The Use of a Linear Flow Channel Reactor to Remove Sulphates in Acid Water Using Sulphate Reducing Bacteria (SRB)

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Abstract**—** Biological sulphate reduction (BSR), using sulphate-reducing bacteria (SRB), is a promising method to treat acidic wastewater emanating from mining activities. This process has been investigated by various researchers in South Africa. During this process, the heavy metals precipitate due to the SRB activity. And the SRB significantly reduces the sulphate content of the treated water. In the present study, sulphate reduction and sulphide oxidation were investigated to lower the sulphate and sulphide concentration in the reactor. A linear flow channel reactor (LFCR) was designed and used in this study. The LFCR was an intermediate size between the original small reactor (2 L) and a large one (25 L). The reactor was operated for 30 days, and the hydraulic residence time (HRT) was varied. The acid mine drainage (AMD) mixture was fed to the reactor along with ethanol to act as a carbon source, and the overflow was recycled into the reactor. The effluent pH did increase from an initial value of 6.8 to 7.6, indicating mainly alkaline conditions. And the sulphate concentration of the effluent decreased overtime, with a high removal efficiency of 97% achieved when recycling the overflow. A floating sulphur biofilm (FSB) was formed after seven days of operation, which indicates bio-sulphur recovery by partial sulphide oxidation. After 30 days, the sulphide concentration increases in the reactor. The sulphide concentration was higher than 1440 mg/L due to the formation of the FSB. Adding a recycling stream and variating the HRT affected the process performance. The BSR process requires prolonged periods to achieve higher removal efficiencies, and other inexpensive carbon sources, for example, lignocellulose waste and glycerol, can be used to achieve a stable performance.

Keywords**—** Bio-Sulphur; Floating Sulphur Biofilm; Hydraulic Residence Time; Linear Flow Channel Reactor; Sulphate-Reducing Bacteria.

I. Introduction

Acid mine drainage (AMD) produced by mines in South Africa (SA) affects the water sources and aquatic life. Primary sources of AMD-contaminated water are neglected mine basins, which discharge high AMD volume (Marais et al., 2020). AMD from diffuse sources (coal discards) linked with coal mining, can also be released into the water systems (Marais et al., 2020). Environmental hazards linked with AMD include elevated levels of acids, metal ions, and sulphates (McCarthy, 2011). In SA, there have been some difficulties with handling and treating AMD at closed mines using inexpensive processes. The challenge for AMD treatment has been the excessive chemical sludge production and low sulphate removal efficiencies when using chemical processes (Speelman et al., 2020).

Three processes have been used for AMD treatment. These processes are membrane filtration, neutralization, and biological methods. Each treatment process has pilot-scale and full-scale treatment systems, which have been used in SA. The treatment of AMD using membrane filtration is based on the application of ultrafiltration, nanofiltration, microfiltration, and reverse osmosis. According to Kefeni et al. (2017), the membrane filtration process was found to be expensive due to fouling. The performance of the process was also affected by membrane fouling (Aslam et al., 2022; Hu et al., 2020; Shahid et al., 2020; Vinardell et al., 2020). The neutralization process is based on the use of alkaline chemicals such as calcium oxide (CaO), limestone ($CaCO_3$), and slaked lime ($Ca(OH)_2$). Conventional activated sludge treatment plants using neutralization for AMD treatment are also expensive due to the high cost of alkaline chemicals, and the process is affected by increased sludge production (Chen et al., 2021; Naidu et al., 2019; Tong et al., 2020). Biological treatment processes have been studied to find a cost-effective treatment process that can remove metals and significantly reduce the sulphate load in AMD. Bioprocesses that have been used include algae strains, sulphate-reducing bacteria (SRB), wetlands, and anoxic ponds (Rambabu et al., 2020; Bwalwa et al., 2017). In SA, Mintek has been conducting pilot-scale tests on using the SRB for AMD treatment. SAVMIN™ and cloSURE™ are two processes that are effective in treating AMD (van Rooyen et al., 2020).

