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HEAVY METALS REMOVAL BY *Rhodospirillum rubrum*

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ABSTRACT

The application of bacterial cultures in wastewater treatment, particularly for addressing heavy metal contaminants, has garnered attention within the scientific community. In the current study, we assessed the metabolic conditions of photosynthetic bacteria, such as *Rhodospirillum rubrum*, which, when employed, exhibit the potential for metal removal (specifically chromium and lead). *R. rubrum* was introduced into 500 mL Erlenmeyer flasks containing *Rhodobacter capsulatus* V minimal medium - RCV medium[1] with initial concentrations of 20 mg/L chromium (VI) and 10 mg/L lead. Under anaerobic conditions and a light intensity of 5,670 lx in the bacterial photosystem, the experiment ran for 216 h. The microorganism demonstrated significant metal removal capabilities, achieving rates of 91% for chromium and 89% for lead. The utilization of these purple non-sulfur bacteria in the recovery of wastewater containing heavy metals presents a viable and sustainable alternative in bioremediation processes.

Keywords: *R. Rubrum*. Purple non-sulfur bacteria. Bioremediation. Heavy metals. Wastewater treatment.

1 INTRODUCTION

Recent industrial progress has resulted in adverse outcomes for humans and the environment. Industrial effluents are discharged into water bodies and soil without treatment, contributing to the risk of contamination. Consequently, activities like metallurgy, mining, electroplating for surface treatment of metals, and the overuse of fertilizers stand out as primary contributors to environmental pollution caused by toxic metals.[2]

Heavy metals like chromium (Cr) and lead (Pb) are recognized as toxic substances that have deleterious effects on organisms. Lead is classically a chronic cumulative substance, and the central nervous system is particularly vulnerable to this metal[3]. Hexavalent chromium (Cr (VI)), when inhaled at high concentrations, can cause allergic reactions, irritation in the lining of the nose, and nasal ulcers. Other diseases generated by this metal include anemia, ulcers in the small intestine and stomach[4]. These metals are non-biodegradable, persisting in the environment for extended periods. Additionally, their carcinogenic properties pose a significant threat to living organisms.[5,6]

Addressing the challenge of heavy metal toxicity represent a significant task for both developed and developing nations. The bioremediation process, closely associated with naturally occurring bacteria, plays a role in removing heavy metals from the environment and mitigating toxicity. Due to their remarkable adaptability and cellular mechanisms, bacteria exhibit tolerance and absorb various types of heavy metals.[7,8]

In comparison to traditional treatment methods, biosorption offers numerous advantages, encompassing high removal efficiency, regeneration capacity, cost-effectiveness, and the potential for metal recovery. The removal of heavy metals from effluent treatment through biosorption is mainly attributed to the occurrence of numerous functional groups on the cell walls of organisms that aid in the absorption, degradation, and neutralization of toxic elements in water. Among these functional groups, amine, hydroxyl, carbonyl, sulfhydryl, and phosphonate are responsible for the adsorption of metals onto the viscous coatings of bacterial[9] polysaccharides. Metals such as lead (II), chromium (VI), and copper (II) can be accumulated via carboxyl and amino groups with the assistance of proton displacement[10]. Biosorption process use of biological materials to create complexes with heavy metals through their ligands or functional groups.[11,12]

Microorganisms used in the remediation of heavy metals is a popular field due to attractive characteristics of bacteria, such as their small size and ability to grow under controlled conditions[13] . The mechanism used by phototrophic bacteria for the removal of metallic particles is the process of biosorption and bioaccumulation. Some bacteria may employ more than one mechanism for the removal of metallic particles. The specific mechanism utilized by the organism depends on the properties of the metal ion present in the environment[14].The current study aimed to investigate the bioremediation of effluents containing heavy metals such as chromium and lead by phototrophic bacteria, *Rhodospirillum rubrum*. The analysis focused on the bacteria's ability to remove metals and cellular growth.

2 MATERIAL & METHODS

Rhodospirillum rubrum (DSM No. 467) was cultured anaerobically in RCV medium[1] at pH 6.8, 30° C and constant light intensity of 2,400 lx.

Stock metal solutions were prepared by dissolving appropriate amounts of the analytical reagent, $K_2Cr_2O_7$ and $Pb(C_2H_3O_2)_2$ in deionized water to obtain the stock standard solutions of 1,000 mg/L metal ions.

The experiments were performed in a 500 mL Erlenmeyer with 400 mL reaction mixtures containing the RCV medium with the heavy metals, chromium (VI) and lead, with an initial pH of 6.8. Initial concentrations of 20.0 mg/L of Cr (VI) and 10.0 mg/L of Pb^{2+} were prepared by diluted different amounts of $K_2Cr_2O_7$ and $Pb(C_2H3O_2)_2$ solutions with deionized water. The anaerobic condition was maintained by injecting Argon gas (99.99 %) for 3 min.

The reactors were in an incubator with 30°C and continuous illumination provided by dimmable lamps at 5,670 lx – an optimal light intensity determined by a study conducted by Sousa et al.[15]. Experiments were performed with and without heavy metals (RCV medium with bacteria) over 216 h. Daily, the cell concentration was harvest and measured to construct the growth curve for *R. rubrum*.

The heavy metal concentrations in the supernatant were determined by an atomic absorption spectrophotometry (Shimadzu - AA-7000, Shimadzu Corporation, Kyoto, Japan). The biomass production of *R. rubrum* in the presence of the heavy metals was measured in terms of an increase in optical density at 650 nm (OD650) using a spectrophotometer (Shimadzu – UV-1240).

