

BIOLOGICAL REMOVAL OF COPPER AND SULFATE FROM THE DRAINAGE OF SUNGUN COPPER TAILINGS BY SULFATE-REDUCING BACTERIA IN A DOWN-FLOW FLUIDIZED BED ANAEROBIC REACTOR

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ABSTRACT

Copper (Cu) mine drainage contains large amounts of heavy metal and sulfate (SO₄) pollutants that can potentially pose serious risks to human and environmental health. Therefore, it is of particular importance to present practical solutions with minimum cost and maximum efficiency for pollutant removal. Anaerobic bioreactors are considered to be the most suitable methods for the treatment of acidic effluents containing heavy metals due to some advantages such as the possible recycling of precious metals, the need for a small area, easy control, and better process prediction. In this experimental laboratory-scale study, the performance of Sulfate-Reducing Bacteria (SRB) was investigated in a Down-Flow Fluidized Bed (DFFB) anaerobic reactor (2.3 L) for SO₄ and Cu removal from the drainage of Sungun copper tailings. To this end, 800 g of activated carbon granules with an average diameter of 0.63 mm, an apparent density of 0.58 g/cm³, and an effluent volume of 2.5 L were introduced into the reactor in a non-fluid form with a height of 35 cm and 6-600 rpm. To cultivate SRB, 100 g of swampy soil with sand and clay existing near the forest and river was added to 5 L of a liquid culture medium and incubated at 30°C for 7 days. In this experiment, calcium sulfate (1.2 g/L) and sodium lactate (8 mg/L) were used as the final electron acceptor and the energy source, respectively. The pH of the final solution was set to about 7.4 (suitable for SRB growth) using NaOH. The analyzed results indicated that the DFFB reactor was an efficient and suitable method for the treatment of effluents containing large amounts of SO₄ and Cu. The concentrations of SO₄ and Cu in the samples decreased by 99.12 and 99.05%, respectively, after 24 h of retention time.

INTRODUCTION

The increasing growth of industries and, consequently, the environmental pollution issue have attracted the attention of many researchers because improper management of wastewater production will increase the potential for the pollution and accumulation of heavy metals in the food chain of living organisms. Accordingly, the issue of Acid Mine Drainage (AMD) production is among the critical environmental issues of copper mines, which may adversely affect freshwater ecosystems according to the high concentrations of heavy metals and sulfate.

Sulfate is one of the most prevalent metal ions in the world. that is naturally present in various running water in the form of soluble and insoluble salts such as barite (BaSO₄), epsomite (MgSO₄·7H₂O), and gypsum (CaSO₄·2H₂O). In addition, copper is a vital element for organisms and its excessive levels inhibit cellular metabolism and damage the nervous system, thereby creating abnormal proteins in living organisms.

According to available studies, considerable research has been devoted to the treatment of acidic effluents with high levels of heavy metals from mine

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drainage, but SO_4 removal has been overlooked due to its average pollution levels. Different physicochemical and biological treatment technologies (e.g., ion exchange, reverse osmosis, and adsorption) are utilized for the removal of heavy metals and sulfates from mine drainage (Fig. 1).

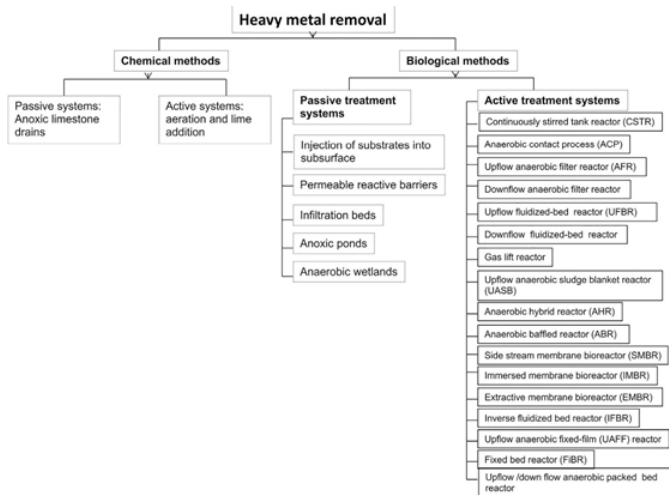


Fig. 1 An overview of different chemical and biological treatment systems for the treatment of wastewater containing heavy metals.

