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Development of Heavy Metals Bioaccumulation on Anaerobic Support System with Sulfate Reducing Bacteria Media

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ABSTRACT

Heavy metals in wastewater come from processes related to heavy metals as raw materials and contaminants. Heavy metals pose a significant threat. Bioaugmentation technique that utilizes communities of microorganisms to bioaccumulation heavy metals from wastewater. However, the application of SRB in anaerobic system installations for wastewater treatment needs to continue to be developed with more practical applications. In this study, the enriched SRB colony source was applied to an anaerobic tank. The grown SRB is used to extract heavy metals from wastewater with the addition of sulfate and supporting nutrients. Throughout the treatment process, the anaerobic system with SRB consistently maintained a sulfate removal efficiency of 87–88%, indicating continued sulfate consumption activity by the SRB colony. Despite the high initial concentration of heavy metals, the system effectively removed > 91% of Pb, Cd, Zn, and Cr on days 15, 30, and 45. Additionally, the system reduced the Cu content by 43.6%, thereby reaching peak metal removal heavy. the level was 85% on day 30 and decreased slightly to 83% on day 45. This study bridges the gap in understanding the application of SRB in wastewater treatment systems with effective performance.

Keywords: anaerobic system with SRB, bioaccumulation, heavy metals wastewater.

INTRODUCTION

In the field of environmental science and wastewater management, the treatment of heavy metals wastewater presents a significant challenge due to its hazardous nature. Contaminants such as heavy metals, organic and inorganic solvents, and sulphate compounds are often found in industrial effluents, posing serious threats to both human health and the environment. As one of the most dangerous substances, heavy metal is highly soluble in aquatic settings, readily absorbed by living things, and difficult to break down by traditional biological treatment (Kinuthia et al., 2020). For these reasons, the presence of heavy metal in laboratory waste is a serious issue in many laboratories. Substances classified as heavy metals have an atomic weight between 63.5 and 200.6 and a specific gravity greater than 5 (Soliman, Moustafa, 2020). Lead (Pb) and copper (Cu) are two of the most prevalent heavy metals found in effluent from chemical industry and laboratories (Amanze et al., 2022; Mosivand et al., 2019).

While it was uncommon to detect cadmium (Cd) and chromium (Cr) in the wastewater, however, the presence of these heavy metals even in a small amount may provide hazardous risk to the environment and human health. As a non-essential heavy metal, due to its high toxicity, Cd(II) and Cr(VI) has been classified as a class I carcinogen (Kapoor et al., 2022; Loomis et al., 2018; Singh et al., 2022). The order of toxicity for certain heavy metals is as follows: cobalt (Co) < aluminum (Al) < chromium (Cr) < lead (Pb) < nickel (Ni) < zinc (Zn) < copper (Cu) < cadmium (Cd) < mercury (Hg) (Mansourri, Madani, 2016). To mitigate the detrimental impact of heavy metals found in common industry wastewater, it is imperative to implement effective wastewater treatment methods specifically tailored for chemical wastewater settings. These methods should be user-friendly and capable of substantially reducing heavy metal concentrations. Various techniques, including chemical precipitation, chemical stabilization, ion-exchange, chemical coagulation, membrane filtration, adsorption, chemical oxidation, and electrochemical treatment, have been explored by scientists for water treatment (Naushad et al., 2015; Obaid et al., 2018; Xu et al., 2013). Each of these techniques offers certain advantages and drawbacks, underscoring the importance of careful consideration in selecting the most appropriate method for addressing heavy metal contamination in laboratory wastewater. In the end, a number of factors, including as the starting concentration of heavy metals, the composition of the wastewater, capital investment and operating expenses, plant flexibility, reliability, and environmental impact, will determine the most efficient approach for treating wastewater (Mosivand et al., 2019).

