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TECHNICAL ARTICLE

Mine Wastewater Treatment with Upflow Anaerobic Fixed Film Reactors

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Abstract

Microbial bioremediation of metals in wastewater by sulfate-reducing bacteria (SRB) has received much attention due to its high efficiency, eco-friendly techniques, and low cost. We investigated upflow anaerobic fixed film reactors (UAFFR) for removal of metals from artificial mine wastewater in the presence of two different carbon sources (lactate and ethanol), a range of temperatures, and pH. The UAFFR had different remediation reactors: R1 (inoculated directly with the sludge), R2 (inoculated with SRB-enriched culture), R3 (inoculated with SRB-enriched culture in combination with a pure culture of SRB species, i.e. *Desulfotomaculum ruminis*, to treat all of the metals together), and R4 (inoculated with an SRB-enriched culture in combination with the *Desulfotomaculum ruminis*, to treat individual metals). Of the four seeded reactors, those supplemented with SRB-enriched cultures (R2, R3, R4) removed the metals more efficiently than R1. The reactors using ethanol as the carbon source and polyhedral hollow balls removed sulfate and metals more efficiently than those with lactate and small pall rings.

Keywords Bioremediation · SRB (sulfate-reducing bacteria) · Toxic metals · Carbon source, Ethanol

Introduction

Contaminants such as lead (Pb), cadmium (Cd), cobalt (Co), copper (Cu), mercury (Hg), arsenic (As), and chromium (Cr) are discharged into the environment as industrial and mining wastes, causing serious soil and water pollution. Many of these accumulate in organisms and can cause health issues (Mishra et al. 2013). This is a serious problem for ecosystems and public health in the affected areas (Kuzmanović et al. 2016; Mohmand et al. 2015; Qing et al. 2015).

Dissolved metals and other potentially toxic elements are removed from wastewater by physical andchemical methods, such as filtration, activated carbon adsorption, ion exchange, precipitation, ultrafiltration, electrodialysis, and reverse osmosis (Erdem et al. 2004). However, these methods have disadvantages such as unpredictable metal ion removal and production of toxic sludge that can be difficult to dewater and dispose of. These disadvantages become more prominent at low metal concentrations in contaminated groundwater, mine tailings effluents, and other industrial wastewaters. Therefore, innovative treatment technologies are needed to remove metals from wastewater.

There has been growing interest in remediation technologies based on biological immobilization of metal ions

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through microbial reduction and precipitation (Gadd 2004). Sulfate-reducing bacteria (SRB) reduce and precipitate metals as metal sulfides, which form stable precipitates due to their low solubility. The SRB reduce sulfates to sulfides and also produce bicarbonates. The sulfides reacts with metals cations to form stable metal sulfides that are immobilized in the bioreactor while bicarbonates acts as buffers (Blowes et al. 2000; Johnson and Hallberg 2005a; Logan et al. 2005). SRB have been used to remove metals from wastewater, acid mine drainage, and contaminated groundwater plumes in in-situ permeable reactive barriers (Benner et al. 1999; Janssen and Temminghoff 2004; Wang et al. 2000; Waybrant et al. 2002), as well as ex-situ in bioreactors (Dvorak et al. 1992; Elliott et al. 1998) or constructed/natural wetlands (Moreau et al. 2013).

For decontamination of high sulfate- and metal-containing waters, passive treatment systems are preferred due to their low cost, low required maintenance, and minimal hazardous waste production (Berghorn & Hunzeker 2001; Waybrant et al. 2002). Sulfate-reducing bioreactors provide an attractive passive treatment approach because they are typically inexpensive to construct and require a minimal input of resources once in operation (Benner et al. 1999; Johnson and Hallberg 2005b).

The use of SRB in an up-flow anaerobic fixed film reactor (UAFFR) showed great promising results for the removal of metals from industrial wastes (Kiran et al. 2017a). This easy, comparatively inexpensive, and efficient method can be used by maintaining optimal environmental conditions for SRB growth. It is important that SRB be the dominant bacteria in the reactor to achieve high efficiencies.

While lactate is the preferred carbon source for SRB growth in industrial wastewater treatment (Bharati and Kumar 2012), ethanol may be a more efficient carbon donor for metal removal. The aim of the study was to design and use UAFFR with optimized features, i.e. surface area, feed, SRB species, etc., to treat artificial mine wastewater containing high levels of sulfates and metals with increased efficiency and at low cost.

