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# Review Article
# Microscopic Investigations on the Biosorption of Heavy Metals by Bacterial Cells: A Review

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## Abstract

Industry has been the major contributor of pollution since decades, destroying the environment and natural resources. Among the types of pollutants, heavy metals are engendering major threat to the biosphere. A significant number of biosorption studies on the removal of heavy metals from aqueous solutions have been conducted worldwide. Nearly all of them have been aimed at optimizing biosorption parameters to obtain the highest removal efficiency while the rest of them are concerned with the biosorption mechanism. The mechanism and chemical nature of heavy metal sequestration by bacteria were investigated by scanning electron microscopy (SEM) with energy dispersive X-ray (EDX) analysis and atomic force microscopy (AFM). Atomic force microscopy used in the tapping mode elucidated the morphological changes in bacterial cells following heavy metal binding. Morphological assessment and quantification of heavy metals within the bacterial cells were performed by scanning electron microscopy and energy dispersive X-ray analysis.

Key words: Biosorption, bacterial cells, scanning electron microscopy (SEM), energy dispersive x-ray (EDX) analysis, atomic force microscopy (AFM)

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## INTRODUCTION

Industrial operations such as electroplating, steel manufacturing, leather tanning, wood preservation, ceramics, glass manufacturing, chemical processing and fertilizer applications release alarmingly higher amounts of heavy metals into the natural environment[1,2]. Heavy metals in low concentration are essential to all living organisms. However, in high concentrations, they cause severe toxic effects in exposed plants, animals and humans. They do cause severe neurological and physiological damage to human body. Three kinds of heavy metals are of concern, including toxic metals (Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co and Sn), precious metals (Pd, Pt, Ag, Au and Ru) and radio-nuclides (U, Th, Ra and Am)[3,4]. Among these that reach hazardous levels are the heavy metals which comprise of lead, chromium, mercury, uranium, selenium, zinc, arsenic, cadmium, silver, gold, nickel and copper[5,6]. Therefore, the effective removal of heavy metal ions from wastewater is very important and has attracted attention and considerable research.

Various conventional technologies such as precipitation, oxidation, reduction, adsorption, filtration, flocculation, sedimentation, osmosis, ion-exchange and biosorption have been used in treating the wastewater contaminated with heavy metals[7-9]. Among the available techniques, biosorption plays a significant role in the removal of heavy metals due to excellent adsorbability, eco-friendliness, cost-effectiveness and easy availability of biosorbents[10]. Various biosorbents like bacteria, fungi, yeasts and agricultural products have been used for biosorption[11]. Bacteria are most commonly used as biosorbents owing to their small size, ubiquity and ability to grow under controlled conditions and resilience to a wide range of environmental conditions[12]. There is a high degree of heterogeneity among different bacterial species with reference to surface binding sites, binding strength for metal ions and binding mechanism[13]. The cell wall as the first component of contact has carboxyl, phosphate and hydroxyl groups[14].

Biosorptive efficiencies of different bacterial strains have been reported by Vijayaraghavan and Yun[15]. *Bacillus, Micrococcus*, *Arthrobacter*, *Sphingomonas* and *Microbacterium* are common genera including metal-tolerant Gram-negative and Gram-positive species[16]. A gene cluster, *czr*, involved in cadmium and zinc resistance was identified in *Pseudomonas aeruginosa* (*P. aeruginosa*) CMG103[17]. Rani *et al.*[18] reported that *Bacillus* sp., *Pseudomonas* sp. and *Micrococcus* sp., were identified as efficient strains that were resistant to Cu, Cd and Pb, respectively. Nickel adsorption by dried cells of *Enterobacter agglomerans* SM38 was found at optimum pH and the removal reached 25.2% while for *Bacillus subtilis* WD90, nickel removal was 27%[19]. Certain species have mechanisms of chromate tolerance and resistance such as *Enterobacter cloacae*, *Desulfovibrio vulgaris*, *Pseudomonas aeruginosa*, *Cupriavidus metallidurans*, *Ralstonia metallidurans*, *P. putida*, *Escherichia coli*, *Caulobacter crescentus*, *Shewanella oneidensis*, *Bacillus firmus* and *Burkholderia cepacia*. The mechanisms by which these microorganisms reduce Cr (VI) are variable and are species dependent[20]. A multi-site surface complexation phenomenon governed by the formation of U-carboxyl and U-H-phosphoryl surface species has been reported for U sorption onto *Bacillus subtilis* by Fowle *et al.*[21]. Previous reports by Sar and D'Souza[22,23] and D'Souza *et al.*[24] have shown that *Pseudomonas* strain (MTCC 3087) has a high efficiency for metabolism independent accumulation of uranium and thorium which may find a potential application in the removal and recovery of metals from industrial effluents.

The present review has been undertaken to decipher the possible mechanism and chemical nature of heavy metal sorption by bacteria. The mechanism of interaction of heavy metals with bacteria has been elucidated by reviewing the analytical techniques such as scanning electronic microscopy equipped with EDX and atomic force microscopy.