Addressing this issue, recent research efforts have been directed towards innovative and sustainable treatment technologies to treat heavy metals-containing laboratory wastewater. One such promising approach involves the utilization of anaerobic bioreactors bioaugmented by sulphate reducing bacteria (SRB). SRB communities able to reduce sulphate to sulfide which was an efficient precipitant to remove heavy metals in the wastewater by forming metal sulfide (Magowo et al., 2020). The majority of SRB are classified into 23 genera within the Deltaproteobacteria group, with gram-positive SRB found predominantly in the Clostridia class (Ayala-Parra et al., 2016; Cruz Viggi et al., 2010). SRB communities can be readily isolated from muddy soil in oxygen-depleted settings, such as sediments within contaminated aquatic environments where the presence of hydrogen sulfide gas imparts a distinctive odor.

Several previous studies have successfully demonstrated the potential heavy metals removal by SRB communities and the metabolic pathway of SRB in treating heavy metals (Ayangbenro et al., 2018; Guo et al., 2022; Neria-González, Aguilar-López, 2021; Wu et al., 2022; Xu and Chen, 2020). Santini et al., demonstrated 96% Pb removal after 50 days of treatment process by SRB communities incubated in the temperature range of 18–30 °C with straw and ethanol as carbon source. Another research required longer HRT of 500 days to attain 98% of Cu removal and 70.59% of Fe removal in treating acid mining water using SRB communities (Pinto et al., 2018). Study from Li et al. reported the removal efficiencies of Cu, Zn, Pb and Cd reached 76.3%, 95.6%, 100% and 91.2%, respectively, after treatment using SRB communities and sodium lactate as nutrient (Li et al., 2017). Despite there have been a number of research exploring the capabilities of SRB communities in treating heavy metals, however, the application to the pilot scale wastewater treatment plant was still limited. Hence the present study was aimed to assess the performance of SRB communities, that was obtained from local environment, in the pilot scale wastewater treatment plant treating heavy-metals containing laboratory wastewater.

MATERIALS AND METHODS

Enrichment and cultivation of sulphate-reducing bacteria

Sediment samples were randomly taken from five sampling points of a river in Batubulan village, Gianyar Regency, Bali Province, Indonesia. As much as 50 grams of black sediment in each sampling point was obtained and stored in cooling box immediately after sampling. Enrichment of SRB was performed using simplified Postgate B liquid media as described in our previous study (Suyasa et al., 2022). In the current study, the enrichment media was composed of 3.5 g/L sodium lactate, 2.0 g/L magnesium sulphate, 0.2 g/L ammonium chloride, 0.5 g/L potassium dihydro phosphate, 0.5 g/L ferrous sulphate and 0.1 g/L ascorbic acid. Sulfuric acid was added to ensure the acid condition of the media (pH 4.0) before sterilization process. The enrichment media was sterilized at 121 °C for 15 minutes and then kept at 37 °C for 5 minutes before stored in refrigerator.

A total of 200 grams of black sediment was put into a 1 L Erlenmeyer flask mixed with enrichment media, then subsequently sealed, and incubated at room temperature for 10 days. Following that, 250 ml of the suspension from the Erlenmeyer flask was taken for the second phase of culture by adding it to a fresh 500 ml Erlenmeyer flask that had been filled with enrichment media and incubated at room temperature. In order to maintain the quality of SRB cultures, this procedure was repeated every three weeks. The production of a black precipitate (ferrous iron), which emerged a few days after inoculation, served as a defining feature of the SRB growth phase (Seong et al., 2007). Thereafter, Postgate B medium was used to develop the SRB inoculum cultures in 1 L transparent glass vials, where they were then incubated at 30 °C for 24 hours without light. In the absence of the SRB communities, tubes that did not display a blackish hue change were taken into consideration. The population density of SRB was estimated using the 3 series tube MPN technique in order to conduct a microbial evaluation.

Experimental setup

The anaerobic system with SRB was built to treat heavy metals wastewater of Laboratory service of Faculty of Math and Natural Sciences, Udayana University. The anaerobic system with SRB was designed with a series of treatment units consisting of equalization tank, anaerobic reactor, constructed wetland, and effluent tank (Figure 1). The anaerobic reactor is composed of a cylindrical tank, made of fiberglass, with total volume of 800 L in the form of an airtight column to preserve anaerobic environment within the reactor that supports the growth of SRB. The reactor was also equipped with the inflow and outflow monitoring system, drainage system, and the section to inoculate the SRB colonies.

