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Semi-pilot Scale Biological Removal of Metals and Sulfate from Industrial AMD in Fluidized-bed Reactor

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ABSTRACT

Acidic mine drainage (AMD) contains large amounts of heavy metal ions and SO_4^{2-} , which can pose serious risks to human and environmental health. Anaerobic bioreactors are considered to be suitable methods for the treatment of acidic effluents due to some advantages such as the need for a small area, easy control, and simultaneous removal of sulfate and metals even in low concentrations. In this study, sulfate-reducing bacteria (SRB) performance was investigated in a semi-pilot scale down-flow fluidized-bed (DFFB) anaerobic reactor for SO_4^{2-} and metals removal from the Sungun copper tailings AMD. The results indicated that utilizing SRB in the DFFB anaerobic bioreactor was an efficient, cost-effective, and environmentally friendly method for the treatment of effluents containing large amounts of SO_4^{2-} and metals. All contaminants except Cr showed more than 70% removal after 24 h. The SO_4^{2-} and Cu which had the highest initial concentrations showed removal efficiencies of 98.64% and 98.75%, respectively. Besides the removal of hazardous contaminants, the alkalinity of effluent increased remarkably. Also, the SRB had acceptable stability even after six consecutive cycles due to using AC granules as a support in the reactor which is an important parameter in industrial applications.

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1. Background

The increasing growth of industries and environmental pollution issues have attracted many researchers' attention (Sahinkaya et al., 2017, Venkatesan and Rajagopalan, 2016). So the use of eco-friendly methods has grown exceedingly in the last decades (Arjaghi et al., 2021). Sulfate, hazardous metals, and metalloids are thought to be the main contributors to environmental contamination in mining and metallurgy wastewater (with pH near 2-3) (de Matos et al., 2018). Accordingly, acid mine drainage (AMD) production is among the critical environmental issues of copper mines, which may adversely affect freshwater ecosystems due to the high concentrations of heavy metal ions and sulfate.

Sulfate is one of the most prevalent salts in the world, which is naturally present in various running water in the form of soluble and insoluble salts such as barite (BaSO_4), epsomite ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), and gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) (Neculita et al., 2007, Niya, 2021, Mobar and Bhatnagar, 2021, Mobar and Bhatnagar, 2022). Even though sulfate could be a chemically inactive, non-volatile, and non-toxic compound, high sulfate concentrations can be harmful to nature within the normal sulfur cycle imbalance. Besides, heavy metal ions, such as copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), cadmium (Cd), and lead (Pb), raise genuine contamination in the environment (Feng et al., 2019). These heavy metal ions can diffuse into the food chain of animals and eventually into human food, which can pose serious risks to human health (Rajaei et al., 2020, K. Kabir and Hosain, 2021, Elsayied Abdein, 2022, Baruah, 2021, Barth, 2021).

Different physicochemical and biological treatment technologies are utilized for the removal of heavy metal ions and sulfates from mine drainage (Kim et al., 2017, Kubendiran et al., 2021, Nejatbakhsh et al., 2022). Common methods like chemical precipitation, ion exchange, reverse osmosis, adsorption, and membrane separation can't perform well in low sulfate concentrations. Also, these methods are not usually cost-effective.

So, biotechnological methods are introduced as potential candidates in wastewater treatments (de Matos et al., 2018, Sun and Khayatnezhad, 2021, Wang et al., 2021, Wang et al., 2022, Guo et al., 2022). Due to various benefits including the removal of low concentrations and generation of denser sludge (with a high sedimentation ability), low volumetric sludge production, and high stability of metal deposits, biological approaches are more attractive than physicochemical methods. Biological removal of sulfate and metal ions is divided into two common categories; inactive methods, which include wetlands and anaerobic ponds, and bioreactor methods. Although they require a lot of maintenance, biological reactors are now regarded as the most effective technology since they take up little space, are easy to regulate, and offer improved process prediction in addition to benefits such as the continuous and simultaneous removal of sulfur and heavy metals (Torbaghan and Torghabeh, 2019).

A down-flow fluidized bed (DFFB) reactor offers a singular benefit for the purification of precious metals according to its one-step procedure. The return flow of the fluid in this

reactor moves the carrier materials and the biofilm that is atop the reactor. The created metal sulfide precipitates at the bottom of the reactor are separated from the biomass during the treatment of effluents containing metals and SO_4^{2-} in the DFFB. This provides the recovery of the metal in a single-use bioreactor. The first research to examine the application of DFFB for metal purification was (Gallegos-Garcia et al., 2009). Despite the high metal removal and efficiency, the accumulation of acetate resulted in maximum COD and SO_4^{2-} removal rates of just 54% and 41%, respectively. In another study, high SO_4^{2-} reduction efficiency (up to 85%) and COD reduction (90%) were reported in an up-flow fluidized bed reactor (UFBR) (Kaksonen et al., 2003).