On the contrary of physicochemical processes, that consumes chemicals and energy, biological processes can be an economical and environmentally friendly method to remove SO_4 and heavy metals from effluents such as mine drainage (Sahinkaya, et al., 2017). In the anaerobic bioreactor method, SO_4 reduction happened by Sulfate-Reducing Bacteria (SRB), which is mediated by the metal's removal, in particular copper, through metal sulfide precipitation. Most of the metal sulfides that form because of sulfide reactions with metals are stable in anaerobic treatment systems. SRBs are anaerobic bacteria for which a pH of 5.7-8.7 is the best condition. Low-pH water reduces the efficiency and capacity of metal treatment. SRB has a major impact in the prevention of detrimental environmental effects. SRB and Sulfate-Oxidizing Bacteria (SOB) are two general categories for sulfur cycle bacteria. SRB is crucial for removing SO_4 and heavy metals, and SOB is crucial for decreasing sulfide. SRBs are obligate anaerobes and chemotrophic bacteria that utilize simple organic molecules as a carbon source. Heavy metals are removed by SRB through three steps. Firstly, SO_4 as the last electron acceptor is reduced by above-mentioned bacteria and converted to sulfide. Then, the sulfide resulting from SO_4 reduction reacts with heavy metals, forming a metal precipitate. At last, excess sulfide is finally oxidized by SOB or is converted to elemental S by manual addition of an oxidant. A down-flow fluidized bed reactor offers a singular benefit for the purification of precious metals because it is a 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 re-

actor and is separated from the biomass during the treatment of effluents containing metals and SO_4 in the DFFB. This provides the recovery of the metal in a single-use bioreactor (Figs. 2a-2h).

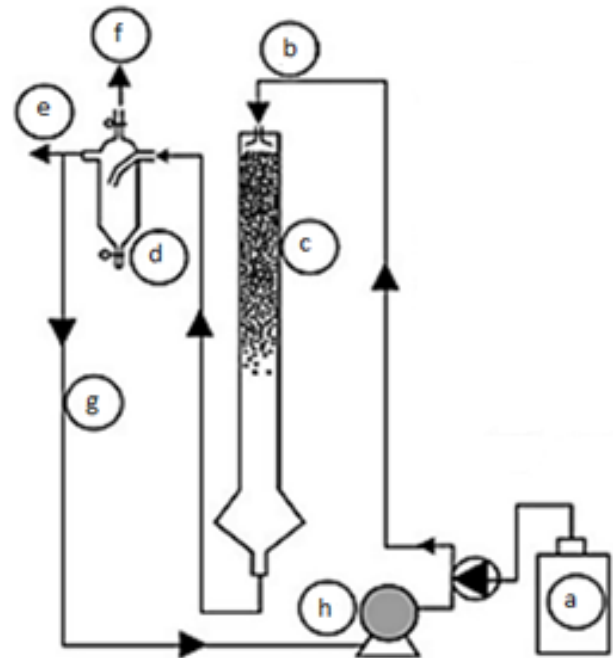


Fig. 2 Schematic of a down-flow fluidized bed reactor. **Note:** (a) Feed reservoir; (b) Influent; (c) Fluidized bed; (d) Liquid-solid-gas separator and water level adjuster; (e) Biogas outlet; (f) Effluent; (g) Recirculation flow; (h) Pump

The first research to examine the application of DFFB for metal purification was. Despite their study's metal removal, efficiency was quite high, the accumulation of acetate resulted in maximum COD and SO_4 removal rates of just 54% and 41%, respectively. In another study, high SO_4 reduction efficiency (up to 85%) and DOC reduction (approx. 90%) were reported in an Up-Flow Fluidized Bed Reactor (UFBR).