Material and Methods

This research study was divided into two different parts: (1) with lactate and ethanol as carbon sources, using SRB consortium from sludge; and (2) in combination with a single SRB species. In the first phase, lactate was used as the substrate in all of the reactors under different operating parameters, i.e. hydraulic retention time, temperature, and pH. In the second phase, ethanol was used as the carbon source and the removal of sulfates and metals were monitored under different operating conditions. In the third and fourth phases, both the above steps were repeated while the reactors were further inoculated with SRB species *Desulfotomaculum ruminis* in addition to the SRB already there. It was observed that whenever any change was made in the feed or mode of operation, a minimum of five days were required to achieve steady state conditions. The system was operated for about four to six weeks to collect sufficient data under steady state conditions for each run. For reproducibility, all the experimentation and operations were repeated.

The bioreactors were designed using AutoCAD 2012 (supplemental Fig. S–1). The UAFFR were constructed from Plexiglas®, a transparent material. This helped with the observation of SRB growth and its distribution along the reactors' height. Each reactor was filled with polypropylene pall rings and polyhedral balls with a porosity of 80% (supplemental Fig. S–2). The reactors were kept airtight using rubber stoppers and screws to control and close all the openings. The UAFFR outer layer had a water jacket connected to a controlled temperature water bath. Two pumps were used to provide flow into the fixed film reactors.

Artificial Mine Wastewater, Chemicals, and Water

Artificial mine wastewater was synthesized based on the mine water reported by Din et al. (2015) and used in the reactors. The water contents were kept the same except for the metal and sulfate concentrations, which were gradually increased in steps to optimize the bacterial acclimation and prevent any shock from a sudden increase in metal and hydrogen sulfide production. The water was supplemented with $CaSO_4{\cdot}2H_2O$ (1.27 g/L), $MgSO_4{\cdot}7H_2O$ (2.0 g/L), NH_4Cl (1.0 g/L), $K_2HPO_4{\cdot}3H_2O$ (0.5 g/L), Na_2SO_4 (5.26 g/L), and sodium lactate (3.5 ml) (70% solution) or ethanol to aid the growth of bacteria. Ethanol quantity was calculated per the recommendations given by Bomberg et al. (2017).

Only analytical grade chemicals were used to prepare the artificial wastewater. All the components except for the $FeSO_4{\cdot}7H_2O$, were dissolved in distilled/deionized water, diluted to the required volume, and mixed thoroughly. The water was sparged with 80% N_2 + 20% CO_2 for 10–15 min. Then the $FeSO_4{\cdot}7H_2O$ was added and mixed thoroughly and continuously sparged with 80% N_2 + 20% CO_2. Finally, the pH was adjusted to the required levels (Asadi et al. 2008).

Inoculum Preparation

Sludge samples were collected from two wastewater treatment plants: the Shougang sewage treatment plant (treating wastewater from metals processing industries) and the Qinghe wastewater treatment plant (Beijing Drainage Group, treating sewage wastewater). Sludge bacteria were cultured anaerobically twice in Postgate's medium to obtain SRB-enriched cultures (Atlas 2005), and the

SRB community structure was identified later by polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE). These cultures were then used for further inoculation of the bioreactors. Out of the four reactors, the first reactor was inoculated directly with the sludge (300 mL). The second one was inoculated with SRB-enriched cultures (300 mL), and the third one was inoculated with SRB-enriched cultures in combination with 100 mL of a pure culture of a SRB species, *Desulfotomaculum ruminis*, which was provided by the Institute of Microbiology, Chinese Academy of Sciences (CAS), Beijing, China. The fourth reactor was first inoculated with SRB-enriched cultures and then with *Desulfotomaculum ruminis*, to treat individual metals. After inoculation, the systems were operated in batch mode for two weeks to promote biofilm growth. The artificial wastewater was subsequently fed to the reactors.