Before discharged into the anaerobic system, as much as 10 L of laboratory wastewater was sampled using jerry cans and underwent water quality examinations to analyze the initial concentration of biochemical oxygen demand (BOD), chemical oxygen demand (COD), Total Suspended Solids (TSS), total dissolved solids (TDS), sulphate ion, and several heavy metals parameter such as Pb, Cd, Zn, Cu and Cr. Prior to the treatment process, 1 L of SRB cultures was firstly introduced to the anaerobic reactor combined with fermented compost as co-digestion and enrichment media (15% from the total volume of the reactor) and then incubated for 72 hours. After 72 hours of acclimatization, laboratory wastewater was discharged to the anaerobic tank with total retention time of 15 days. Water quality parameters such as BOD, COD, TSS, TDS, pH, sulphate ion, and heavy metals were analyzed every 15 days until day 45. The removal efficiency of the anaerobic system unit, developed by the current study, was expected to be around 50–80%.

RESULTS AND DISCUSSION

SRB active suspension

SRB active suspension was produced during activation in a mixture of soil/water sediments and postgate B solution for 15 days. The growth medium consisted of 65% Postgate B solution, 30% fermented compost liquid, and 5% active suspension liquid. The change in color into a

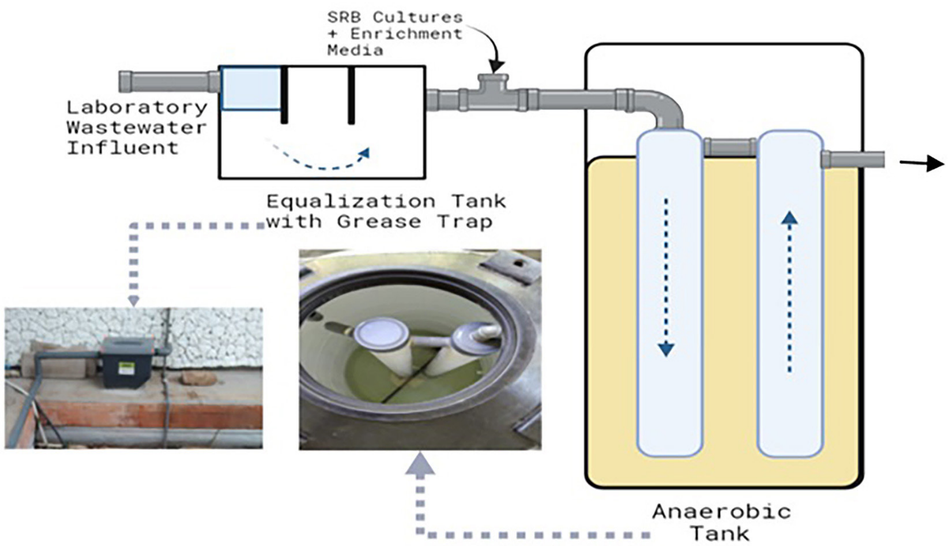


Figure 1. Schematic diagram of the aerobic tank

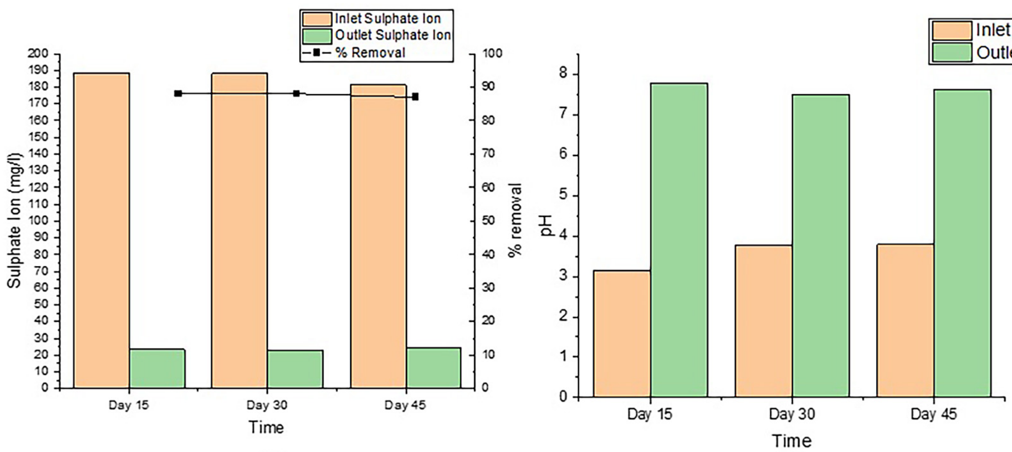
dark black suspension during activation process indicates SRB increasing activity in the solution. SRB uses hydrogen and sulphate as electron acceptors to degrade organic compounds and volatile fatty acids (VFA), ultimately producing sulphides gas and bicarbonate ions (Sudiartha and Imai, 2022; Wang et al., 2022). To ascertain the population density of SRB cells in the SRB solution, MPN 3 series tube analysis was conducted. According to the findings of the MPN analysis, there were around 1.1 × 10^5 CFU/ml of SRB cells in total. The number of cells was determined to be adequate for the wastewater treatment operation since the minimum threshold was 10^4 CFU/ml (Seong et al., 2007).

Removal of sulphate concentration and its effect on pH

