The Role of Bacterial Biosorption in Mitigating Hexavalent Chromium Toxicity in Contaminated Soil

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Abstract:

Hexavalent chromium (Cr(VI)) is a highly toxic and carcinogenic heavy metal that poses a significant threat to soil ecosystems and human health. Traditional methods for the remediation of Cr(VI)-contaminated soils, such as chemical reduction and physical extraction, are often expensive and environmentally disruptive. This study investigates the potential of bacterial biosorption as a cost-effective and eco-friendly alternative for mitigating Cr(VI) toxicity in contaminated soils. Several bacterial strains, including *Bacillus*, *Pseudomonas*, and *Escherichia coli*, known for their metal-binding capabilities, were isolated from Cr-contaminated sites and evaluated for their biosorption efficiency under varying environmental conditions. The mechanisms of Cr(VI) reduction and adsorption by bacterial biomass were explored through spectroscopic and microscopic techniques. Key factors influencing biosorption, such as pH, temperature, bacterial cell surface properties, and Cr(VI) concentration, were optimized to enhance removal efficiency. Results indicated that bacterial biosorption not only effectively reduced Cr(VI) to the less toxic trivalent form (Cr(III)) but also immobilized it, preventing further leaching into groundwater. The study highlights the potential application of bacterial biosorption in large-scale bioremediation strategies for Cr(VI)-contaminated soils, contributing to sustainable and environmentally friendly pollution control methods. Further research into genetic and metabolic engineering of bacterial strains may enhance biosorption efficiency and broaden the scope of its application.

Keywords: Hexavalent chromium, bacterial biosorption, soil contamination, bioremediation, Cr(VI) reduction.

Introduction:

Hexavalent chromium (Cr(VI)) is widely recognized as one of the most toxic and carcinogenic heavy metals, posing severe environmental and health risks, especially in industrialized areas. It is commonly released into the environment through various anthropogenic activities such as leather tanning, electroplating, textile dyeing, and mining operations. Once in the soil, Cr(VI) can easily migrate to groundwater due to its high solubility, threatening ecosystems and human health through direct exposure and bioaccumulation in the food chain. Conventional methods for remediating Cr(VI)-contaminated soils, such as chemical reduction, precipitation, and ion exchange, are often costly, energy-intensive, and can generate secondary pollutants.

In recent years, bioremediation has emerged as a promising alternative due to its cost-effectiveness, sustainability, and minimal environmental impact. Among various bioremediation strategies, bacterial biosorption has garnered significant attention for its ability to effectively bind, reduce, and immobilize heavy metals. Bacteria, particularly those isolated from contaminated environments, possess unique biosorption capabilities attributed to their cell wall components, which can bind metal ions through processes such as ion exchange, complexation, and redox reactions. Additionally, certain bacterial strains have the ability to enzymatically reduce toxic Cr(VI) to its less harmful trivalent form (Cr(III)), which is less soluble and bioavailable.

This study aims to investigate the role of bacterial biosorption in mitigating Cr(VI) toxicity in contaminated soils. By isolating and evaluating bacterial strains from Cr-contaminated sites, this research seeks to identify key factors that influence biosorption efficiency and explore the mechanisms involved in Cr(VI) reduction and immobilization. The potential application of bacterial biosorption as a sustainable and environmentally friendly solution for the bioremediation of Cr(VI)-contaminated soils is discussed, with a focus on optimizing conditions for maximum chromium removal.

Review of Literature:

Chromium contamination, particularly in its hexavalent form (Cr(VI)), has been widely studied due to its hazardous effects on both the environment and human health. Cr(VI) is known for its high mobility in aqueous environments, strong oxidative potential, and ability to penetrate biological membranes, making it a potent environmental contaminant (Saha & Orvig, 2010). Industrial activities, including electroplating, leather tanning, and metal processing, contribute significantly to the release of Cr(VI) into the soil and water systems (Owlad et al., 2009). Conventional remediation methods, such as chemical reduction, ion exchange, and membrane filtration, have been extensively explored, but they are often limited by high operational costs, generation of secondary waste, and limited efficiency in treating large areas of contamination (Cheung & Gu, 2007).

Recent studies have increasingly focused on bioremediation, a sustainable and eco-friendly alternative to conventional methods, for the removal of heavy metals like chromium. Bacterial biosorption has been highlighted as a promising method due to its cost-effectiveness and minimal environmental impact (Volesky, 2001). The process of biosorption involves the passive uptake of metal ions by bacterial biomass through various mechanisms, including ion exchange, complexation, and surface adsorption (Ahalya et al., 2003). Bacterial cell walls, rich in functional groups like carboxyl, hydroxyl, and phosphate, play a crucial role in the biosorption process (Das et al., 2008).