Operational Parameters

There are several operational parameters that affect the performance of biological anaerobic fixed film reactors seeded with SRB. These include hydraulic retention time (HRT), contact surface area, pH, temperature, and oxidation–reduction potential (ORP). The bacterial growth depends on temperature, pH, redox potential, and carbon substrate availability, whereas HRT is known to greatly influence the performance of SRBs (Vasquez et al. 2016). It has been shown that an increase in flow/influent velocity deaccelerates metal removal (Lappan 1987) while an increase in HRT prolongs wastewater treatment time and increases the cost. Thus, the designed HRT should maintain a good reactor performance and be economically feasible.

Based on the literature, the most suitable HRT for optimum performance of the UAFFR is in the range of 10 to 30 h. Wang et al. (2005) and Chen and Neibling (2014) concluded that a retention time of about 11 h was suitable to maintain the desired recommended bacterial growth and metal removal, especially when SRB was fed with lactate as the organic carbon source, while six hours of residence time was insufficient for significant sulfate reduction (Bayoumy 1997; Bi et al. 2019; Chen and Neibling 2014; Kemp 2004; Lappan 1987; Maree and Strydom 1985). In this study, HRTs of 5, 10, 15, 20, 30, and 40 h were used (Singh et al. 2011; Slattery 2019; Wang et al. 2005). In addition, packing materials of polypropylene polyhedral hollow balls and pall rings were used to increase the surface area, to retain more biomass while maintaining sufficient contact between the biomass and the wastewater. The reactors were operated at a temperature range of 20 to 35 °C and a pH range of 5.0 to 8.0, consistent with Postgate (1984).

Bioreactor Operation

Before operation, all four reactors, the support media, and the tubing were sterilized. The substrate was pumped from the glass feed container to the inlet of the reactor by means of variable speed peristaltic pumps. These pumps were calibrated at the beginning of the experiment and checked regularly. The N_2 gas was continuously bubbled through the feed bottle to purge the dissolved oxygen as well as mix the substrate. The reactors' temperature was kept between 20 and 35 °C with the help of circulating water in the reactor's water jackets. The HRT of the reactors was adjusted by the flow rate of the pumps. The effluent flowing at the outlet near the top of each reactor was collected in separate vessels. The gas produced in the reactors by the SRB (mainly H_2S) and coming out of the exhaust at the top of the reactors was passed through a zinc acetate solution to absorb the H_2S gas and to reduce the foul smell of the exhaust. The reactors were operated for six months and were fed with the artificial wastewater.

Sampling and Chemical Analyses

Samples were collected from the effluent's collection vessels 2–3 days after achieving the steady state conditions. For sulfate analysis, sample were collected in polyethylene bottles and stored at 4 °C.. For metal analysis, samples were filtered with 0.45 µm pore size membrane filters, acidified with 50% HNO_3 to a pH < 2.0, and then stored at 4 °C. Samples from the reactors were similarly analyzed for sulfate and metals contents.

The samples were prepared and diluted as 100%, 50%, 25%, 10% and 5% and analyzed for nine metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin Elmer Optima 7000 DV, USA). Sulfate was measured by the turbidimetric method while sulfides were measured iodometrically (Eaton et al. 2005).

Wastewater samples were analyzed in triplicates. Samples reproducibility was found at 90 ± 5% confidence level and mean values were used for further results interpretation. For the ICP-OES calibration and data confirmation, a blank was used with two standards after every 5 samples. The metal standards were prepared from stock Fluka Kamica (Buchs, Switzerland) solutions of 1000 mg/L with deionized water.

Data Analysis

Statistical manipulation of the data was carried out using computer software like MS Excel (Office 2010) and Sigma plot 12.5 for the arithmetic means, standard deviation, ranges, and data presentation.

Results and Discussion

The UAFFRs were studied for removal of metals in two ways: (1) with a substrate containing all of the nine metals and (2) a substrate containing one metal at a time. The removal efficiency was greater for a single metal than metals in a combined state (Tables S–1 and S–2). The reactors were more efficient in metals removal, even at low HRT (five hours). The quantitative removal efficiency for metals in combined state was in the order of Fe ≥ Cu > Zn > Cd ≥ Co > Cr > Ni > Mn > Pb (Fig. 1). The removal rate for Co was the same as that of Cd.

Figure. 1 will be placed near here during the final printing process.