During the treatment process, the anaerobic system with SRB strongly supported high sulphate-reducing behavior, demonstrated by the eager decrease in sulphate concentrations in the effluent. As seen in Figure 2a, the sulphate concentration decreases from the level of 188.81 mg /l to 23.41 mg/L on day 15, achieving an 88% sulphate removal efficiency. This removal efficiency indicated that about 88% of sulphate was consumed by SRB colonies and reduced to sulfide ions. The anaerobic system with SRB maintained its sulphate removal performance around 87–88% area during the 45-days treatment process, signifying a sustainable and consistent sulphate-consuming activity by SRB colonies. Along with the decrease in sulphate concentration during the treatment day, the pH levels showed a significant increase in the anaerobic system with SRB effluent. Sulphate concentration was the main contributor on acid condition in the influent wastewater as it forms a strong acidic solution when bonds with hydrogen ions (H^+) in the water. Hence, the removal of sulphate results in a more buffer condition (pH around 7) in the wastewater. As depicted in Figure 2b, on day 15, the pH level surged from acid condition, pH 3.15, to buffer condition of pH 7.79 after 88% of sulphate ions were removed. There was a slight fluctuation in the pH levels on day 30 and 45 associated with a modest decrease in influent sulphate ion concentrations, yet the pH levels remained in the buffer zone area.

Anaerobic system with SRB performance in removing solids and organic matters

Solids and organic matters are two important water quality parameters as these parameters regulate the growth of SRB and may affect the heavy metals removal efficiency (Kushkevych et al., 2019). As seen in Figure 3a, the influent TSS concentration fluctuated around the range 27–38 mg/L with the peak concentration on day 30. However, despite fluctuation in the influent TSS concentration, the anaerobic system with SRB managed to remove up to 88% suspended solids and maintain the effluent concentration of TSS in the range of 4.4–4.5 mg/L. In contrast to TSS removal, the anaerobic system with SRB depicted a lower treatment performance in removing the TDS concentrations (Figure 3b). With the influent TDS concentration ranging around 710–803 mg/L, the anaerobic system with SRB was



(a) (b)

Figure 2. Sulphate ion removal (a) and pH changes (b) during wastewater treatment process in the pilot scale wastewater treatment plant