Reaction solution (30 mL) was sampled from each flask and then centrifuged at 8,000 rpm for 15 min, after 216 h. Thereafter, the supernatant was collected in separate clean test tubes, filtered, and acidified with 1M HNO_3. The heavy metal concentrations in the supernatant were determined by flame atomic absorption spectrometry (Shimadzu brand, model AA-7000). All experiments were performed in triplicate. The percent of Cr (VI) and Pb^{2+} removal efficiency (R) was calculated by using the following Eq. (1):

$$R\ (\%) = [(C_0 - C)/C]*100 \qquad (1)$$

where C_0 is the initial heavy metal concentration (mg/L), and C is the residual heavy metal concentration (mg/L).

3 RESULTS & DISCUSSION

Bioremediation is a promising biotechnological approach for treating wastewater contaminated with heavy metals. It has gained prominence in scientific literature due to its effectiveness and cost-effectiveness, offering a more sustainable alternative.[16,17]

The removal efficiency of heavy metal ions, Cr (VI) and Pb (II), were investigated using the association of purple nonsulfur bacteria (PNSB), *Rhodospirillum rubrum*.

As shown in Table 1, *R. rubrum* was able to remove 91% chromium and 89% lead after 216 h of experiment.

Table 1 The percent removal of 20 mg/L of Cr and 10 mg/L of Pb in RCV medium with R. *rubrum*.

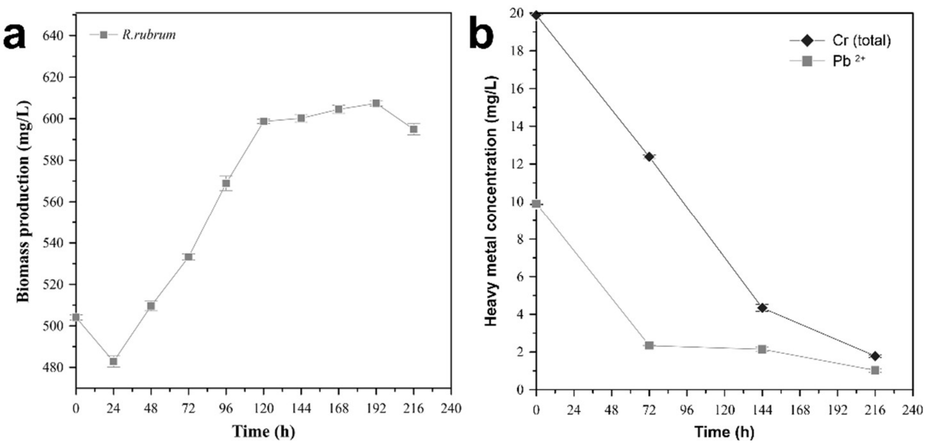
Time (h)	R_{Cr} (%)	R_{Pb} (%)
0	0.0	0.0
72	15.1	75.2
144	65.5	77.5
216	91.1	89.2

R_{Cr}: Chromium removal efficiency; R_{Pb}: Lead removal efficiency.

With a concentration of 20 mg/L of Cr (VI) and 10 mg/L of Pb, the evaluated PNSB were able to reduce 99.9 % of Cr (VI) in all experiments. This reduction of Cr (VI) to its less harmful form (Cr (III)) is possible due to detoxification mechanisms involving the enzyme chromium reductase or non-enzymatic processes present in various microorganisms, particularly in non-sulfur purple bacteria.[15,18]

The Figure 1 shows (a) the biomass production of *R. rubrum* under heavy metal stress and (b) the heavy metals concentrations during the process of absorption by the microrganism.

Figure 1 Biomass production of *R. rubrum* under heavy metal stress (a). Cr(total) and Pb^{2+} during the experiment with *R. rubrum* in 216 hours (b).



The growth curve was obtained according to biomass production under stressful conditions daily. With three metals in the RCV medium, *R. rubrum* was able to growth about 24% compared with the initial biomass concentration.

Su et al.[12] investigated metal removal (Cd, Pb, and Hg) by employing the non-sulfur purple bacteria *R. sphaeroides* SC01. Their findings indicated that lead removal surged to 98 % for concentrations of around 160 mg/L of the metal, implying that SC01 boasts remarkable resistance to Pb and holds promise for lead removal from industrial effluents.

Sousa et al.[15] conducted an assessment of an unusual photosystem's efficacy in eliminating hexavalent chromium and total chromium contamination from effluent through bioremediation, utilizing Rhodobacter capsulatus. The results revealed complete elimination of hexavalent chromium. Regarding total chromium, the highest removal rates were observed under 5,670 lx illumination, particularly at low initial concentrations of hexavalent chromium: 10 mg/L (80.2%), 30 mg/L (89.3%), and 60 mg/L (77.0%). In comparison to other reporters from the literature, it becomes apparent that the removal efficiencies achieved in this study hold promise.

3 CONCLUSION

It was observed that the non-sulfur purple bacteria, *R. rubrum* demonstrated efficiency in removing heavy metals from the synthetic effluent produced in the study.

Based on these results, we propose that *R. rubrum* has high removal efficiency of Cr(total) and Pb, with 91% and 89%, respectively, and the microorganisms can be used to treat wastewaters contaminated with heavy metals.

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