Given the diversity of wastewater, the UAFFR experiments were performed with varied parameters. The reactors were seeded with sludge cultures and SRB-enriched cultures, carbon sources, supporting media, temperature, and pH, to increase the probability and efficiency of metal and sulfate removal from the mine wastewater. Results revealed that the reactors seeded with SRB-enriched cultures (R2, R3, R4) treated the artificial mine wastewater more efficiently than the reactor seeded with only sludge (R1), as shown in Fig. 2. The reactor inoculated with only sludge would have had a consortium of bacteria in addition to SRB, which could have inhibited or reduced the SRB activity. The reactors with SRB-enriched cultures were likely more efficient due to a higher proportion of SRB in the consortium. This is consistent with the findings of Kieu et al. (2011). Sulfate removal efficacy was also evaluated for SRB-enriched cultures in isolation and in combination with *D. ruminis*, and this showed higher efficiencies for SRB-enriched cultures in the combined state (Fig. 3).

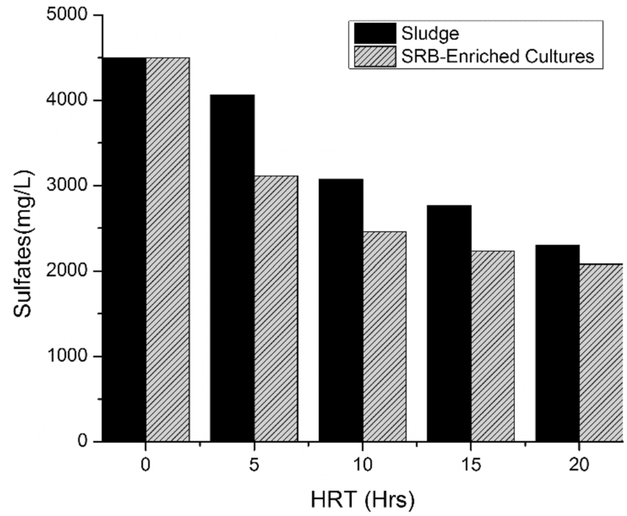

Fig. 2 Comparative sulfates reduction by Sludge bacteria and SRB-enriched cultures in UAFFR

The reactors were studied both with lactate and ethanol as carbon sources (Fig. 4a and b). The SRB in the reactors demonstrated greater sulfate and metal removal efficiency for ethanol than with lactate, in contrast with the observations of Bharati and Kumar (2012). Ethanol is preferred as a carbon source/electron donor for SRB for reasons such as ease of availability and low cost. Moreover, ethanol is more effective in stimulating sulfide production than lactate (White and Gadd 1996). With an M/S^{2-} ratio < 1, sulfate reduction produces enough sulfides for efficient precipitation of metals, but the SRB growth rate is decreased (Kiran et al. 2017b; Villa-Gomez et al. 2015). Bacterial growth is inhibited in the beginning with

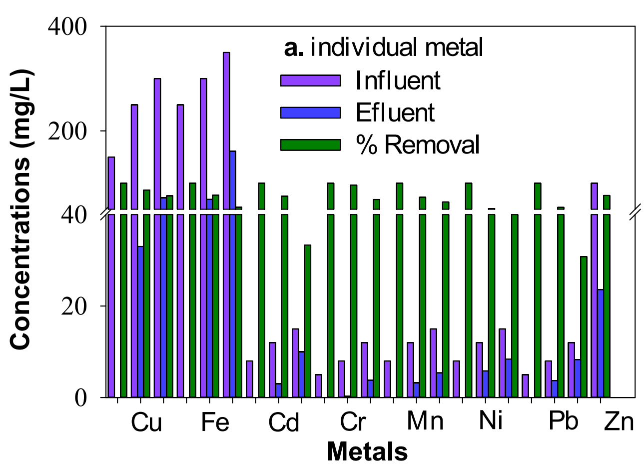

Fig. 1 Treatment of Mining Wastewater for metals in UAFFR using ethanol as carbon source