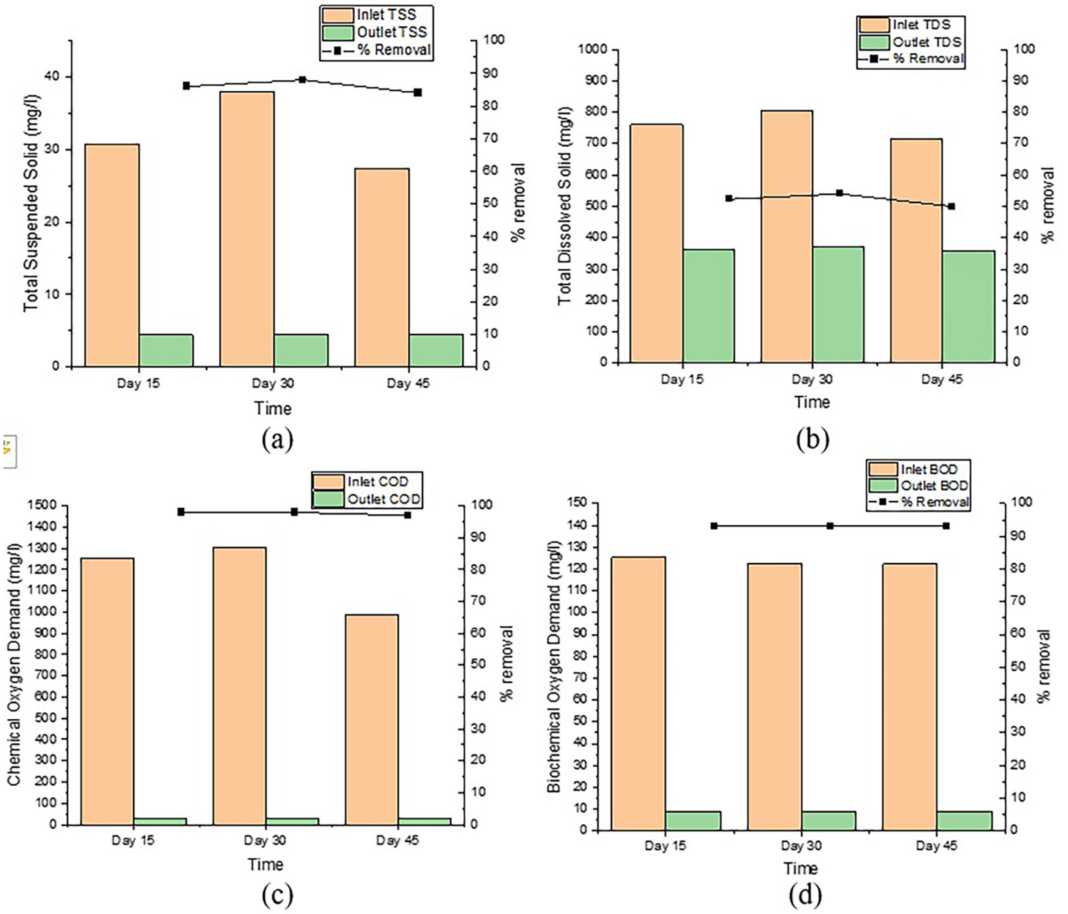


Figure 3. Influent and effluent concentration of TSS (a) TDS, (b) COD, (c) BOD, (d) during the treatment of the laboratory wastewater

only capable of removing a maximum of 54% of TDS concentration. The high TDS concentration in the present study was mainly attributed to the high concentration of sulphate in the laboratory wastewater, while the other substances were potentially sodium, potassium, calcium, magnesium, chloride, bicarbonates and heavy metals in dissolved forms (Chen et al., 2021).

In spite of low TDS treatment efficiency, the anaerobic system with SRB demonstrated a remarkable potential in removing organic matters by achieving average removal efficiency of 98% in COD removal and 93% in BOD removal, as seen in Figure 3c and 3d respectively. In the present study, it was observed that the concentration of BOD in the laboratory wastewater was much lower than COD confirmed by the rocks bottom BOD/COD ratio around 0.093–0.123. This ratio also suggested that there was more non-biodegradable organic matter contained in the wastewater than digestible organic matter. The common level of BOD/COD ratio in order to achieve optimum biochemical degradation was 0.5–0.8 depends on the microbial communities involved in the treatment process (Dhall et al., 2012; Kumar et al., 2010; Oladipo et al., 2017). With the low BOD/COD ratio yet remarkable organic matters removal efficiency in the current research, indicates that the SRB communities managed to survive and perform organic degradation even with lack of biodegradable substrates.