Numerous research conducted throughout the world identified the amounts of SO_4 and heavy metals in AMD investigated the simultaneous treatment of SO_4 and heavy metals using suicidogenic bioreactors. They introduced biological treatment systems to be a promising method for the treatment of heavy metal-contaminated effluents due to the precipitation of heavy metals by SRB and the advantages such as low maintenance and setup costs, very high efficiency in metal removal and recovery, even at very low concentrations, lower volume, and ease of operation.

Investigated the removal of SO_4 and heavy metals from AMD using SRB. They found that the reduction of SO_4 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. Complete removal of zinc by SRB lasted 24 hour, while it continued for 360 hour for native bacteria isolated from

the mine soil.

Investigated the SO_4 reduction in AMD in two up-flow reactors loaded by 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 by approximately 51 and 31 mg/L, respectively. In this study, copper precipitation was completed at a $\text{pH} < 2$ for 35 min (Venkatesan, et al., 2016).

A study on the biological removal of copper and SO_4 from artificial wastewater by SRB in an AFBR showed desirable SO_4 reduction and copper precipitation using this method. Moreover, the production of alkalinity during the process could tailor the system to facilitate the treatment of acidic effluents containing SO_4 and Cu.

The effluent of the Sungun copper mine-processing complex has destructive consequences for the surrounding environment as it contains heavy metal copper 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 city), environmental threats are much higher than other dams. Therefore, any leakage from the dam, even in little quantities will lead to serious environmental hazards due to the nature of the tailings stored in the dam reservoir. The mine, particularly the processing plant of this complex, obviously has negative environmental effects on the rivers and forests of Arasbaran, which are located adjacent to the mine and registered as the biosphere in the global lists. 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 cycle.

In this study, the biological removal method by SRB was used in a DFFB anaerobic reactor for the first time to remove and reduce pollutants (copper and sulfate) from the acidic drainage of Sungun copper mine tailings.

MATERIALS AND METHODS

Sungun Porphyry Copper Complex is located in East Azarbaijan province at the coordinates of $46^\circ 43' \text{ E}$ and $38^\circ 43' \text{ N}$, 130 km northwest of Tabriz (neighborhood of Azerbaijan and Armenia Republics) in the 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. As shown in Fig. 3, a downward DFFB was produced using 800 g of activated carbon granules as the carrier with an average diameter of 0.63 mm and a density of 0.58 g/cm^3 . To feed the system, an effluent volume of 2.3 L was introduced into the reactor in a non-fluid form, and the return flow was adjusted in such a way to maintain the bed height at about 100 cm in the fluid state. 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 .

Shows the performance and specifications of the DFFB reactor for SO_4 removal.



Fig. 3 The setup of a down-flow fluidized bed bioreactor on a laboratory scale.

Reducing bacteria

To the activation of the reactor, the sludge of the anaerobic digestive unit of the municipal wastewater treatment plant was used for SRB source. To culture SRB in Fig. 4, 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 g), 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 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.