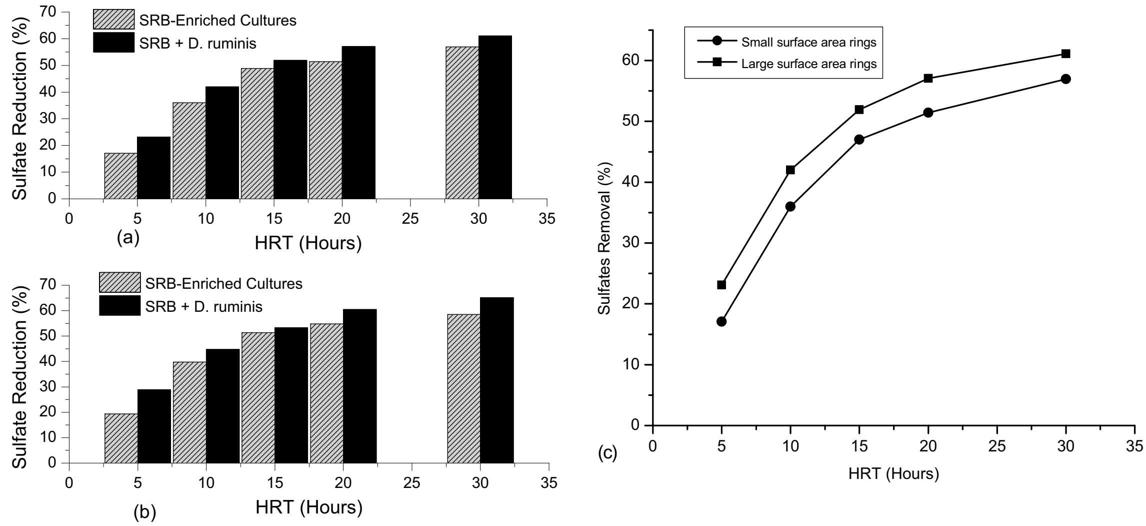

Fig. 3 Comparative sulfates reduction by SRB-enriched cultures and in combination with D. ruminis in UAFFR **a** substrate with eight metals, **b** substrate with a single metal **c** Reactors containing polyhedral hollow balls and pall rings

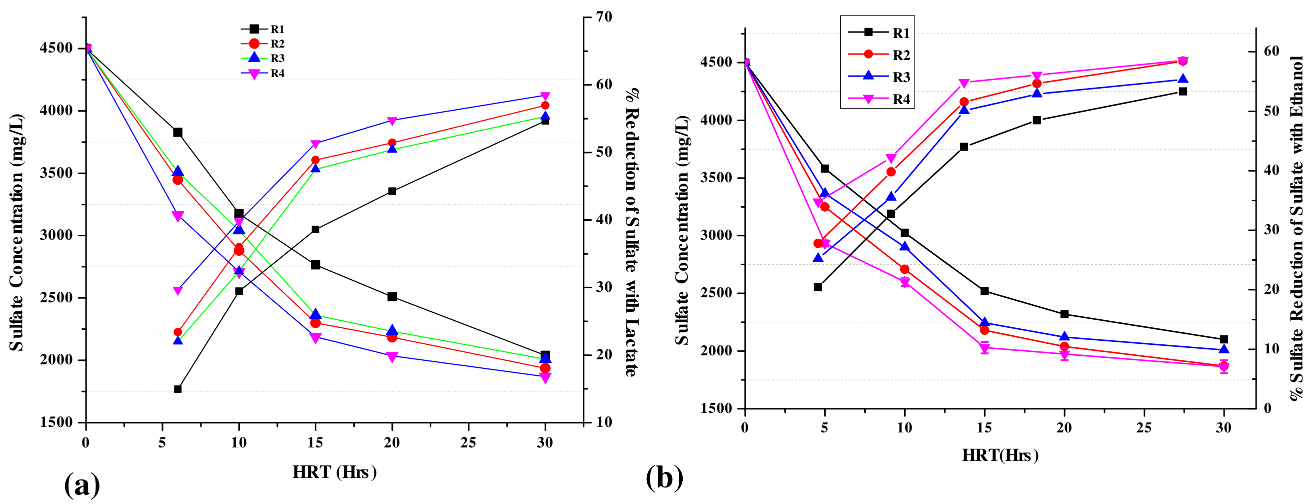

Fig. 4 Sulfate reductions by SRB in UAFFR **a** Lactate as carbon source **b** Ethanol as carbon source