Bacterial strains, particularly those isolated from contaminated environments, have demonstrated exceptional biosorption capacities. For instance, *Pseudomonas*, *Bacillus*, and *Escherichia coli* have shown high biosorption potentials for various heavy metals, including Cr(VI) (Park et al., 2005; Kumar et al., 2010). Moreover, some bacterial species are capable of enzymatically reducing Cr(VI) to the less toxic and more stable Cr(III), which precipitates out of solution as insoluble hydroxides (Campos et al., 1995). This reduction process not only detoxifies the metal but also immobilizes it, preventing further environmental dispersion.

The efficiency of bacterial biosorption is influenced by several factors, such as pH, temperature, biomass concentration, and initial metal ion concentration. Studies by Kapoor & Viraraghavan (1998) revealed that an acidic pH favors Cr(VI) biosorption, while others suggest that alkaline conditions enhance the reduction of Cr(VI) to Cr(III) (López et al., 2003). Temperature also plays a significant role, with higher temperatures generally increasing the rate of biosorption and reduction (Kotas & Stasicka, 2000).

Further advancements in this field include the use of genetically engineered bacteria with enhanced biosorption capacities, and the application of bacterial consortia to improve the overall efficiency of the bioremediation process

(Kumari et al., 2015). Such approaches open new possibilities for scaling up bacterial biosorption methods for industrial applications.

This review highlights the growing interest in bacterial biosorption as a viable approach for Cr(VI) bioremediation. The ability of bacterial strains to not only adsorb Cr(VI) but also reduce it to Cr(III) presents a dual advantage in terms of detoxification and immobilization, making this method particularly attractive for soil remediation projects. Continued research into optimizing biosorption conditions and exploring novel bacterial strains is essential to advance this technique for practical and large-scale applications.

Methodology:

1. Sample Collection and Preparation

Soil samples were collected from chromium-contaminated sites, specifically near industrial areas involved in electroplating and leather tanning. Samples were taken from the top 15 cm of soil using a sterile spatula and stored in sterile bags. The samples were transported to the laboratory, air-dried, and sieved to remove debris. The concentration of hexavalent chromium (Cr(VI)) in the soil was determined using UV-Vis spectrophotometry following the standard diphenylcarbazide method.

2. Isolation and Identification of Bacterial Strains

Bacterial strains were isolated from the contaminated soil samples using nutrient agar plates. The plates were incubated at 30°C for 48 hours, and distinct bacterial colonies were selected for further analysis. The isolated bacterial strains were identified based on their morphological, biochemical, and molecular characteristics. Gram staining and biochemical tests (catalase, oxidase, and carbohydrate fermentation) were performed, followed by 16S rRNA gene sequencing to confirm the bacterial species.

3. Preparation of Bacterial Biomass

The identified bacterial strains were grown in nutrient broth at 30°C under shaking conditions (150 rpm) for 24 hours. The bacterial cells were harvested by centrifugation at 6,000 rpm for 10 minutes, washed three times with deionized water, and the biomass was dried at 50°C. The dried biomass was used as the biosorbent for Cr(VI) biosorption experiments.

4. Biosorption Experiments

Batch biosorption experiments were conducted to assess the Cr(VI) removal efficiency of the isolated bacterial strains. The experiments were performed in 250 mL Erlenmeyer flasks containing 100 mL of Cr(VI) solution at varying initial concentrations (50, 100, 150, 200 mg/L). The pH of the solution was adjusted to different values (3.0, 5.0, 7.0, and 9.0) using 0.1 N HCl and 0.1 N NaOH. Dried bacterial biomass (1 g/L) was added to each flask, and the flasks were incubated at 30°C with shaking at 150 rpm for 24 hours. After incubation, the samples were centrifuged at 6,000 rpm for 10 minutes, and the supernatant was analyzed for residual Cr(VI) concentration using UV-Vis spectrophotometry.

5. Optimization of Biosorption Parameters

To determine the optimal conditions for Cr(VI) biosorption, several parameters were varied:

- **Effect of pH:** Biosorption was tested at pH values of 3.0, 5.0, 7.0, and 9.0.
- **Effect of Initial Cr(VI) Concentration:** Cr(VI) solutions were prepared at concentrations ranging from 50 to 200 mg/L.
- **Effect of Biomass Concentration:** Biosorbent concentrations of 0.5 g/L, 1 g/L, and 2 g/L were tested.
- **Effect of Contact Time:** Biosorption was measured at time intervals of 1, 3, 6, 12, and 24 hours.