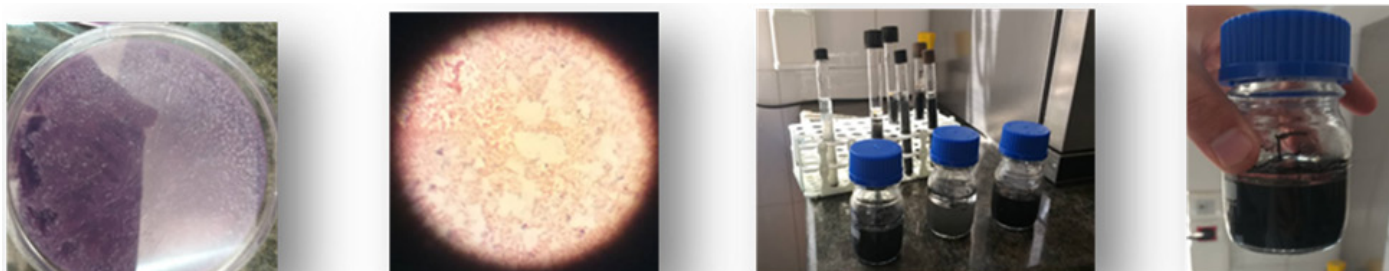


Fig. 4 Stages of SRB culture in the laboratory.

Laboratory conditions

All serum bottles used for activity measurement were fastened with rubber seals and aluminum bottles with a ratio of N₂/CO₂=20%/80% were washed for 3 min to provide lower air pressure. The activity of methanogens was measured in 60 mL serum bottles (including 25 mL of a base medium and 3 mL of carriers). Acetate was adjusted to 1 g/L of COD). Sulfidogens were measured in 120 L bottles containing 50 mL and 5 mL of carriers (1 g/L of COD propionate as the substrate and 50 mL of bromoethane sulfonic acid for the inhibition of methanogens).

Experimental procedure

The samples were taken out to perform experiments at 4°C by measuring the parameters of alkalinity, Volatile Suspended Solids (VSS), Total Suspended Solids (TSS), Chemical Oxygen Demand (COD), SO₄, and Cu of the effluent samples. The efficiency of the bioreactor for Cu and SO₄ removal was evaluated within 24 hour. For this purpose, the reactor was set up with a lactate-to-SO₄ ratio (COD/SO₄) of 0.67 and pH=7.5. The properties of the biofilm produced on the carriers were determined through the activity test (Neculita, et al., 2007; Johnson, et al., 2005).

Employing turbidimetry and a UV-Visible spectrophotometer, the system's influent and residual SO₄ levels were evaluated. An atomic absorption spectrometer was used to measure the amount of residual copper (Shimadzu UV-1601). TSS, VSS, alkalinity, Electrical Conductivity (EC), pH, and COD were determined using gravimetry, a furnace and gravimetry, EDTA titration (National Standard Organization of Iran), an electrical conductivity meter, a pH meter, and oxidation with potassium bichromate, respectively.

Data analysis

In this study, the Chi-squared (X²) statistical test and SPSS software were used for the experimental design and the statistical analysis of data. This test is a valid non-parametric statistical test that can be used to determine the systematic relationship between two variables.

RESULTS AND DISCUSSION

Removal of SO₄ and Cu

The DFFB anaerobic reactor was operated under SO₄ and

Cu removal conditions for 24 h. The sulfate injection rate was adjusted in such a way to reach an SO₄ concentration of 3900 ppm in the solution after the stabilization step. Results indicated that, depending on the various inputs of Cu and SO₄ more than 60% of Cu and SO₄ were removed during the 8-h retention time, with average SO₄ and Cu removal rates of 98.38% and 98.05%, respectively, in 24 h retention time, indicating a significant increase. Compares the average removal rates of both pollutants.

According to previous research, the optimal removal of copper may occur at a pH of about 6 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 copper removal from heavy metals.

As a result, it can be assumed that SRB has a significant impact on the precipitation of heavy metals as metal sulfide. Studies on the kinetics of chemical reactions indicate that significant copper removal may result from the extremely low solubility of copper sulfide precipitate. The strong affinity of sulfides for reactions with copper and other heavy metals may also be a factor. Cu and SO₄ removal rates dropped drastically for retention times of less than one day so that SO₄ and Cu were removed by 43.59% and 47.5%, respectively, for a retention time of 4 h, which is confirmed by the results of other studies.