The biological sulphate reduction (BSR) process is a bioprocess that has been used by recent studies to remove heavy metals and sulphates from the AMD. The process uses sulphate-reducing bacteria (SRB) supplemented with a carbon source. The SRB activity is promoted by carbon sources such as ethanol, lactate, acetate, and cow manure (Magowo et al., 2020; Marais et al., 2020; Sahinkaya et al., 2019; Zhou et al., 2020). In the presence of SRB and organic matter (CH_2O), sulphate is reduced to sulphides (hydrogen sulphide gas) and bicarbonates, given by Reaction (1). The pH of the effluent is

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then increased by the production of bicarbonate (Yurtsever et al., 2016; Sahinkaya et al., 2019). In Reaction (2), metals are removed through the precipitation of metal sulphides.

$$2CH_2O + SO_4^{2-} \rightarrow H_2S\,(g) + 2HCO_3^{-} \quad (1)$$

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

Different sulphidogenic bioreactors have been used to treat AMD. These bioreactors are membrane (AnMBR), fluidized bed (FBR), sequential batch (SBR), upflow sludge (UASB), and packed bed reactors (Costa et al., 2021; Miranda et al., 2022; Sahinkaya et al., 2018; Yan et al., 2020). According to Huisman et al. (2006), advantages of the BSR process in these bioreactors include less production of sludge, an increase in metal recovery potential, reduction or elimination of alkali addition, and metal sulphide compounds have low solubility products. Other advantages as found in recent studies include, the increase in the pH due to alkalinity, sulphate removal percentages that are greater than 90%, and 99% removal of heavy metals (Oztemur et al., 2020; Sahinkaya et al., 2019; Siddiqui et al., 2020).

The linear flow channel reactor (LFCR) was initially designed by Mooruth (2013) to enhance the BSR process for sulphur recovery and heavy metal removal. The reactor was also developed to overcome the sulphate formation by sulphide oxidation. Mooruth (2013) used a larger LFCR (25 L) to treat AMD. Recently, a smaller LFCR was developed at the University of Cape Town (UCT), and it was used for different studies (Horn et al., 2021; Marais et al., 2020; Marais et al., 2020; van Hille et al., 2021). Inside the reactor, the floating sulphur biofilm (FSB) forms within two days when treating AMD. The FSB concept is described as the surface layer consisting of sulphide (Molwantwa, 2008), where it is required to maintain a microenvironment suitable for partial sulphide oxidation (Marais et al., 2020). After several weeks, bio-sulphur forms in the biofilm due to the presence of sulphate-oxidizing bacteria (SOB).

This study will evaluate the performance of a linear flow channel reactor (LFCR) used to treat AMD by applying the BSR process. The activity of the mixed SRB will be used to evaluate the efficiency of the sulphate reduction process. The performance of the reactor will be determined in terms of heavy metal, sulphate, and sulphide concentration. Finally, the effect of the formation of floating sulphur biofilm (FSB) and the recycling rate on the performance of the reactor will be evaluated.

II. MATERIALS AND METHODS

A. *Acid mine drainage and inoculum*

Acid mine drainage (AMD) in a 25 L container was supplied by Mintek. This AMD was collected by Mintek from the coal mines in Mpumalanga. The AMD was stored at room temperature (25 ºC), and the main characteristics of this AMD are given in TABLE I. AMD was added to the reactor when there was a high COD (greater than 5 g/L) or a high pH (greater than 7.8).

The inoculum was supplied by Mintek. This inoculum consisted of a mixture of different sulphate-reducing bacteria (SRB) communities and was similar to the one that was used in Mintek's studies (du Preez, 2021; van Rooyen et al., 2020). The main components of the medium that consisted of inoculum were as follows: sulphate was 1400 mg/L, and sulphides were 260 mg/L. AMD and the inoculum were mixed in a ratio of 70% AMD to 30% inoculum, and the mixture was fed into the reactor along with the carbon source.

TABLE I
KEY CHARACTERISTICS OF THE AMD FROM MINTEK.

Characteristic	Value
pH	2.9
Sulphate (SO_4^{2-}) (mg/L)	3451
Hydrogen sulphide	0.001
COD (mg/L)	22
Fe (mg/L)	52.4
Mn (mg/L)	11.6
Cu (mg/L)	0.30
Zn (mg/L)	0.59
Ni (mg/L)	0.22

B. *Configuration of the linear flow channel reactor*

The linear flow channel reactor (LFCR) was developed at North-West University (NWU) and used to treat the AMD obtained from Mintek. The reactor had a total volume of 10.6 L and a working volume of 9 L. The LFCR was constructed from Perspex with a nominal thickness of 11 mm and is shown in **Fig. 1**. Four inlet and outlet ports were used, respectively. The AMD mixture was fed to the reactor using the uppermost inlet port, and the effluent was collected using the uppermost outlet port. Also, there were twelve sampling ports on the reactor. However, only the ports in the middle were used to collect samples. Finally, a peristaltic pump was used to pump the feed into the reactor.