Heavy metals removal

To determine the anaerobic system with SRB capabilities in removing heavy metals concentrations in the laboratory wastewater, the aqueous phase of both influent and effluent were taken and analyzed for the heavy metal content. During the study, Zn was detected as the most frequent metals found in the laboratory wastewater with the highest concentrations compared to the other metals content. As seen in Figure 4a–d concentration of Pb, Cd, Zn and Cr were dramatically decreased to the level below 0.235 mg/L which correspond to > 91% removal of the initial concentrations in the influent on day 15, 30 and 45. Cd, Zn, and Cr were heavy metals with the highest concentration detected in the influent laboratory wastewater which fluctuated around 0.014–0.235 mg/L (Cd), 0.036–0.800 mg/L (Zn), and 0.020–0.143 mg/L (Cr). Despite the high concentrations, the anaerobic system with SRB managed to exhibit a total removal for those heavy metals resulting in 0 mg/L of Cd, Zn and Cr contents in the effluent.

In contrast, the removal of Cu content in the wastewater showed a slower rate than the other heavy metals removal (Figure 4e). For instance, on day 15, the anaerobic system with SRB was only capable of eliminating 43.6% of Cu content and advanced to a maximum removal rate of 85% on day 30 yet declined to 83% on day 45. The elimination of metal was ascribed to the precipitation of insoluble metal sulfides as a result of the sulfides created by the biological activities of SRB. The deposition of Cu increased significantly following to the removal of sulphate ion into sulfides by SRB communities thus forming CuS that has the lowest solubility in the water compared to the other heavy metals; log K_s equivalent to -40.94 (Jong and Parry, 2003). However, in the present study, as the maximum Cu removal rate was 85% signified that there was potentially inadequate amount of sulfides were produced during the sulphate ions conversion, consequently lower CuS deposits were formed.

COD to sulphate ratio and its effect on heavy metal concentrations

The removal efficiency of heavy metals by SRB may be correlated with the amounts of the organic substrate as well as the type of organic substrate. Hence, it is important to analyze the ratio between available substrates (represented by COD) and the concentration of sulphate in the wastewater. According to previous research, differences in the ratio of organic substrate to sulphate (COD/sulphate) have a significant impact on how effectively heavy metals are removed by SRB cultures (Liu et al., 2018; Najib et al., 2017; Xu and Chen, 2020). The COD/sulphate ratio affects the removal of heavy metals and sulphate by inflicting the competition between SRB and other microorganisms, due to shared carbon sources. SRB craves similar substances as the other microorganisms to grow, such as H_2, acetate, propionate and butyrate (McCartney and Oleszkiewicz, 1993). Several previous studies have been highlighting the immense competition between SRB and other microorganisms, especially methanogen, in craving available substrates (Chen et al., 2019; Shi et al., 2020; Sudiartha et al., 2022; Sudiartha and Imai, 2022). Theoretically, all the electrons will shift to sulphate when the ratio of COD/sulphate is below 0.67, once the ratio exceeds over 0.67, there will be more competition between SRB and other microorganisms for the

