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Short Communication

Bioprecipitation of Toxic Metal Ions by Highly Metal-Resistant Dissimilatory Sulfate-Reducing Bacteria using Pressmud Hydrolysate as Growth Substrate

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ABSTRACT

Environmental impact of metallic ions is well studied thus necessitates their removal from anthropogenically impacted regions. Chemical neutralization is frequently employed but has drawbacks such producing secondary pollutants and being expensive. Exploitation of metals-resistant sulfate-reducing bacteria in biological waste management offers benefits such as decreased secondary pollution, lower costs, and improved performance. The current study is focused on using pressmud hydrolysate as source of energy and carbon for sulfate-reducing bacteria to precipitate toxic metal ions from effluent. Results have shown that the bioprecipitation of Cd was minimal (37% at 5 ppm and no precipitation at 20 ppm), while Cu showed maximum bioprecipitation at lower concentrations (91% at 5 ppm) but reduced effectiveness at higher concentrations (13% at 20 ppm). Cr had the least negative impact on SRB and could efficiently precipitate higher concentrations of this metal (99% at 5 ppm and 17% at 20 ppm). This study also discussed the influence of growth environment including temperature and pH, on the remediation potential of the microbes. Optimal temperature of 30°C and pH of 7.0 ± 0.5 were found to be favorable for effective metal ion removal. The outcomes of present study will contribute for designing the effective bioremedial systems to be used for the mitigation of metal pollution.

The ongoing invasion of various metals in the surroundings is primarily attributed to human activities, particularly mining and diverse industrial practices (Genchi *et al*., 2020; Adnan *et al*., 2022; Razzak *et al*., 2022). These metallic ions pose both direct and indirect threats to human health and environment (Hussain and Qazi, 2016; Zaynab *et al*., 2022; Powolny *et al*., 2023; Sun *et al*., 2023). Consequently, it becomes necessary to remove metallic ions before they come into contact with the environment. Several methods have been employed for this purpose, with chemical neutralization being the most commonly used approach (Bashir *et al*., 2019; Rajendran *et al*., 2022; Chen *et al*., 2023). However, there are limitations associated with this method, such as the generation of secondary pollutants and high costs (Geng *et al*., 2020; Qasem *et al*., 2021).

Biological treatment presents an appealing alternative for the remediation of harmful metallic ions. Biological treatment offers various advantages, including reduced generation of secondary pollutants, compatibility with the natural environment, lower operational costs, and improved performance (Hussain *et al*., 2019; Batool *et al*., 2019). So, the utilization of metals-resistant sulfate-reducing bacteria (SRB) has been demonstrated as an encouraging approach for the simultaneous management of sulfates and metal ions. These bacteria convert metal ions into their corresponding sulfides by using sulfates

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as terminal electron acceptors, thereby facilitating their removal (Hussain *et al*., 2016; Xu and Chen, 2020). Different studies have shown the efficient utility of SRB for the remediation of simulated and/or real wastewaters loaded with heavy metals (Hussain *et al*., 2016, 2019; Muneeb *et al*., 2020).

Several investigators have recognized diverse categories of waste materials that can serve as energy and carbon sources for metal precipitation through biological sulfate reduction (Hussain *et al*., 2014a, b). Pressmud is one of the sugar industry wastes and can be used for the economical cultivation of SRB. Pressmud is spongy, soft, brownish, and amorphous material that comprises coagulated colloids, fiber, and sugar including albuminoids, wax of cane, soil particle and inorganic salt (Saranraj and Stella, 2014). Proximally, it contains protein (1.68 g/L), ash (18.25%), and moisture (70-75%). Pressmud comprises abundant quantity of cellulose (22.3%) and hemicelluloses (21.67%) (Pawar *et al*. 2017). The current experimentation was designed for the precipitation of selected toxic metal ions from artificially prepared metal-rich wastewater while using pressmud hydrolysate as sole source of carbon and energy.

Materials and methods

Desulfovibrio baculatum-HAQ8 employed for the experimentation was obtained from the Microbial Biotechnology Laboratory, Institute of Zoology, University of the Punjab. Initially, the SRB culture was grown and preserved in Postgate B medium. It was also revived in the same medium.

Pressmud was obtained from Pattoki Sugar Mill, dried in dry oven at 60°C, thoroughly ground, and passed through a sieve to achieve a very fine powder of approximately 1 mm particle size. The achieved biomass was treated with 0.5% H_2SO_4 and then autoclaved (Rattanapoltee and Kaewkannetra, 2014). Afterwards, the powdered pressmud was dried, and stored in airtight container for use in future experiments.