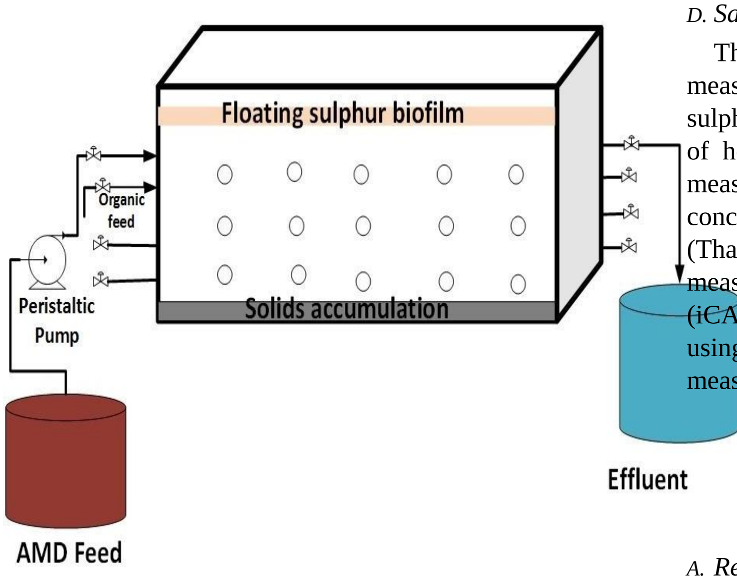

Fig. 1 The linear flow channel reactor used, showing also the 15 sampling ports.

C. *Reactor operation*

A mixture containing acid mine drainage (AMD) and inoculum was pumped into the LFCR at a flow rate of 2 L/d. The feed had a sulphate and sulphide concentration of 5 g/L and 0.2 g/L, respectively. Alongside the reactor feed, ethanol was added to the reactor as a carbon source. The LFCR was operated at a temperature of 23 ºC, and the pH was controlled between 6 and 7.9. The COD/sulphate ratio was kept between 0.67 and 2. When the ratio was below 0.67, more ethanol was added to increase the COD. And for the COD/sulphate ratio higher than 2, AMD was added to increase the sulphate concentration.

The hydraulic residence time (HRT) was varied throughout the operation of the reactor. The HRTs that were used are given in TABLE II. The process had a recycling stream, where the effluent was recycled into the reactor throughout the week. However, the effluent was not recycled during the weekend to monitor the effect of the recycling rate on the process performance and the formation of the floating sulphur biofilm (FSB). The recycling rates that were used are given in TABLE II. A peristaltic pump was used to recycle the effluent. And the effluent was recycled 4 times per day.

TABLE II
HYDRAULIC RESIDENCE TIMES AND RECYCLING RATES THAT WERE USED

Period	HRT (days)	Recycling rates (L/h)
Day 0-8	1.5	2.5
Day 9-20	2	2.2
Day 21-30	3	2

D. *Sample analysis*

The performance of the reactor was monitored by measuring the following parameters: pH, sulphate and sulphide concentration, influent COD, and the concentration of heavy metals. The pH of the influent and effluent was measured using the metrohm pH meter. Sulphate concentration was measured using UV-spectroscopy (Thangiah, 2019), and heavy metals concentration was measured using the Thermo Fischer Scientific, ICP-OES (iCAP 6000). The concentration of sulphide was determined using methylene blue method (Apha, 2001). The COD was measured using the Hanna multi-photometer instrument.

III. RESULTS AND DISCUSSION

A. *Reactor performance*

1) *Floating sulphur biofilm*

The floating sulphur biofilm (FSB) is essential for providing a suitable environment for the biological sulphate reduction (BSR) process. The FSB started to form in the reactor after 7 days, as shown in Fig. 2 a). According to Sahinkaya et al. (2019), the biofilm consists of metal sulphides that formed in the reactor as per Reaction (2), where sulphides are precipitated to metal sulphides. The FSB was fully developed after 9 days, as shown in **Error! Reference source not found.** b). The structure of the FSB was disrupted when recycling the overflow, as shown in **Error! Reference source not found.** c and d).

Fig. 2 The formation of the floating sulphur biofilm as time progresses. A) after 7 days. B) after 12 days. C) after 25 days. D) after 30 days.