SRB and sulfide-oxidizing bacteria (SOB) are two general categories of sulfur cycle bacteria. SRB is crucial for removing SO_4^{2-} and heavy metals and SOB is crucial for decreasing sulfide. SRBs are obligate anaerobic and chemotrophic bacteria that utilize simple organic molecules as carbon source. In the anaerobic bioreactor method, SO_4^{2-} reduction happened by sulfate-reducing bacteria (SRB), which is mediated by the metal's removal through metal sulfide precipitation (Sahinkaya and Gungor, 2010, Han et al., 2017). Most of the metal sulfides that form because of sulfide reactions with metals are stable in anaerobic treatment systems. A pH of 5.7-8.7 is the best condition for SRBs. Low-pH water reduces the efficiency and capacity of metal treatment. SRB has a major impact on the prevention of detrimental environmental effects. Heavy metals are removed by SRB through three steps. Firstly, SO_4^{2-} as the last electron acceptor is reduced by the above-mentioned bacteria and converted to sulfide. Then, the sulfide resulting from SO_4^{2-} reduction reacts with heavy metals, forming a metal precipitate. At last, excess sulfide is finally oxidized by SOB or converted to elemental S by the manual addition of an oxidant (Tang et al., 2009, Kusumawati et al., 2017, Carlier et al., 2019).

Numerous research conducted throughout the world for SO_4^{2-} and heavy metals removal from AMD. Yildiz et al. (Yildiz et al., 2019) investigated the SO_4^{2-} reduction in AMD in two up-flow reactors loaded with acetate and ethanol, respectively. The outcomes demonstrated that after 148 days of operation, parallel reactors in ethanol and acetate reactors reduced 2000 mL of SO_4^{2-} by approximately 51 and 31 mg/L, respectively. In this study, copper precipitation was completed at a pH < 2 for 35 min. Kiran et al. (Kiran et al., 2017) investigated the simultaneous treatment of SO_4^{2-} and heavy metals using sulfidogenic bioreactors. They introduced biological treatment systems to be as promising methods for the treatment of heavy metal-contaminated effluents. Hwang et al. (Hwang and Jho, 2018) investigated the removal of SO_4^{2-} and heavy metals from AMD using SRB. They found that the reduction of SO_4^{2-} and heavy metals by SRB had a higher removal efficiency than native bacteria isolated from the mine soil. Sulfate was reduced by these bacteria in 24 h, but this lasted for 360 h by native bacteria isolated from the mine soil. According to previous research, the optimal removal of copper may occur at a pH of about 6 (Nobari et al., 2019), and SRB can be used as a permanent purifier for long-term bioremediation at the

contamination site. An anaerobic reactor was used to purify polluted water in a coal mine by removing 95% of copper, zinc, and nickel, indicating that this system was highly efficient in heavy metals removal (Dvorak et al., 1992).

All in all, the study on the biological removal of metals and SO_4^{2-} from artificial wastewater by SRB in anaerobic fluidized-bed reactors showed desirable SO_4^{2-} reduction and metals precipitation. Moreover, the production of alkalinity during the process could tailor the system to facilitate the treatment of acidic effluents (Hajizadeh et al., 2017).

However, the pilot and semi-pilot scale studies on the real AMDs can be further investigated and could be a big step in the way of industrialization of this method. The effluent of the Sungun copper mine-processing complex has destructive consequences for the surrounding environment as it contains heavy metal ions and sulfate. Since the Sungun copper mine tailings dam is located upstream of agricultural lands and villages, as well as a tributary of the Sattar Khan dam (Ahar, Iran), environmental threats are much more vital here. Neutralization and treatment of this effluent not only reduced its negative and destructive effects but also reused by recovering the water in the effluent and returning it to the plant. In this study, the biological removal method by SRB was used in a semi-pilot scale DFFB anaerobic reactor for the first time to remove and reduce pollutants from the acidic drainage of Sungun copper mine tailings. This method was used as a cost-effective and environmentally friendly approach for the simultaneous removal of sulfate and six different metals (Cu, Zn, Cr, Ni, Cd, Pb). The stability of SRB with the aid of AC granules in the reactor as well as other important parameters including COD, volatile suspended solids (VSS), total suspended solids (TSS), and alkalinity changes were also investigated.

