (2012) observed 99% Cu and Zn removal while treating AMD in a sulfidogenic reactor with different organic carbon sources. Jiménez-Rodríguez et al. (2009), using a semi-continuous reactor with whey as carbon source for AMD treatment, found 96.1% and 79% Cu and Zn removals, respectively. In this study, Cu removal was also shown to be more effectively removed than Zn, for the same carbon source.

Greater deviations of sulfide concentrations were noticed within the same phase. Still, the same average sulfide concentration was observed in all phases, even with less sulfate removal (Fig. 2g and Fig. 3). These variations can result from the method of analysis adopted, in which the sulfide sample is collected at the reactor's outlet, having contacted the atmosphere, even if for a short period of time. As sulfide is very volatile, instant losses to the environment may occur.

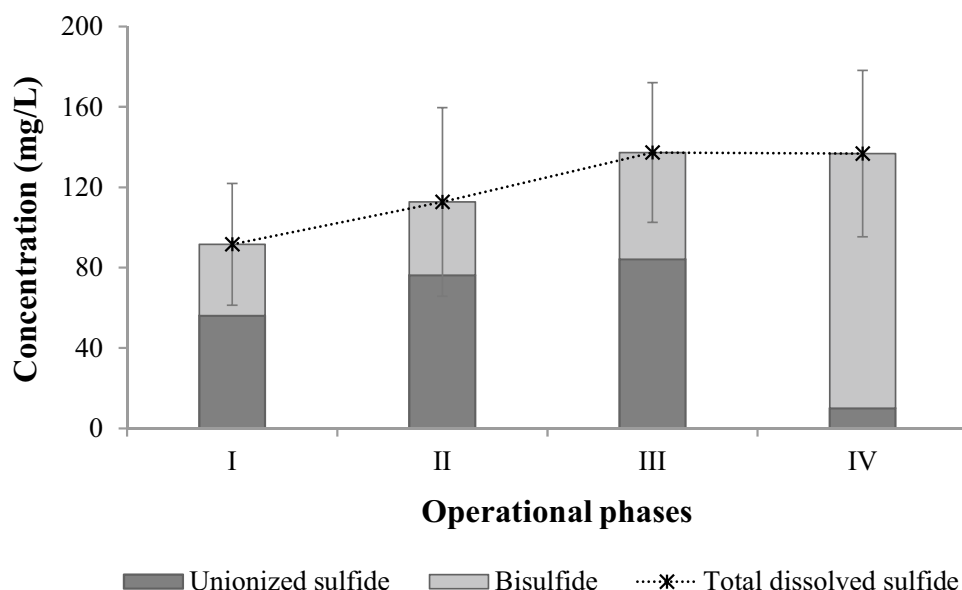
The sulfate kinetics were determined through profiles at the end of each operational phase; each profile resulted in a kinetic adjustment of the first order, done with Software Origin 8.0, following the kinetic adjustment equation (Eq. 1). The sulfate removal behavior in the kinetic adjustment can be seen in Fig. 2. The residual sulfate ( $S_f$ ), apparent kinetic constant ( $k_{ap}$ ), and equation adjustment ( $R^2$ ) data obtained in each phase can be seen in Table 3.

The data presented shows that the adjustments obtained for all of the operational phases were satisfactory. Besides that, kinetics constants of  $\approx 0.1$  and  $0.2$  ( $\text{h}^{-1}$ ) were observed, except for phase 2 ( $3.57 \text{ h}^{-1}$ ). In that phase, the reactor was operated with a HRT of 16 h and COD/ $\text{SO}_4^{2-}$  ratio of 0.8, considered the most severe for the system, as previously discussed. However, in this study, this phase presented the highest kinetic constant; sulfate consumption occurred much faster than in the other phases. Nevertheless, the residual sulfate (833.15 mg/L) was one of the highest. This indicates that the sulfate consumption occurred quickly, but with low efficiency. This low conversion of sulfate was already discussed as a collateral effect of the severity of the operational parameters to the system and the SRB. The lower kinetic constants of the last two operational phases indicate the impact of the phase II parameters, which the system recovered from, as already discussed, though not enough to reestablish the sulfate removal in phase I.



**Fig. 2** Results of (a) effluent pH, (b) sulfate removal, (c) COD removal, (d) iron removal, (e) copper removal, (f) zinc removal, and (g) sulfide concentration in each reactor's operational phase

**Fig. 3** Concentrations of total dissolved sulfide and its distribution in bisulfide ( $\text{HS}^-$ ) and unionized sulfide ( $\text{H}_2\text{S}$ )



**Table 3** The sulfate kinetic adjustment parameters

Phase	$S_f$ (mg/L)	$k_{ap}$ ( $\text{h}^{-1}$ )	$R^2$
I	$323.30 \pm 49.52$	$0.19 \pm 0.03$	0.96
II	$833.15 \pm 13.51$	$3.58 \pm 1.47$	0.95
III	$838.57 \pm 112.11$	$0.10 \pm 0.03$	0.96
IV	$706.06 \pm 85.49$	$0.09 \pm 0.02$	0.95

## Conclusion

A HRT of 16 h and  $\text{COD}/\text{SO}_4^{2-}$  ratio of 1.0 (phase I) as well as the same HRT, but a  $\text{COD}/\text{SO}_4^{2-}$  ratio of 0.8 (phase II) generated unfavorable conditions for the SRB, which led to a gradual sulfate removal decrease and affected alkalinity and COD removal. COD removal improved in phase III (HRT of 24 h and  $\text{COD}/\text{SO}_4^{2-}$  ratio of 0.8), which increased by 5%, but this did not translate to an improvement in sulfate removal, which still decreased in phase III. Only in phase IV (HRT of 24 h,  $\text{COD}/\text{SO}_4^{2-}$  ratio of 0.8) with 200 mg/L of iron was the system reestablished, increasing COD and sulfate removal, as well as decreasing the medium acidity. Iron removal was constant throughout all operational phases, with values above 99%.

Finally, in this study, the UASB reactor treating AMD with whey as the carbon source performed satisfactorily. However, caution regarding the operating parameters is necessary, since success in a reactor depends on interconnected factors and end up being directly or indirectly affected by the alteration of a single parameter.

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