For bioprecipitation by SRB, the experimentation was conducted in triplicates in 120-mL sterile serum bottles following Hussain and Qazi (2016). Postgate B medium was used, and pH was maintained at 7.5±0.5. The composition (g/L) of Postgate B medium was: $CaSO_4$, 1.0; $FeSO_4.7H_2O$, 0.5; KH_2PO_4, 0.5; $MgSO_4.7H_2O$, 2.0; NH_4Cl, 1.0; Ascorbic acid, 0.1; yeast extract, 1.0; Sodium lactate, 3.5 mL, Thioglycolic acid, 0.1 mL). Four concentrations viz. 1, 5, 10, and 15 ppm of Cd, Cu, and Cr were made individually by using the same medium. The metal solutions and the medium was autoclaved separately. Sodium lactate was replaced by pressmud hydrolysate (as growth substrate) in these experiments. 5% (v/v) inoculum of freshly cultured SRB containing approximately 1.8×10^6 colony-forming units (CFU) per mL was used. Control vials along with the same concentrations of all three metals used in the experimental vials without inoculation were included. To prevent oxygen diffusion in the inoculated media, a sterilized film of liquid paraffin (3-5 mm) was added. The vials were vacuum-packed with butyl rubber plugs (Hebei Xiangyi International Trading Co., China) and aluminum crimp seals (Zhejiang Aijiren Technology, Inc., China) to ensure the complete evacuation of air. All the vials were placed in an incubator at 30°C for 15 days.

For data analysis at regular intervals of 5 days, a 5-mL sample was taken from each vial using a sterile syringe and then filtered using high-quality filter paper (Whatman Cat No. 1001917, U.K.). The concentrations of metals in these withdrawn samples after acidification with nitric acid were determined by atomic absorption spectrophotometer (CE-2041, U.K.). After obtaining the concentrations of metals that remained unprecipitated, the bioprecipitation of the metals was performed by using the formula

$$\text{Bioprecipitation (\%)} = \frac{([\mathrm{M}]\mathrm{t} = 0 - [\mathrm{M}]\mathrm{t} = \mathrm{t}) \times 100}{[\mathrm{M}]\mathrm{t} = 0}$$

where [M]t = 0 stands for the concentration of dissolved metal immediately after inoculation and [M]t = t stands for the concentration of dissolved metal at measure time.

The data obtained from the experiments were subjected to analysis using GLM procedures. To compare the means, Duncan's Multiple Range test was used, and data were analyzed statistically by using SAS 9.1 software (Cary, 2002). Significance between means was determined at a level of $P < 0.05$.

Results and discussion

The present study was designed for the precipitation of randomly selected toxic metal ions from simulated metal-loaded wastewaters by SRB using pressmud hydrolysate as source of energy and carbon. The study revealed that bioprecipitation of Cd appeared minimum even at lowest concentration (5 ppm) of the added metal (37%) after an incubation period of 15 days (Fig. 1A). Cd removal became more challenging as metal content increased from 10 to 20 ppm (Fig. 1A, B, C). No precipitation of Cd was observed at 20 ppm of the added metal (Fig. 1D). Similar results were reported in earlier studies (Utgikar *et al*., 2003; Cabrera *et al*., 2006; Azabou *et al*., 2007). Several studies like Utgikar *et al*. (2002) and Sani *et al*. (2003) have concluded that heavy metals have toxic effects on microbes when present in high concentrations, resulting in abnormal metabolic activities and even death. At low concentrations, heavy metals have been proven to stimulate microbial growth. At higher concentrations, metallic pollutants can compete with cations, denature proteins, and inactivate enzymes (Utgikar *et al*., 2002).