2. Materials and Methods

2.1 Study area and feed specification

Sungun Porphyry Copper Complex is located in East Azerbaijan province at the coordinates of $46^\circ 43'$ E and $38^\circ 43'$ N, 130 km northwest of Tabriz (a neighborhood of Azerbaijan and Armenia Republics) in northwest Iran. In this deposit, there is a reserve of copper sulfide (> 500 million tons) with a grade of 0.76% copper and 0.01% molybdenum. The drainage of this complex includes many metals and other contaminants which are shown in table 1.

Table 1. Sungun complex drainage specification

Parameters	Concentration	Unit
Cu	20	mg/lit
Zn	1.7	mg/lit
Cr	0.24	mg/lit
Ni	0.83	mg/lit
Cd	0.41	mg/lit
Pb	0.28	mg/lit
SO_4^{2-}	3900	mg/lit
EC	3900	$\mu\text{s}/\text{cm}$
COD	380	mg/lit
TSS	2255	mg/lit
VSS	70	mg/lit
Alkalinity	820	mg/lit

2.2 DFFB construction

As shown in Fig.1, to construct the anaerobic reactor, a glass container with a volume of 5 liters was used.

A magnetic stirred heater was used to heat and mix the contents inside the reactor to prevent sedimentation. The chamber was filled using 800 g of activated carbon granules as the carrier with an average diameter of 0.63 mm and a density of $0.58 \text{ g}/\text{cm}^3$. The volume of wastewater that could be placed inside it was 2.3 liters. Inert gas (nitrogen) was used to purge the reactor during discharge. The gas produced by the bacteria during the process was entered through a separate tube into two one-liter glass containers that were connected (Pascal-connected containers). One of the glass containers was filled with distilled water containing 30 ml of sulfuric acid and 200 grams of sodium sulfate (to prevent gas from escaping from the aqueous solution). The other vessel, which was calibrated in terms of S_2H milliliters, was installed at a lower height than the other one, so due to the difference in height between the two glass vessels, this solution is equivalent to the volume of produced gas (Celis-García et al., 2007, Celis et al., 2009).

2.3 SRB cultivation

For the activation of the reactor, the sludge of the anaerobic digestive unit of the municipal wastewater treatment plant was used as the SRB source. To culture SRB, (Fig 2), sodium lactate (1.75 g), beef extract (0.5 g), peptone (1 g), Na_2SO_4 (0.75 g), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (1 g), K_2HPO_4 (0.25 L), and CaCl_2 (0.05 g) were added to 1 L of distilled water, and the pH was set at 5.7-8.7. The culture medium was sterilized at 121°C and 1.2 bar. Then, 0.329 g of ammonium sulfate was separately sterilized in 10 mL of distilled water and 1 mL of this solution was added to 10 mL of the SRB medium. Sodium ascorbate (1 g) was dissolved in 10 mL of distilled water, sterilized separately, and 1 mL was added to 10 mL of the SRB medium. 5 mL of the sludge was inoculated into the medium and placed in the reactor at 27°C . To ensure the growth of SRB after 7-10 days and observation of discoloration (black), a sample was taken from the reactor and stained by Gram staining. The presence of Gram-negative curved bacteria indicated a multitude of SRB in the medium (Torbaghan and Torghabeh, 2019).

2.4 Experimental procedure

To feed the system, the 2.3 L reactor was filled with 100 cc volumes of effluent and the return flow was adjusted in such a way to maintain the bed height at about 100 cm in the fluid state. Feed specifications and also schematic and characteristics of the reactor were introduced in the last sections. Calcium sulfate (1.2 g/L) and sodium lactate (8 mg/L) were utilized as the last electron acceptor and the energy source, respectively. The final solution pH was set at about 5.7-8.7 (suitable for SRB growth) using NaOH, and the reactor temperature was set at 27°C (Hao et al., 2014).