2) *COD reduction in the reactor*

The COD concentration of the effluent is shown in Fig. 3. The COD in the reactor was attributed to the change in the concentration of ethanol in the reactor. The COD of the ethanol can easily be calculated or given as the degree of reduction of ethanol, where 12 electron mol is multiplied by 8 mol of ethanol to get 96/46 g COD/g ethanol, equalling 2.09 g COD/g ethanol (Jwara et al., 2022). The COD of the ethanol was calculated to be between 2-3 g/L. The COD of the effluent was very high for the first three days due to the slow growth of the sulphate-reducing bacteria (SRB) and the addition of 20 mL of ethanol. As time progressed, the COD concentration suddenly decreased due to the decreasing ethanol concentration, as the SRB started to use ethanol as a carbon source for growth. The rapid decline in the COD concentration from day 9 was due to the SRB already adapting to the acidic conditions inside the reactor. Throughout this period (day 3 to day 9), the COD declined by 55%.

After day 15 and day 21, adding a mixture of AMD and inoculum led to a slight increase in the effluent COD. The AMD has a COD concentration of zero, so the inoculum affected the COD concentration inside the reactor. From day 15 to day 26, there was a slower decline in the COD concentration due to the slow growth of the SRB (lag phase) (Otzemur et al., 2020). Also, this slow decline led to higher COD/sulphate ratios, which were above 5. Between day 25 and day 30, there was a significant decline in COD concentration, as it declined by 72%. According to van Hille et al. (2014), the SRB was in the high-performance phase, so more ethanol was used by the SRB to decrease the COD in the reactor.

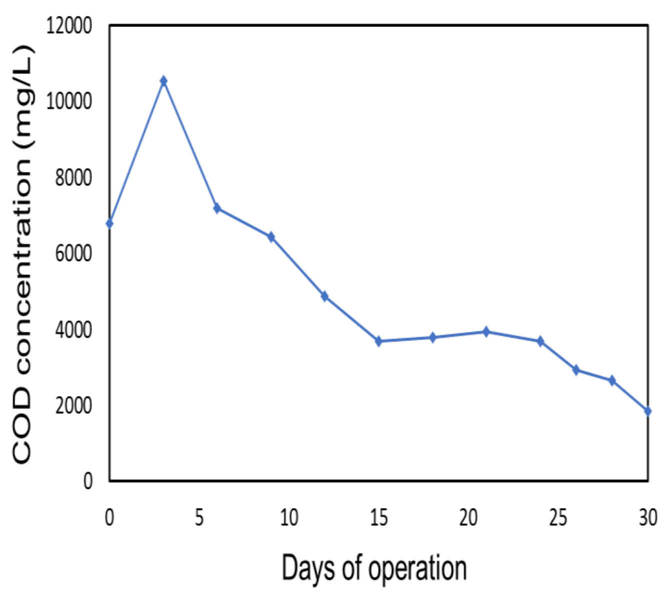

Fig. 3 COD concentration in the LFCR during the operation for 30 days.

3) Sulphate removal

The initial sulphate concentration in the feed was the same as the sulphate concentration of the AMD (5.5 g/L). The sulphate concentration of the influent and effluent are shown in **Fig. 4**. During the first 5 days of operation, the sulphate concentration of the effluent started to decrease steadily. The growth of the SRB was slow during this period, and the COD/sulphate ratio was between 0.67 and 2. The SRB was in the lag phase (van Hille et al., 2014). Between day 6 and day 9, there was a significant decline in the sulphate concentration from 2490±5 mg/L to 1150±10 mg/L due to the fast growth of the SRB, as it already adapted to the conditions in the reactor, and the SRB was in the high-performance phase (van Hille et al., 2014). Also, the floating sulphur biofilm (FSB) was starting to develop fully, so the efficiency of the BSR began to increase (Marais et al., 2020). However, the COD/sulphate ratio was very high during this period. The removal percentage of the sulphate found in the effluent increased to 78%.

On day 12 and day 18, AMD (2L and 750 ml, respectively) was added to the reactor to increase the sulphate concentration. From day 18 to day 30, the sulphate concentration was below 600 mg/L. The sulphate removal percentages were above 90% during this period, with the highest being 97% on day 30. The COD/sulphate ratios increased from 5 to 12. It can be stated that the sulphates were efficiently removed from AMD after 30 days.