In the present study, comparing copper removal percentages at various retention times showed that the copper removal rate was 4.75% at 20 h retention time, and about 9.25 and 20.25% for 16 and 12 h, respectively. For 8 h, it was 36.25% less than the removal rate at the 24 h retention time. In other words, the acceptable removal efficiency was obtained for removal times of half a day and even 8 h.

The SO₄ removal rates were 2.02% in the retention time of 20 h and about 10.02 and 10.20% for 16 and 12 h retention times, respectively. For 8 h, the removal rate was 33.26% less than that of 24 h, suggesting that the removal rate was acceptable for retention times of less than one day. It is noteworthy that a rise in metal sulfide deposits has the potential to obstruct the reactor bed, which reduces the access of bacteria to the substrate, thereby reducing SO₄ reducibility by these bacteria and consequently the system efficiency. According to Figs. 5A and 5B the removal rates of SO₄ and Cu are very different at low

retention times, but the removal rates of these two are almost the same at higher retention times probably due to a longer time for bacteria to reduce SO_4 at a higher retention time (Kaksonen, et al., 2007; Davarpanah, et al., 2019; Jalali, et al., 2000).

COD Removal

The maximum and minimum average reduction and removal rates of COD were about 88.95% and 26.32% for retention times of 24 h and 4, respectively (Fig. 5C). The COD/ SO_4 ratio is a key parameter in regulating SO_4 reduction, resulting from the competition of SRB and methanogenic bacteria for monomeric compounds (lactate, acetate, amino acids, etc.). The COD/ SO_4 ratio also shows the electron flow rate during SO_4 and methane production reduction. Reduction of one mole of SO_4 generally requires 0.67 mol of COD producer or electron donor. A decrease in this ratio means that large amounts of SO_4 are available. Thus, the organic matter required for biomass is not available to reduce sulfate, and thus it is necessary to add an external source of organic matter (preferably a carbon source) as the electron donor. In fact, very high levels of this ratio mean that methane producers and SO_4 reducers compete for acetate. The removal efficiency and COD of the effluent decreased with increasing the SO_4 concentration. This result can be attributed to the decreased activity of most anaerobic bacteria due to toxicity resulting from SO_4 reduction, which increases the organic load and causes important effects, such as reduced methane production, H_2S production (toxicity for methanogenic bacteria), and formation of H_2S (odor production), on anaerobic treatment.

Alkalinity changes

In removal by the DFFB anaerobic bioreactor, the amount of alkalinity increased with increasing retention time so that alkalinity increased by 28.05% after 12 h. An increase of about 50% was observed in the retention time of 20 h, and the maximum increase in alkalinity was 78.29% after 24 h. Increased values of alkalinity in the treated effluent from the reactor (Jong, et al., 2003).

Due to the importance of alkalinity in the SO_4 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 reduction steps by SRB, and an extremely low retention time reduces reactor capacity in alkalinity production. The generated alkalinity can balance the acidity of the system's input solutions hence it can be utilized to treat highly acidic effluents. Besides, the removal efficiency decreased with increasing the concentration compared to lower concentrations, resulting from the effect of SO_4 toxicity on these concentrations.

Removal of TSS and VSS

TSS and VSS influents were about 480 and 70 mg/L, respectively the average reductions of TSS and VSS with the influent concentrations mentioned for different re-

tention times. Accordingly, the removal rates for TSS and VSS are about 78.29 and 89.71%, respectively, in 24-h retention time, and 33.33 and 68.57%, respectively, for 4-h retention time. Figs. 5D-5G shows the removal rates of VSS and TSS, respectively.