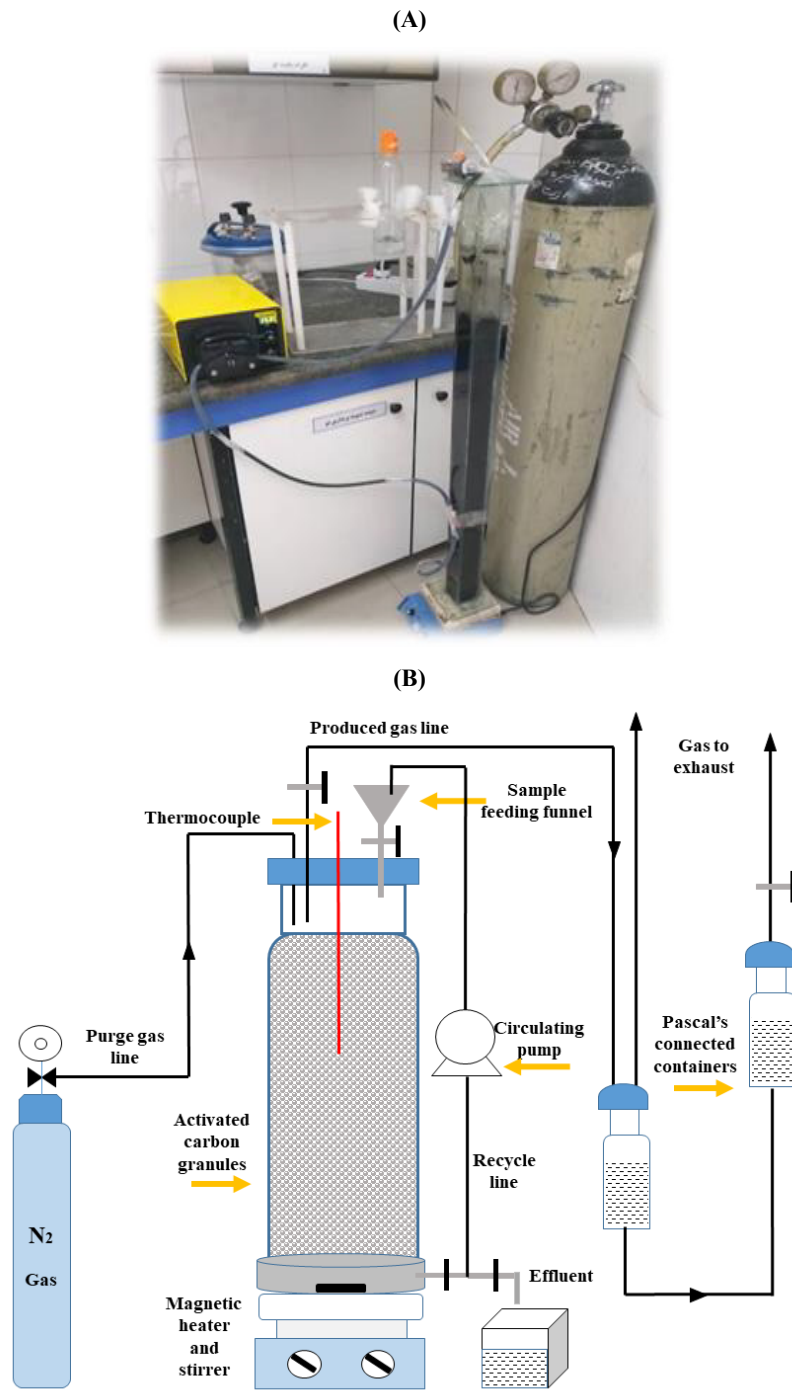


Fig 1. The a) setup and b) schematic of a down-flow fluidized bed bioreactor

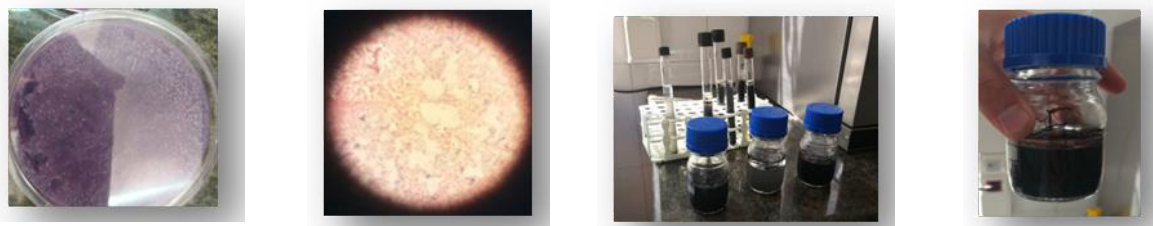


Fig 2. Stages of SRB culture in the laboratory

The efficiency of the bioreactor to remove metals and SO₄²⁻ was evaluated every 4 hours to 24 hours by the valve installed at the outlet of the reactor by sampling method. For this purpose, the reactor was set up with a lactate (COD)/SO₄²⁻ ratio of 0.67. According to the importance of the COD/ SO₄²⁻ ratio on the bioreactor's efficiency, this ratio was calculated every 4 h time step and is regulated if it was needed (by adding lactate). The same procedure was repeated two times and average values were reported as the final results.