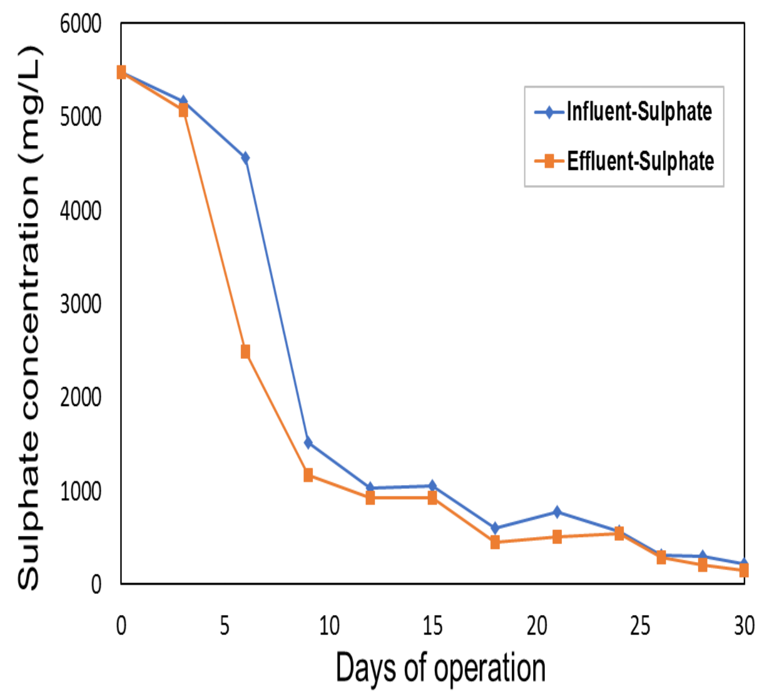

Fig. 4 Concentration of sulphate present in the influent and effluent in the LFCR after 30 days of operation.

4) Sulphide production

The sulphide concentration of the influent and effluent are shown in Fig. 5. Initially, the sulphide concentration was 95 mg/L. As time progressed, the sulphide concentration of the effluent increased significantly to 650 mg/L as the SRB started to reduce the sulphate in the reactor. Thus, the formation of the FSB had an impact on the increasing sulphide concentration, as the FSB blocks the sulphide gas from escaping (Mooruth, 2013; Molwantwa, 2008). The addition of AMD on day 12, day 21, and day 26, led to a decrease in the sulphide concentration of the effluent. The highest sulphide concentration of 1440±10 mg/L was observed after 18 days of operation.

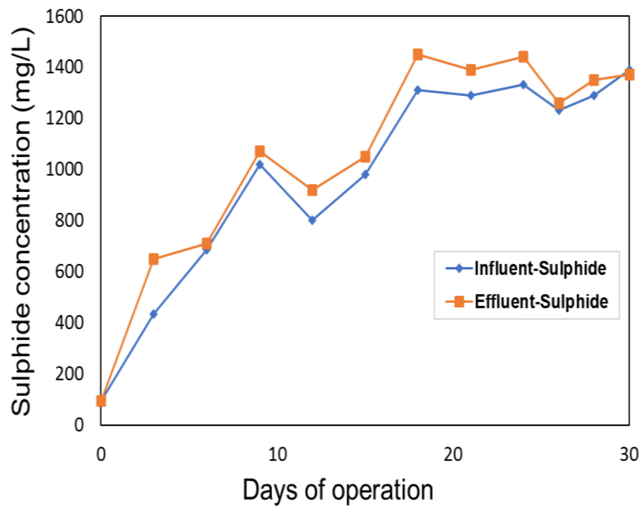

Fig. 5 Sulphide concentration of the reactor influent and effluent during the operation of the reactor for 30 days.

5) pH in the reactor

The pH of the influent and effluent are shown in Fig. 6. The initial pH of the reactor influent was different from the pH of the AMD. Mixing the AMD with the inoculum increased the pH to 6.8±0.4, which was visibly seen by the change of colour of the mixture, which was dark. From day 1 to day 9, the pH of the effluent increased to 7.3±0.5. The bicarbonate produced in the reactor increased pH, as shown in Reaction **Error! Reference source not found.**) (Sahinkaya et al., 2018). The significant decline in pH was due to the addition of AMD, but overall, the pH of the effluent was between 6.8 and 7.3 during the 30 days of operation, with the pH of the effluent consistently above that of the influent.