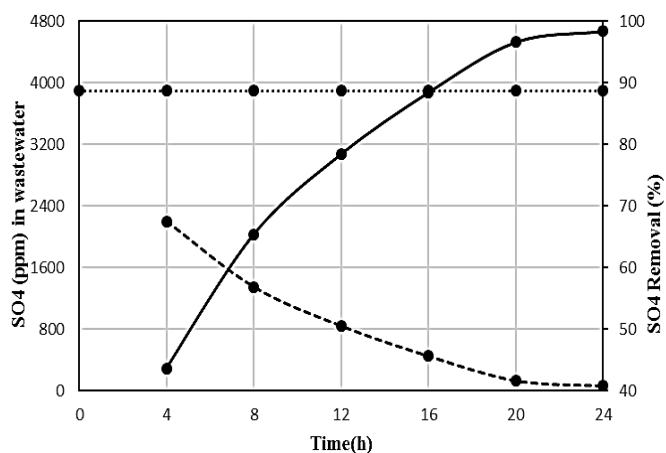


Fig. 5A Influent concentrations and removal efficiencies of SO_4 . **Note:** (.....●.....) Influent SO_4 ; (-●-) Effluent SO_4 ; (—●—) SO_4 Removal.

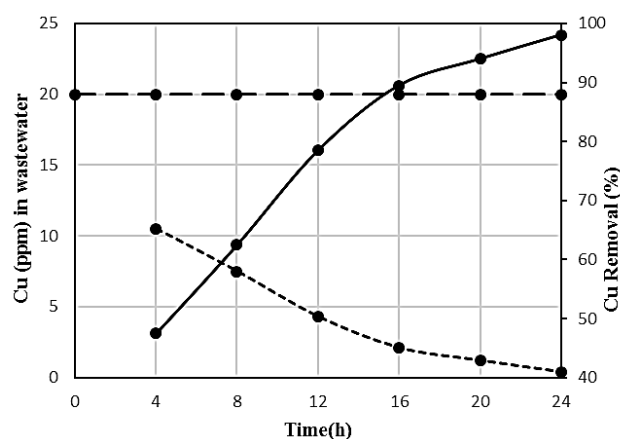


Fig. 5B Influent concentrations and removal efficiencies of Cu. **Note:** (.....●.....) Influent Cu; (-●-) Effluent Cu; (—●—) Cu Removal.

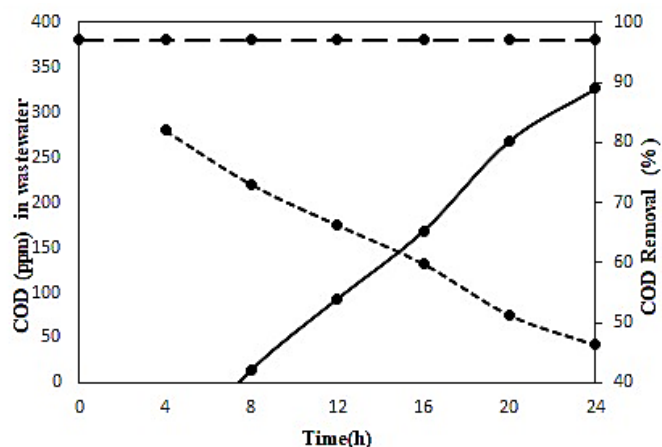


Fig. 5C Influent concentrations and removal efficiencies of elevated EC. **Note:** (.....●.....) Influent COD; (-●-) Effluent COD; (—●—) COD Removal.

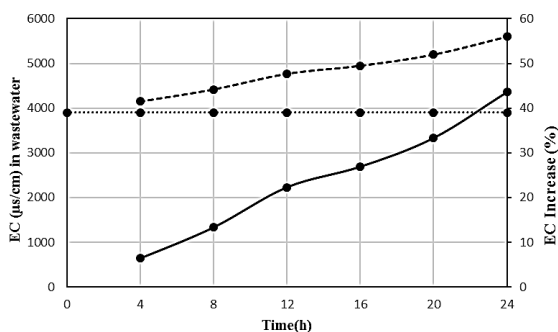


Fig. 5D Influent concentrations and removal efficiencies of TSS influent concentrations. **Note:** (—●—) Influent COD; (-●-) Effluent COD; (—●) COD Removal.