2.5 Parameters' measurement equipment and procedure

The samples were taken out to measure the parameters of pH, alkalinity, VSS, TSS, COD, SO₄²⁻, and metals of the effluent samples. Turbidimetry and a UV-Visible spectrophotometer were used to evaluation of the system's influent and residual SO₄²⁻ levels. An atomic absorption spectrophotometer (AAS) was used to measure the concentration of the metals (Shimadzu UV-1601, 190 – 1100 nm wavelength and 50W halogen lamp and deuterium lamp light source). The gravimetric method was used for measuring total suspended solids (TSS) and volatile suspended solids (VSS). Measurement of carbonate and bicarbonate was performed by titration method, which requires the determination of carbonate and bicarbonate concentrations in terms of calcium carbonate to calculate alkalinity. The alkalinity of water is obtained due to the presence of one of the compounds of carbonates, bicarbonates, and hydroxides. Also, the pH was measured by a pH meter. To measure COD, 20 ml of a test sample and 10 ml of potassium dichromate solution were poured into the distillation flask along with the boiling stone. Then 30 ml of sulfuric acid containing silver was added to the solution. The solution was boiled with the help of a reflux device, then cooled and the volume of the solution inside the balloon was set to about 150 ml. Finally, the solution was titrated with ferro-ammonium sulfate solution in the presence of 3 drops of ferrobin detector. The indicator changed color from blue to red at the endpoint of the reaction. Simultaneously, a control sample containing 20 ml of distilled water was measured. The value of COD was determined using the following relationship:

$$COD = \frac{(a - b) \times f \times 200}{V}$$

Where a is the volume of ferro-ammonium sulfate used for the test sample, b represents the volume of ferro-ammonium sulfate used for the control sample, c is the titration factor of ferrous ammonium sulfate solution and V is the sample volume.

2.6 Stability of SRB

The stability of SRB in the reactor and its efficiency in the long term is one of the important parameters, especially in industrial applications. This issue was investigated by repeating the removal procedure six times with the same condition, same SRB, and fresh feed. The sulfate concentration was measured to evaluate and discuss this factor after each cycle.

3. Results

3.1 Removal of metals and SO₄²⁻

The DFFB anaerobic reactor was used for SO₄²⁻ and metals removal in 24 h. The effects of retention time and initial contaminant concentration were discussed according to the results shown in fig 4 (a-g) and table 2. As can be seen from the results, the overall trend of removal percentages indicated that the removal percentage increased with increasing of the initial contaminant concentration. Except for Cr (which has the lowest initial concentration) with 66.67% removal, other metals and sulfate reached above 70% removal after 24h indicating the acceptable performance of the method to simultaneous removal of seven various contaminants from real AMD. It is noteworthy that, some of the removed metals had very low concentrations which probably couldn't be eliminated with other methods like adsorption at least with low price and low time consumption (de Matos et al., 2018).

3.2 COD removal

The maximum and minimum average reduction and removal rates of COD were about 91.57% and 26.32% for retention times of 24 h and 4, respectively (Fig 4 (b)). The COD/ SO₄²⁻ ratio is a key parameter in regulating SO₄²⁻ reduction, resulting from the competition of SRB and methanogenic bacteria for monomeric compounds (lactate, acetate, amino acids, etc.). The COD/ SO₄²⁻ ratio also shows the electron flow rate during SO₄²⁻ and methane production reduction. Reduction of one mole of SO₄²⁻ generally requires 0.67 mol of COD producer or electron donor. A decrease in this ratio means that large amounts of SO₄²⁻ are available. Thus, the organic matter required for biomass is not available to reduce sulfate. So, it is necessary to add an external source of organic matter (preferably a carbon source) as the electron donor. Very high levels of this ratio mean that methane producers and SO₄²⁻ reducers compete for acetate (Henry and Prasad, 2000, Singh et al., 2011). To prevent this issue, this ratio was checked every 4h and by adding lactate (if needed) kept constant to have the best performance during the process.

Table 2. Initial and final concentrations and max removal percentages of metals and sulfate

Metal ions	Zn	Cr	Ni	Cd	Pb	Cu	SO ₄ ²⁻
Initial C (ppm)	1.7	0.24	0.83	0.41	0.28	20	3900
Final C (ppm)	0.4	0.08	0.18	0.12	0.08	0.25	53
Max removal (%)	76.47	66.67	78.31	70.73	71.42	98.75	98.64