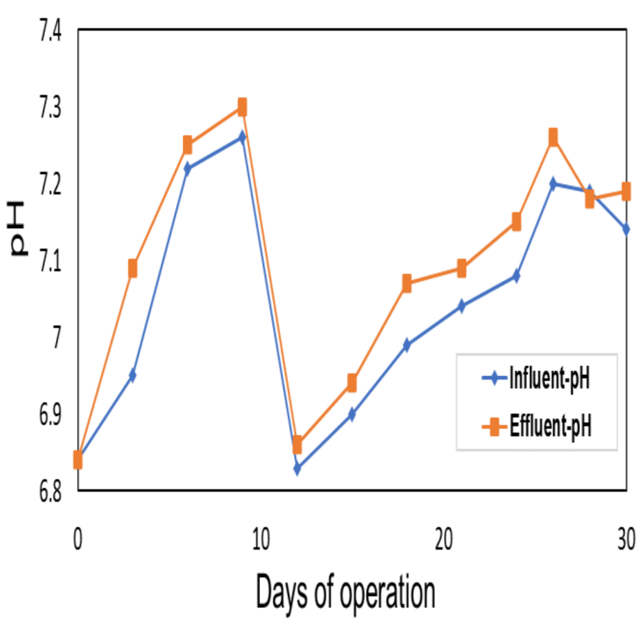

Fig. 6 pH of the influent and effluent during the operation of the reactor for 30 days.

6) Heavy metal removal

The concentrations of the heavy metals (Fe, Mn, Zn, Cu, and Ni) in the effluent are shown in Fig. 7. The reactor feed had an initial high concentration of Fe and Mn. Other heavy metals (Ni, Cu, and Zn) had low concentrations due to precipitation of these during the mixing of AMD and the inoculum. It took about 3 days to remove Ni, Cu, and Zn from the effluent. After 4 days, Fe was also removed from the effluent. However, the concentration of Mn decreased slowly compared to other heavy metals. After 12 days, Mn concentration decreased from 10.8 mg/L to 9 mg/L. In conclusion, the SRB can remove heavy metals efficiently, but Mn requires more time to be removed from the effluent

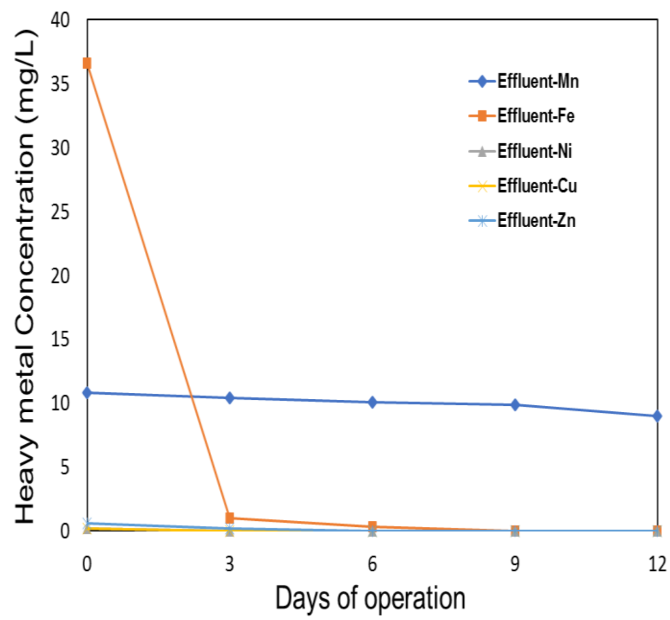

Fig. 7 Heavy metals (Fe, Mn, Zn, Cu, and Ni) concentration in the effluent during the operation of the reactor

IV. Conclusion

The performance of the linear flow channel reactor (LFCR) was efficient for 30 days of operation. The formation of the floating sulphur biofilm (FSB) had an impact on the process performance. A fully developed FSB increased the biological sulphate reduction (BSR) efficiency. The COD concentration of the effluent decreased steadily, and the sulphate concentration decreased rapidly. A high COD removal percentage of 72% was recorded on day 28, and the highest sulphate removal percentage was 97% on day 28. However, the COD/sulphate ratio cannot be controlled to be between 0.67 and 2. The pH of the effluent was between 6 and 8. The sulphide concentration increased rapidly, and 1440±10 mg/L was the highest concentration recorded. Heavy metals such as Fe, Zn, Cu, and Ni were removed from the treated AMD. However, Mn was not removed from the effluent, and there was a Mn concentration of 9 g/L after 12 days. In conclusion, more days are required to remove the COD, Mn, and sulphide from the reactor.

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