ethanol as compared to lactate (Kousi et al. 2018), but then increases when the metals and sulfate are removed through precipitation (Kaksonen et al. 2004). Ethanol feed was gradually increased to avoid shocking the already established SRB biofilms. Studies have shown that H_2S toxicity is dependent on the bacterial species present because hydrogen-utilizing SRB have greater tolerance for high H_2S concentrations than other SRB groups (Icgen and Harrison 2006; Kaksonen et al. 2004; Oyekola et al. 2009). As revealed by the PCR-DGGE investigation, the SRB community in the reactors mainly consisted of *Desulfovibrio, Desulfomicrobium*, and *Desulfotomaculum* species, which produce H_2S more efficiently in the presence of ethanol than in lactate (Postgate 1984).

The reactor's sulfate removal efficiency was also evaluated for two types of support media, (1) plastic polyhedral hollow balls and (2) pall rings, for bacterial growth and biofilm formation. The reactors containing the polyhedral balls showed greater sulfate removal efficiency than the small pall rings (Fig. 3c). The polyhedral hollow balls provided greater surface area for biofilm formation and hence more contact area for the bacteria in the liquid media.

The UAFFRs were also studied for pH and temperature changes. From pH 6.0 to 8.0, the removal efficiency was normal (Fig. 5) but beyond these limits, the sulfate and metal removal rates decreased. Fluctuations in pH seriously affect SRB activity, which in turn greatly affects sulfate consumption and the production of acetate and H_2S. Therefore, pH

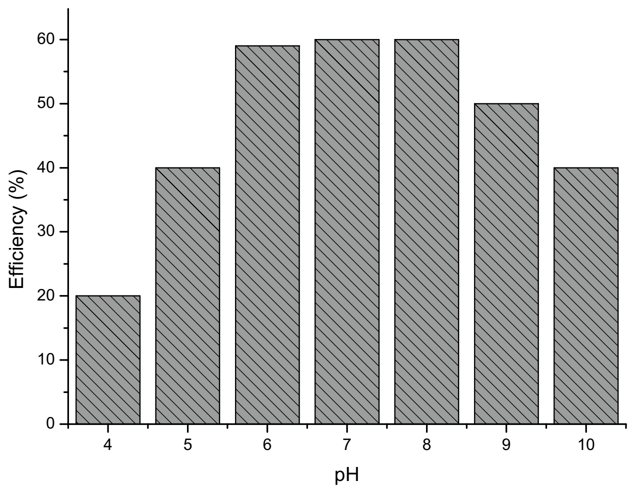

Fig. 5 Reactors overall efficiency at different pH level of the feed

control greatly affects metal removal (Xu and Chen 2020). At a pH < 4, the SRB were inactive and sulfate reduction apparently stopped. The SRB might need much more time to adapt to the low pH environment. The results of low or no SRB activity at low pH were consistent with those reported by Kieu et al. (2011). The reactors were evaluated for temperatures from 20 to 40 °C and showed greater efficiency at 35 °C. Higher SRB efficiency at this temperature agrees with the results reported by Sawicka et al. (2012). Studies have shown that the microbial growth rate is closely dependent on temperature (Hao et al. 2016; Jung et al. 2019; Okabe et al. 1992; Zhang et al. 2018). Low temperature results in decreased SRB activity as well as low removal efficiencies for metals and sulfate (Nielsen et al. 2018). The rates of protonation and deprotonation of functional groups, together with the activity of SRB, increases at higher temperatures (Bajpai et al. 2004). High temperatures can reduce the solubility of toxic H_2S in wastewater and help SRB to outcompete other bacteria in wastewater treatment (Kaksonen and Puhakka 2007).

Conclusion

The SRB-enriched cultures removed sulfate and metals more efficiently than a mixture of SRBs and other types of bacteria. Combining the removal of many contaminants slowed down their removal compared to removing them individually. Although the SRB showed better growth with lactate, the H_2S production was greater with ethanol. Furthermore, the reactors containing polyhedral hollow balls showed greater sulfate removal efficiency than the small pall rings. The polyhedral hollow balls provided greater surface area for biofilm formation and hence greater contact of bacteria with sulfates and organic carbon in the liquid media. This UAFFRs setup can be used as a green, low-cost, and in-situ solution for mine wastewater treatment.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10230-023-00929-3.

Data availability The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

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