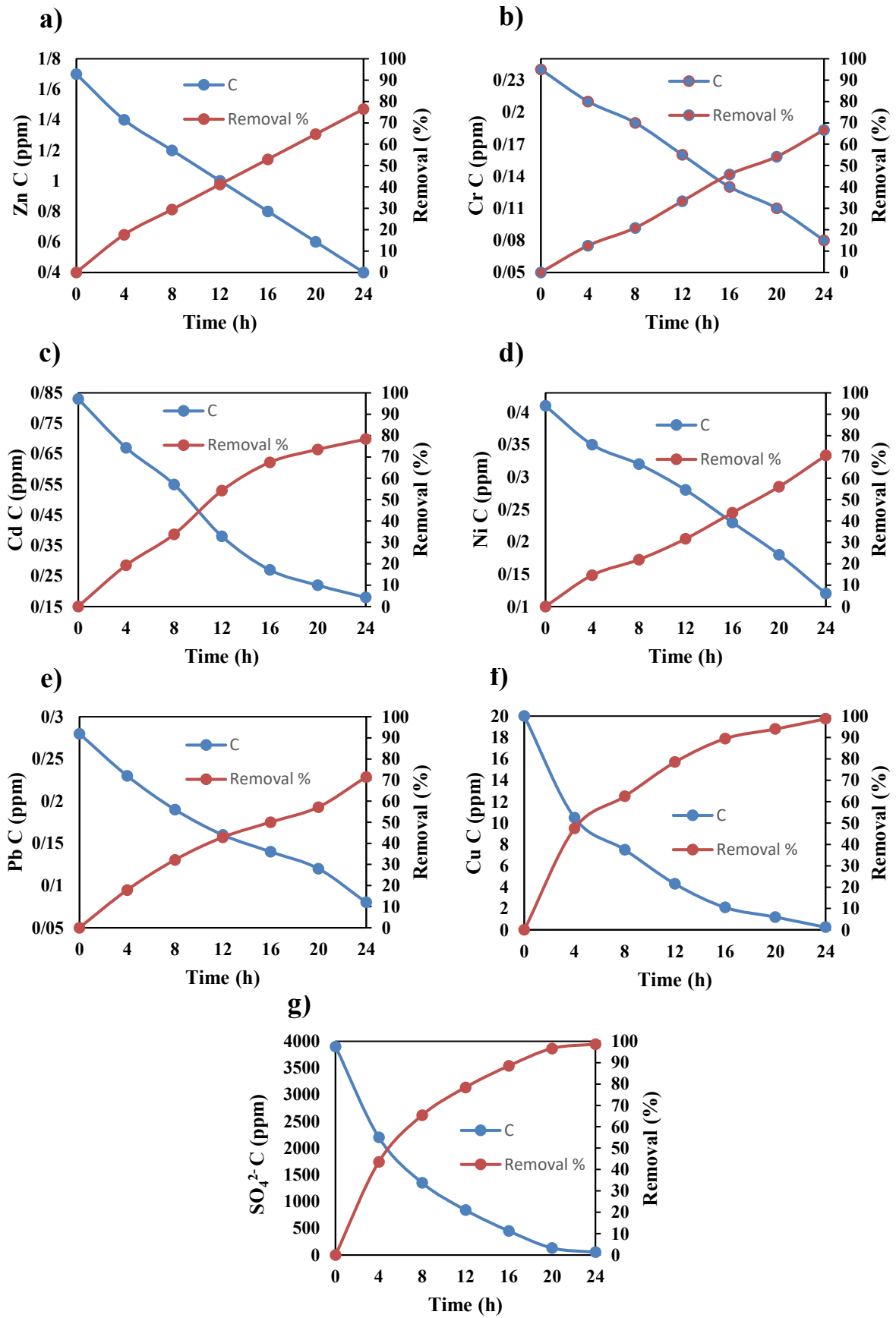


Fig 3. Influent concentrations and removal efficiencies of, a) Zn, b) Cr, c) Cd, d) Ni, e) Pb, f) Cu, and g) SO₄²⁻ at different retention times

3.2 Alkalinity changes

In removal by the DFFB anaerobic bioreactor, the alkalinity increased with increasing retention time (Luptakova and Kusnierova, 2005). Table 3 and Fig 5 (a) show the increased values of alkalinity in the treated effluent from the reactor. The alkalinity increased by 28.05% after 12 h and an increase of about 65% was observed in the retention time of 20 h and the maximum increase in alkalinity was more than 100% after 24 h.

3.4 Removal of TSS and VSS

TSS and VSS influents were about 480 and 70 mg/L, respectively. Table 3 represents the reductions of TSS and VSS with the influent concentrations mentioned for

different retention times. Fig 5 (c and d) show the removal rates of VSS and TSS, respectively. Accordingly, the max removal percentages for TSS and VSS are about 80.04% and 89.71%, respectively, in 24-h retention time, and 20.17% and 68.57%, respectively, for 4-h retention time. Although VSS was not added to the reactor, the influent VSS probably originated from the absorption of lactate by TSS. However, the low overall VSS in the effluent samples may indicate system adaptability. Some of the VSS in the effluent solution could be attributable to the presence of microbial masses, no significant bacterial death, and their non-separation from the attached growth medium beds (Torbaghan and Torghabeh, 2019).