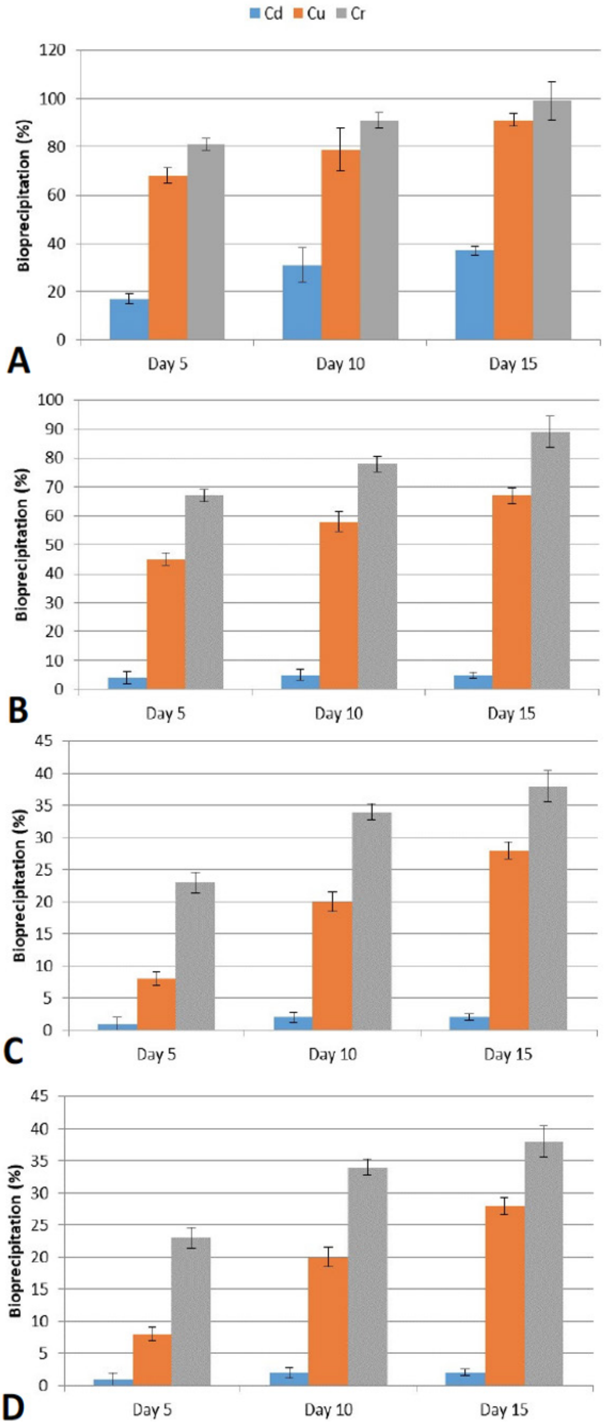

Fig. 1. Bioprecipitation of toxic metal ions at 5 ppm (A), 10 ppm (B), 15 ppm (C), and 20 ppm (D) of the added metal.

Bioprecipitation of Cu appeared maximum than that of Cd. Bioprecipitation of Cu reach maximally to 91% at 5 ppm and reduced to 13% at 20 ppm of the added metal (Fig. 1A-D). Alexandrino *et al.* (2011) reported similar findings that the activity and tolerance level of SRB at higher Cu concentration (500 ppm) was at maximum level. However, in that study, the activity and tolerance level of SRB were high possibly because they were procured from an environment in which the concentration of copper was more than 1000 ppm. In this case, Cu seems to be more toxic for SRB than Cr, as high precipitation of Cr was shown by SRB even at its higher concentration (20 ppm). The findings of this study related to toxic concentration are quite similar to the results reported earlier by Cabrera *et al.* (2006). The difference in cell deactivation may occur due to the association of the metallic pollutant with the microbe culture.

Compared to Cd and Cu, Cr had the least negative impact on SRB due to their ability to efficiently precipitate higher concentrations of this metal (17% at 20 ppm). Cabrera *et al.* (2006) reported that the elevated levels of metal (8.5 ppm or higher) exerted inhibitory effects on bacterial cultures, particularly in *Desulfovibrio vulgaris*. Additionally, the tolerance levels varied among different bacterial isolates obtained from various locations using different isolation methods. This highlights the need for a comprehensive study aimed at isolating and preserving diverse novel microorganisms by subjecting them to varying selective pressure.

In the experiments conducted during anaerobic incubation, it was observed that all the microbes of experimental and control groups exhibited maximum metal uptake within the first five days (1-5). Subsequently, during the following five-day period (5-10), the microbes once again demonstrated the highest metal uptake. However, during the final five days (10-15) of anaerobic incubation, the metal removal appeared to decrease significantly. Results of Hussain and Qazi (2016) are in accordance with our findings.

In the latter half of the incubation, the bacterial cells exhibited maximum metallic removal and demonstrated a greater ability to precipitate metallic ions as they entered the logarithmic growth phase. However, after depleting all the available nutrients in the medium, the bacterial cells entered the stationary phase, during which they were unable to precipitate metallic ions from the medium due to nutrient exhaustion. Consequently, during the terminal period, the potential for remediation dropped to zero due to the unavailability of nutrients. Similar findings were reported by Hussain and Qazi (2012), and Pandian *et al.* (2014) who examined the removal efficiency of *Pseudomonas aeruginosa* against metallic ions.

The current study revealed that the growth conditions, including temperature and pH, had a positive influence on the remedial potential of microbes towards metallic ions. It was observed that the most effective remediation of metallic ions occurred at 30°C and pH 7.0±0.5. This correlation between the ideal growth conditions (temperature and pH) and the efficacy of remediation has also been reported by Hussain *et al*. (2014b) and Aslam *et al*. (2016) involving different microbes and waste treatment scenarios.

Statement of conflict of interest

The authors have declared no conflict of interest.

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