**Scanning electron microscopy and EDX:** In general, the characterization of biosorbents by scanning electron microscopy offers topographical and elemental information of the solids with a large depth of field, allowing different specimen parts to stay in focus. The SEM also has high resolution and higher magnification is possible for closely spaced materials. In addition to its capacity to produce clear image, it is useful in showing the topographical details of biosorbents[25-30]. However, SEM has limitations on its lowest detectable particle size and its inability to detect trace elements in a substance.

The assessment of morphological changes as a result of chromium accumulation within the bacterial strain, *Acinetobacter* sp. has been conducted using SEM by Srivastava and Thakur[30]. Using SEM pictures, they claimed that chromium was uniformly bound on the cell wall surface of the bacteria. Morphological transformation due to exposure of the cells to chromium was also evident. The SEM studies revealed that before Cr (VI) biosorption, the cells appeared to be plump with smooth surfaces in a loosely-bound form. After interaction with Cr (VI), precipitates in the form of round globules and amorphous substances aggregated all over the cell surface of *P. aeruginosa*[31].

The Cr (VI) uptake mechanism of living cells of *Ochrobactrum anthropi* and the influence of bacterial culture medium on the Cr-immobilization process was reported by Li *et al.*[32]. It was found that Cr-immobilization ratio of bacteria in Tris-HCl buffer was higher than that of LB medium. Scanning electron microscopic analysis revealed that bacterial cells without chromate treatment were plump and with smooth surfaces, indicating that the components at bacterial surfaces were uniformly distributed both in Tris-HCl buffer and LB medium. When the bacteria were treated with chromium in Tris-HCl buffer, obvious changes were noticed[32].

Morphological assessment and quantification of chromium within bacterial strains was performed by scanning electron microscopy and energy dispersive X-ray analysis. Chromium treated bacterial strain, *Bacillus cereus* showed morphological changes in response to chromium as analyzed with SEM. In the control, bacterial strain was rod-shaped and elongated and there was no peak of chromate at 5.4 keV after 24 h incubation which was determined by energy dispersive X-ray analysis, while treated bacterial strain showed some changes in morphology. It became small, round-shaped with uneven edges on cell wall and there was a peak of chromium at 5.4 keV in EDX analysis[11].

The surface morphology of *B. cereus* without and with sorption of arsenic (III) and chromium (VI) ions during biosorption process was studied with the help of SEM-EDX. In the absence of sorption of arsenic (III) and chromium (VI) ions, rod-like shape with a smooth surface of *Bacillus cereus* cells was noticed[33]. The shape changed into a spindle-like structure after arsenic (III) and chromium (VI) ions sorption[34].

The surface morphology of dead cells of *Bacillus salmalaya* was analyzed through SEM before and after adsorption. The morphological aspects of dead cells before chromium adsorption were long, thin and rod-shaped. After adsorption of chromium, changes in morphology were observed. This can be attributed to the covering of the cell surface with chromium ions, which looked like fat, spongy and plumped. This finding agrees with the elemental analysis through EDX spectra of dead cells. The percentages of chromium were presented as additional peaks and demonstrated that the chromium ions were attached to the surface[35].

The process of uptake and retention of the heavy metals by the cell wall of *Bacillus* sp. has been studied with EDX analysis[36]. The sulphate reducing bacteria in copper sulphate solution and their efficacy of removal of copper from those solutions was also investigated. SEM images of the unstained bacteria confirmed that the minerals formed were associated with the cell surface. Energy dispersive X-ray spectra confirmed the precipitation of copper as copper sulphide. Cells exhibited a high degree of capsule production in copper solution[37]. This change in cell shape due to heavy metal exposure is an adaptive mechanism to resist the toxicity of heavy metals. The surface area is a significant parameter for the biosorption process of heavy metals by bacteria as surface area is directly proportional to the metabolic rate and growth rate[38].

The comparison of SEM photographs between the copper free and copper loaded *Enterobacter cloacae* showed that copper had undergone remarkable physical disintegration after adsorption in the biosorbent. The cell surface morphology considerably changed after metal biosorption. Moreover, the EDX analysis confirmed the presence of metal adsorbates on the cell mass, enabling direct detection of metals on cells[39].

Electron microscopic observation by Mullen *et al.*[40] exhibited the presence of $Ag^{2+}$ as discrete particles at or near the cell wall of both Gram-positive and Gram-negative bacteria and the presence of silver was confirmed by EDX analysis. To analyze the morphology of the cell surface before and after biosorption, SEM micrographs are often used. With the aid of SEM, Lu *et al.*[41] visualized the surface of metal-loaded *Enterobacter* sp., which appeared to be vague and damaged by heavy metal ions. Vijayaraghavan *et al.*[42] used SEM to exhibit the pattern of *Corynebacterium glutamicum* immobilization within a polysulfone matrix.