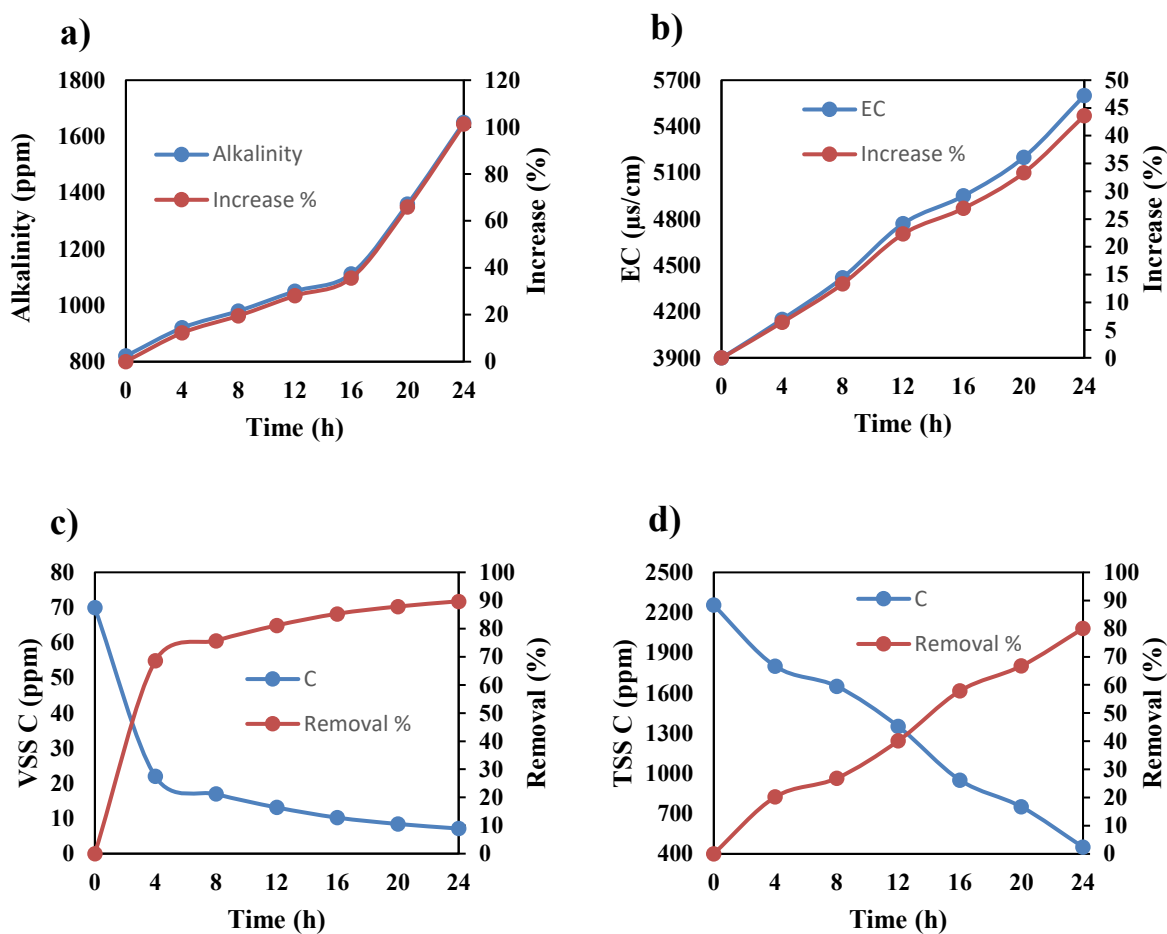


Fig 4. Influent concentrations and removal efficiencies of, a) alkalinity, b) COD, c) VSS, and d) TSS at different retention times

Table 3. Initial and final concentrations and max removal/increase percentages of different parameters

Parameter	Alkalinity	VSS	TSS	COD
Initial value (ppm)	820	70	2255	380
Final value (ppm)	1650	7.2	450	32
Max removal/ increase (%)	101.22	89.71	80.04	91.58

3.6 Stability of SRB

To investigate the stability of SRB in the reactor and the removal efficiency variation over time, six consecutive cycles were designed. As seen in fig 5, results showed that after each cycle the SO_4^{2-} removal efficiency (which represents the overall performance) reduced with an upward trend. However, acceptable results were obtained even after six cycles with a final SO_4^{2-} concentration of