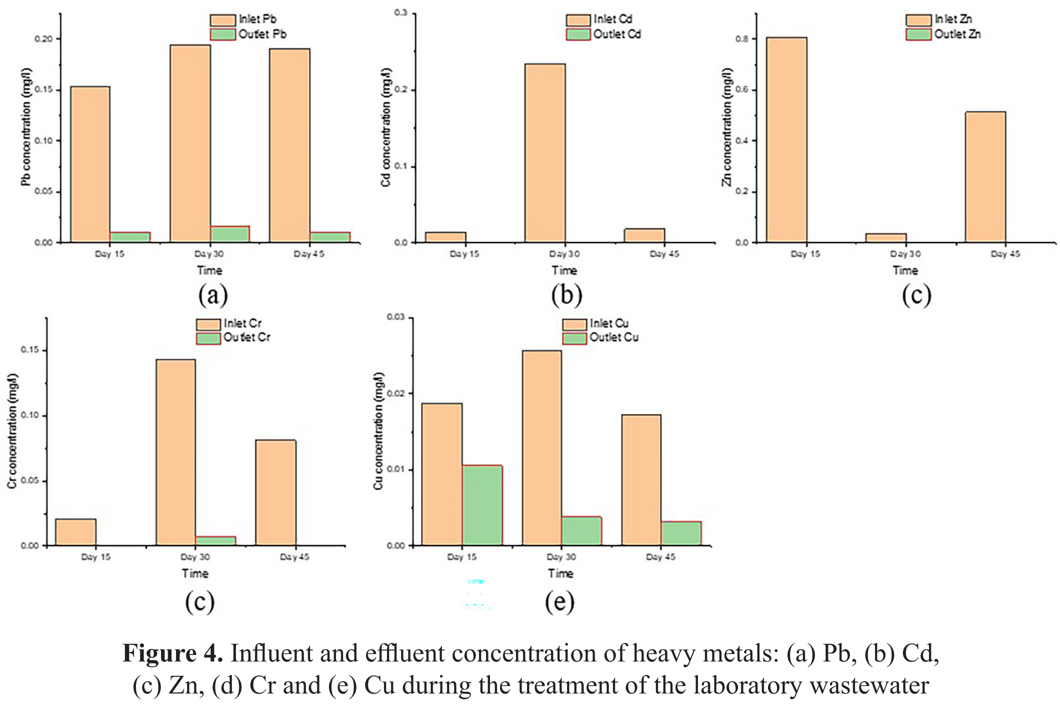


Figure 4. Influent and effluent concentration of heavy metals: (a) Pb, (b) Cd, (c) Zn, (d) Cr and (e) Cu during the treatment of the laboratory wastewater

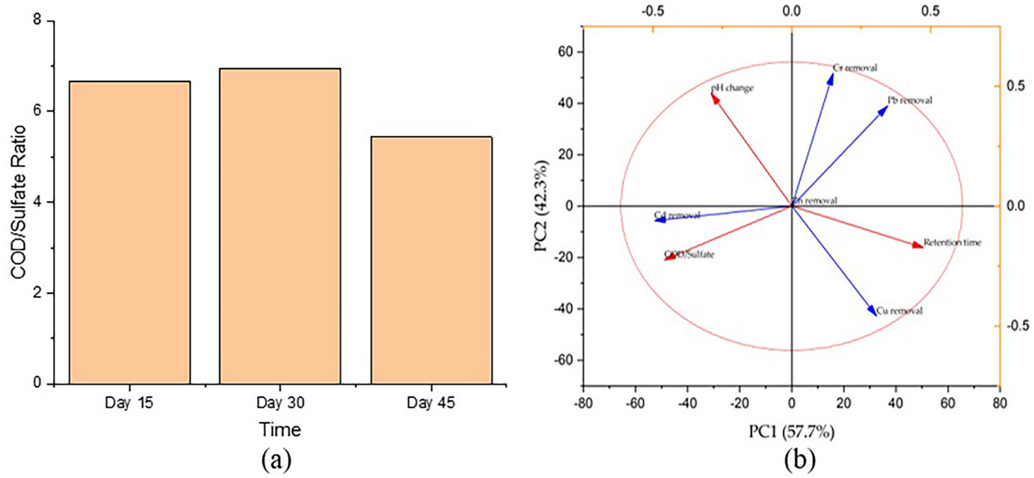


Figure 5. Temporal variation of COD/sulphate ratio (a) and PCA plot of heavy metals removal vs. environmental parameters (b)