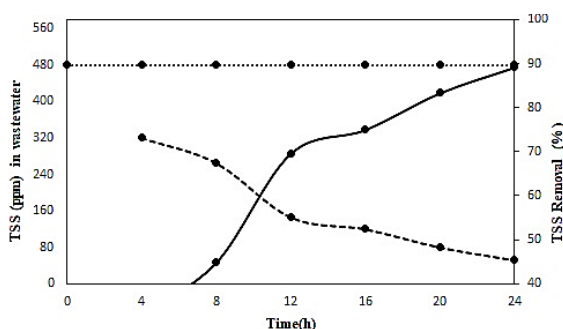


Fig. 5E Influent concentrations and removal efficiencies of Volatile Suspended Solids. **Note:** (—●—) Influent COD; (-●-) Effluent COD; (—●) COD Removal.

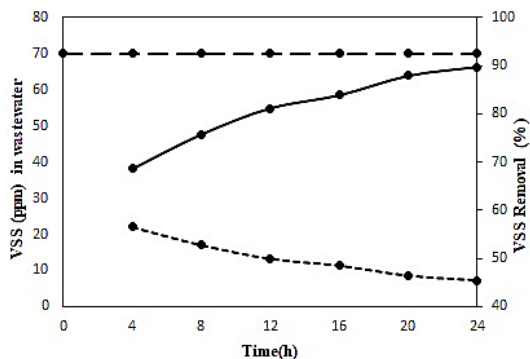


Fig. 5F Influent concentrations and removal efficiencies of increased alkalinity. **Note:** (—●—) Influent COD; (-●-) Effluent COD; (—●) COD Removal.

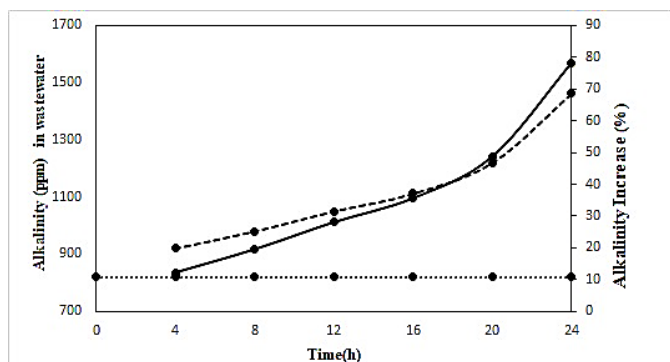


Fig. 5G Influent concentrations and removal efficiencies of at different retention times. **Note:** (—●—) Influent COD; (-●-) Effluent COD; (—●) COD Removal.

Although VSS was not added to the reactor, the influent VSS was probably sourced 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 (Machemer, et al., 1992; Kim, et al., 2017).

CONCLUSION

The present study was conducted on a laboratory scale in an anaerobic reactor to examine the role of SRB in the removal of Cu and SO_4 . The results revealed the good performance of SRB in the removal of Cu and SO_4 . The average removal rates of SO_4 and Cu were 98.38% and 98.05%, respectively, in a retention time of 24 h. A comparison of the present findings with those of other studies indicates that the use of biological methods is one of the most appropriate options for the control of Cu and SO_4 containing effluents.

Using lactate as a carbon source after a bacterial adaptation period (20 h), more Cu and SO_4 removal rates were observed at higher retention times than at lower ones. Some alkalinity was produced during the SO_4 reduction process; therefore, this system can be used for SO_4 and Cu containing effluents of acidic origin and is more cost-effective than other SO_4 removal methods. Additionally, biological methods (e.g., the present study) can be used in a definite range of heavy metals, and higher concentrations are toxic to bacteria. To avoid this, adaptation must be applied for a long time, which is a limitation of this method.

ACKNOWLEDGMENTS

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DECLARATION OF COMPETING INTEREST

The authors declare no conflicts of interest.

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