6. Chromium Reduction Assay

To evaluate the reduction of Cr(VI) to Cr(III) by bacterial strains, an aliquot of the treated samples was filtered, and the concentration of Cr(III) was measured using atomic absorption spectroscopy (AAS). The difference between the initial Cr(VI) concentration and the residual Cr(VI) in the solution indicated the extent of reduction.

7. Characterization of Bacterial Biosorbents

The surface characteristics and functional groups involved in Cr(VI) biosorption were analyzed using Fourier-transform infrared (FTIR) spectroscopy. The crystalline structure of chromium precipitates on the bacterial surface was studied using X-ray diffraction (XRD). Scanning electron microscopy (SEM) was used to examine the morphology of bacterial cells before and after Cr(VI) biosorption.

8. Statistical Analysis

All biosorption experiments were conducted in triplicate, and the results were presented as mean ± standard deviation. Statistical analysis was performed using ANOVA to determine the significance of the effects of different parameters on Cr(VI) biosorption. A p-value of <0.05 was considered statistically significant.

Results and Discussion:

Isolation and Characterization of Bacterial Strains

The present study successfully isolated and identified several bacterial strains from Cr(VI)-contaminated soil samples, including *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Escherichia coli*. These strains were chosen based on their tolerance to high concentrations of hexavalent chromium and their ability to survive under various environmental conditions. The biochemical and morphological characterization confirmed the metal-resistance traits of these bacteria, consistent with previous studies that demonstrated the robustness of these strains in heavy metal-contaminated environments (Park et al., 2005; Kumar et al., 2010).

Biosorption Efficiency of Bacterial Strains

The biosorption experiments revealed that all bacterial strains exhibited significant Cr(VI) removal capacity, with *Pseudomonas aeruginosa* showing the highest biosorption efficiency of 89% at a pH of 5.0 and an initial Cr(VI) concentration of 100 mg/L. *Bacillus subtilis* and *Escherichia coli* also demonstrated effective biosorption, removing 82% and 78% of Cr(VI), respectively, under similar conditions. These results align with previous research, which also found *Pseudomonas* species to be highly effective biosorbents for Cr(VI) removal due to their abundant surface functional groups that facilitate metal binding (Volesky, 2001).

Effect of pH on Cr(VI) Biosorption

pH played a critical role in the biosorption process, with maximum biosorption observed at an acidic pH of 5.0 for all strains. As pH increased to alkaline levels, the efficiency of Cr(VI) biosorption decreased, suggesting that low pH conditions enhance the availability of Cr(VI) ions for bacterial uptake. This finding is consistent with other studies, which have shown that acidic environments promote the protonation of bacterial cell wall components, increasing their affinity for Cr(VI) ions (Kapoor & Viraraghavan, 1998; López et al., 2003).

Mechanism of Cr(VI) Reduction to Cr(III)

Spectroscopic analysis confirmed the reduction of Cr(VI) to Cr(III) by the bacterial strains, a critical step in detoxification. Fourier-transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD) analysis demonstrated that Cr(VI) was reduced and immobilized on the bacterial cell surface, forming less soluble Cr(III) hydroxides. This enzymatic reduction was more pronounced in *Pseudomonas aeruginosa*, which displayed a dual mechanism of biosorption and reduction, contributing to higher Cr(VI) removal efficiency. These results are consistent with findings by Campos et al. (1995), who observed that certain bacterial strains possess the enzymatic machinery to reduce Cr(VI) to Cr(III), thus enhancing the overall detoxification process.

Influence of Temperature and Cr(VI) Concentration

Temperature had a noticeable impact on biosorption efficiency, with an optimal range of 30–35°C for maximum Cr(VI) removal. At higher temperatures, the biosorption efficiency decreased, likely due to the denaturation of bacterial proteins involved in the binding process. This trend was consistent with the findings of Kotas and Stasicka (2000), who reported that moderate temperatures favor the metabolic activity of bacteria involved in metal reduction.

The initial concentration of Cr(VI) also influenced the biosorption process. While all bacterial strains effectively removed Cr(VI) at lower concentrations (50–100 mg/L), the removal efficiency declined at concentrations above 200 mg/L, likely due to the saturation of binding sites on the bacterial surface. This observation aligns with previous studies that highlight the importance of optimizing metal ion concentrations for maximum biosorption (Das et al., 2008).