shared electron donors (Dar et al., 2008; Xu and Chen, 2020). In the current research, the COD/sulphate ratio were recorded at 6.67, 6.95, and 5.44 on day 15, 30, and 45 in the influent channel respectively (Figure 5a). Despite the ratio exceeded the standard COD/sulphate ratio due to high COD concentrations, the competition between microorganisms in craving available substrates was less likely to be occurred due to the low concentrations of biodegradable organic matters (depicted by low BOD/COD ratio). As the main SRB competitor, methanogens are more susceptible towards inhibition due to low biodegradable substrates, while several researches showed the capacity to withstand the food scarcity conditions for SRB communities as it also gains energy from dissimilatory sulphate reduction (Kibangou et al., 2022; Tripathi et al., 2021; Wu et al., 2018). Increased COD/sulphate ratios also enhance the growth of acetogenic bacteria, which are less sensitive to high H_2S that may lead to greater chemical sulphate reduction and metal precipitation, likely in the form of metal sulfides (Icgen and Harrison, 2006; Suyasa et al., 2023).

Previous studies discovered that the higher COD/sulphate ratio accelerates sulphate removal efficiency as well as the removal efficiency of heavy metals by SRB cultures (Piña-Salazar et al., 2011; Ren et al., 2007; Xu and Chen, 2020). In the current research, we found no significant correlation between the COD/sulphate ratio to heavy metal deposition efficiency. As can be seen in Figure 5b, the COD/sulphate ratio shows similar direction and length to Cd removal parameters while also exhibits an inverse direction to Cr and Pb removal. This finding further supports previous discovery that found higher COD/Sulphate increases the abundance SRB communities that enhances sulphate reduction, while Cd precipitates faster during the sulphate reduction process compared to the other heavy metals (Virpiranta et al., 2022). This study marks a stride in comprehending the significance of SRB utilization in pilot scale wastewater treatment plant treating laboratory wastewater that contained heavy metals. However, due to limited resources available, metagenomic analysis of SRB and its correlation to heavy metals removal in the present study could not be performed. In the future, comprehensive metagenomics analysis and functional metabolic analysis may be essential to profoundly study the mechanisms of sulphate reducing process and heavy metals removal by the SRB strain. Furthermore, the impact of the other environmental factors such as COD, TSS, sulphate, COD/sulphate to microbial communities can also be applied to future research in this field or the other research field that also works to explore the dynamic transitions in a microbial population under several circumstances.

CONCLUSIONS

In conclusion, this study demonstrated the successful production of SRB active suspension capable of robust sulphate reduction in a mixture of soil/water sediments and postgate B solution. The growth medium, consisting of 65% postgate B solution, 30% fermented compost liquid, and 5% active suspension liquid, supported a substantial increase in SRB activity, evident from the change in color to a dark black suspension. The population density analysis indicated a significant presence of SRB cells, meeting the required threshold for efficient wastewater treatment. During the treatment process, the pilot scale anaerobic system with SRB exhibited strong sulphate-reducing behavior, achieving an 88% sulphate removal efficiency over 45 days. This reduction in sulphate concentration resulted in a shift from acidic to buffered pH levels in the effluent. Despite fluctuations in influent total suspended solids (TSS) concentration, the anaerobic system with SRB consistently removed up to 88% of suspended solids, maintaining low effluent TSS concentrations. Although the removal efficiency for total dissolved solids (TDS) was lower, the anaerobic system with SRB demonstrated remarkable organic matter removal efficiency, achieving 98% COD removal and 93% BOD removal. In heavy metal removal, the anaerobic system with SRB effectively reduced Pb, Cd, Zn, and Cr concentrations to below 0.235 mg/L, indicating over 91% removal efficiency for these metals. While this research provides valuable insights into SRB-mediated wastewater treatment, limitations in resources prevented comprehensive metagenomic analysis. Future studies should explore these microbial dynamics further, utilizing advanced analytical techniques to unravel the underlying mechanisms and optimize wastewater treatment processes under various environmental conditions.

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