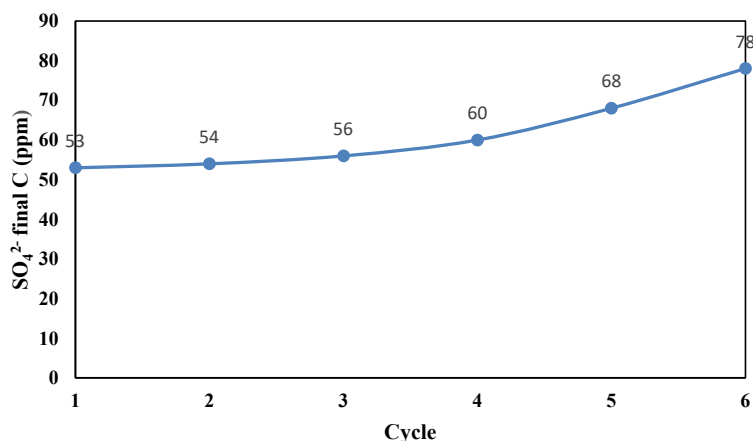


Fig 5. Stability of SRB in six consecutive cycles

4. Discussion

Metals removal curves showed an almost linear trend with time. However, it's not the same for Cu and SO_4^{2-} , which had the highest initial concentrations (20 and 3900 ppm) and also the highest removal percentages of 98.75% and 98.64% respectively. For these two cases (Cu and SO_4^{2-}) in the first 8h, the removal rate was much more than the other 16 h. This phenomenon is attributed to the high initial concentration and availability of contaminants at the beginning of the process. Also, at first 8 h, SRB has higher performance, and a rise in metal sulfide deposits has the potential to obstruct the reactor bed, which reduces the access of bacteria to the substrate, which eventuated in reducing SO_4^{2-} reducibility by these bacteria and consequently decrement of the system efficiency (Machemer and Wildeman, 1992, Jalali and Baldwin, 2000, Jong and Parry, 2003).

Overall, at the end of the 24 h sulfate concentration reaches almost 50 ppm, which is acceptable and after this point, the efficiency of the reaction between metal ions and sulfate reduces remarkably which will affect the performance of the process. So, 24 h was chosen as the optimum retention time for this process.

Due to the importance of alkalinity in the SO_4^{2-} removal process, an increase in this parameter promoted the removal efficiency, which is more evident at high concentrations. The elevated alkalinity indicates that alkalinity production capacity is a function of retention time in the SO_4^{2-} reduction steps by SRB. The generated alkalinity can balance the acidity of the system's input solutions hence

78 ppm. The main reason for the stability of SRB in the reactor is attributed to the presence of AC granules which prevent the loss of SRB. All in all, this method showed an acceptable performance to be used in real AMD treatment. A comparison of the present findings with other studies indicates that the use of biological methods is one of the most appropriate options for the control of Cu and SO_4^{2-} -containing effluents (Davarpanah et al., 2019).

it can be utilized to treat highly acidic effluents. Thereby, not only the was hazardous contaminant removed from the system, but also the acidity of the system was balanced and this prevents the issues that originate from acidic effluent leaching into the environment.

Due to the mutual effects of various parameters on the performance of the selected method, the accurate and complete comparison of the results of this work with other research is not possible. These parameters include the type of reactor, feed specifications, type of bacteria and additives, operating conditions, etc. However, the obtained results showed that the method chosen in this research showed an acceptable performance for removing sulfate and various metal ions that existed in the AMD.

5. Conclusion

In this study, the semi-pilot scale DFFB anaerobic bioreactor was used for the Sungun copper complex's AMD treatment for the first time. The real AMD includes seven various contaminants including six metals (Cu, Cr, Zn, Pb, Cd, and Ni) and SO_4^{2-} in different concentrations. The main obtained results are as follows:

- All of the contaminants except Cr (with 66.67%) reached over 70% removal percentage after 24 h. Also, results indicated that contaminants with higher initial concentration had higher removal percentages.
- The maximum removal belongs to the SO_4^{2-} and Cu with 98.64% and 98.75%, respectively, in a retention time of 24 h. Also, the removal rates of these contaminants were remarkably higher in the first

8 h rather than the other 16 h of the process, which can be attributed to the availability of sulfate at the beginning, the toxicity of SRB, and metal sulfides deposit during the process.

- The alkalinity of the effluent increased during the SO_4^{2-} reduction process; therefore, this system not only removed the hazardous contaminants but also regulated the acidity of flow.
- AC granules were utilized for the stabilization of the SRB in the reactor and as a result, even after six consecutive cycles, the system showed acceptable efficiency which is important parameter in industrial applications.

All in all, the biological method was an effective, cost-effective, and environmentally friendly approach for the treatment of AMD. This method also serves various benefits such as the removal of low concentrations and generation of denser sludge (with a high sedimentation ability), and high stability of metal deposits. So, it can be a potential candidate to use in large-scale industrial applications.

Conflict of Interest

The authors declare no conflicts of interest.

Additional Information And Declarations

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Competing Interests

The author declare there is no competing interests, regarding the publication of this manuscript.

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