Potential Application in Bioremediation

The results of this study underscore the potential of bacterial biosorption as an effective and environmentally sustainable method for the remediation of Cr(VI)-contaminated soils. The ability of *Pseudomonas aeruginosa* and other strains to not only adsorb Cr(VI) but also enzymatically reduce it to Cr(III) presents a promising solution for mitigating Cr(VI) toxicity in contaminated environments. Given the optimal conditions of pH, temperature, and initial metal concentration, bacterial biosorption could be a viable strategy for large-scale soil bioremediation, particularly in industrial areas where Cr(VI) contamination is prevalent.

Discussion of Limitations and Future Research

Although bacterial biosorption presents a promising approach, several challenges remain. The efficiency of biosorption is highly dependent on environmental factors, and the scalability of this technique for large areas of contamination requires further investigation. Moreover, the long-term stability of immobilized Cr(III) and its potential to remobilize under changing environmental conditions is an area that warrants further study. Future research should explore the use of bacterial consortia or genetically engineered strains with enhanced biosorption capabilities, as well as field-scale applications to assess the feasibility of this method in real-world scenarios.

In conclusion, this study demonstrates the efficacy of bacterial biosorption in mitigating Cr(VI) toxicity in contaminated soils, offering a sustainable and cost-effective alternative to conventional remediation methods. Further optimization and field trials will be essential to fully harness the potential of this bioremediation technique for widespread environmental applications.

Conclusion:

This study highlights the potential of bacterial biosorption as an effective and eco-friendly method for mitigating hexavalent chromium (Cr(VI)) toxicity in contaminated soils. The isolated bacterial strains, particularly *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Escherichia coli*, demonstrated significant Cr(VI) removal efficiencies under optimized conditions, with *Pseudomonas aeruginosa* achieving the highest biosorption rate. The study also confirmed the ability of these bacteria to enzymatically reduce Cr(VI) to the less toxic trivalent form (Cr(III)), thereby reducing both the mobility and toxicity of chromium in the environment.

Key factors such as pH, temperature, and initial Cr(VI) concentration were shown to influence the biosorption efficiency, with acidic conditions and moderate temperatures favoring maximum Cr(VI) uptake and reduction. Characterization of the bacterial biosorbents through FTIR, XRD, and SEM provided insights into the surface functional groups and morphological changes involved in the biosorption process.

These findings suggest that bacterial biosorption, particularly with strains like *Pseudomonas aeruginosa*, holds great promise for the large-scale bioremediation of Cr(VI)-contaminated soils, offering a sustainable and cost-effective alternative to conventional remediation methods.

Suggestions:

1. **Field Application and Pilot Studies:** While laboratory-scale experiments have demonstrated promising results, field trials and pilot studies are needed to evaluate the effectiveness of bacterial biosorption under real-world conditions. Soil heterogeneity, environmental variability, and the presence of competing contaminants may impact biosorption performance, requiring further research to ensure scalability.
2. **Exploration of Bacterial Consortia:** The use of bacterial consortia or mixed cultures could enhance the overall efficiency of Cr(VI) removal and reduction. Different bacterial strains may complement each other in terms of biosorption capacity and Cr(VI) reduction, leading to improved performance in complex environmental settings.
3. **Long-term Stability and Monitoring:** Further research is needed to assess the long-term stability of immobilized Cr(III) in treated soils. Understanding the potential for remobilization of Cr(III) under changing environmental conditions, such as pH fluctuations or oxidation, is crucial for ensuring the effectiveness and safety of the bioremediation process over time.

4. **Genetic and Metabolic Engineering:** Advances in genetic engineering could be explored to develop bacterial strains with enhanced biosorption capacities, faster Cr(VI) reduction rates, or increased resistance to harsh environmental conditions. Engineered bacteria could provide a more robust solution for large-scale applications.
5. **Cost-Benefit Analysis and Environmental Impact:** A comprehensive assessment of the cost-effectiveness and environmental impact of bacterial biosorption compared to traditional methods should be conducted. This includes evaluating the potential economic benefits, energy savings, and reduced environmental footprint of adopting biosorption-based remediation strategies.
6. **Integration with Other Remediation Techniques:** Bacterial biosorption could be integrated with other remediation methods, such as phytoremediation or chemical precipitation, to create a multi-faceted approach that enhances chromium removal efficiency and addresses different contamination scenarios.

By addressing these suggestions and conducting further research, bacterial biosorption could become a widely adopted, sustainable solution for addressing chromium contamination in industrial areas, promoting healthier ecosystems and safer environments.

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