The morphological changes due to nickel stress were explored by SEM analysis, which revealed an increase in size of the cells and secretion of extrapolymeric substance after exposure of *Sinorhizobium* sp., BEL5B strain to 3 mM nickel[43]. Similar morphological changes like increased size were also demonstrated in phototrophic bacteria after exposure to metalloid oxyanions as a protection strategy for facing contaminated environment[44]. Helmann *et al.*[45] showed that the effect of metals on cells can be limited by binding metal ions to exopolysaccharide and the cells can survive the metal stress along with normal metabolic activities.

The SEM has been used to explore the microscopic behaviour of microbial sorption. The SEM images of the living cells of *Bacillus* sp., before and after biosorption revealed that surfaces of the cell after biosorption became very rough, with small granular material accumulated and by EDX analysis the small granular material was identified as thorium. SEM and EDX analysis were done with the dead cells and the results were the same as that of live cells[46]. The modifications of bacterial cell surface and precipitation of heavy metals on the cells were revealed by SEM and EDX (Table 1). The shape of *Pseudomonas aeruginosa* MCCB 102 was modified after the adsorption of the heavy metals Zn, Cu, Cd and Pb[47].

Table 1: Scanning electron microscopy and EDX analysis of biosorption of heavy metals by bacteria

| Metals | Bacterial strain | References |
|---|---|---|
| Chromium (IV) | *Acinetobacter* sp. | Srivastava and Thakur[30] |
| | *Pseudomonas aeruginosa* | Chatterjee *et al.*[31] |
| | *Ochrobactrum anthropi* | Li *et al.*[32] |
| | *Bacillus cereus* | Naik *et al.*[11], Ray *et al.*[33] and Giri[34] |
| | *Bacillus salmalaya* | Dadrasnia *et al.*[35] |
| Arsenic (III) | *Bacillus cereus* | Ray *et al.*[33] and Giri[34] |
| Copper | *Bacillus* sp. | Beveridge and Murray[36], Jalali and Baldwin[37], |
| | *Enterobacter cloacae* | Suriya *et al.*[39] and Zolgharnein *et al.*[47] |
| | *Pseudomonas aeruginosa* | |
| Silver(II) | Gram-positive and Gram-negative bacteria | Mullen *et al.*[40] |
| Nickel | *Sinorhizobium* sp., BEL5B | Jobby *et al.*[43] |
| Thorium | *Bacillus* sp. | Lan *et al*[46] |
| Zinc | *Pseudomonas aeruginosa* | Zolgharnein *et al.*[47] |
| Cadmium | *Pseudomonas aeruginosa* | Zolgharnein *et al.*[47] |
| Lead | *Pseudomonas aeruginosa* | Zolgharnein *et al.*[47] |

Table 2: Atomic force microscopic studies on the biosorption of heavy metals by bacteria

| Metals | Bacterial strain | References |
|---|---|---|
| Lanthanum | *Escherichia coli* | Peng *et al.*[54] |
| Nickel | *Escherichia coli* | Wang *et al.*[55] |
| Uranium | *Pseudomonas* sp. | Krueger *et al.*[56] |
| Thorium | *Pseudomonas* sp. | Krueger *et al.*[56] |
| Lead | *Bacillus cereus* | Pan *et al.*[57] |
| Chromium | *Ochrobactrum anthropi* | Li *et al.*[32] and Giri[34] |
| | *Bacillus cereus* | |

**Atomic force microscopy:** The atomic force microscopy is an ideal tool for determining changes in cellular morphology. AFM imaging can be performed using the tapping mode, where, the tip makes intermittent contact with the sample as the tip is oscillated near its resonance frequency. The tapping mode has the advantages like avoiding damage by the tip and reduced lateral forces[48].

Atomic force microscopy is used to investigate the materials at an atomic scale and to measure the interatomic forces and electromagneic forces[49]. The three-dimensional view in AFM can be used to analyse the surface details, which is more advantageous than that of scanning electron microscope[50]. AFM can be an effective tool in determining the physical properties of adsorbent chain, including shape, persistent length and end-to-end distances of the chain[51]. AFM analysis is extensively used for the topological characterization of biosorbents from different sources[51,52]. The adsorption mechanisms of the biosorbent for $Pb^{2+}$ and $Zn^{2+}$ could be the combined action of electrostatic interaction, complexation and ion-exchange between biosorbent functional groups and metal ions[53].

Atomic force microscopic studies revealed that lanthanum binding to *Escherichia coli* substantially changed the structure of outer cell membrane responsible for cell permeability[54]. Wang *et al.*[55] reported a similar change in cell dimensions and roughness following nickel exposure in *E. coli* cells. Cell surface properties in Gram-negative bacteria are regulated by surface proteins and lipopolysaccharides. Interaction of radionuclides like uranium and thorium with such molecules through strong metal binding ligands (phosphate and carboxyl groups), leads to a change in surface architecture as reflected by an increase in surface roughness. Another reason for increased roughness is rupturing of the bacterial cell following U/Th accumulation[56].

Pan *et al.*[57] showed AFM images of *Bacillus cereus* cells exposed to different concentrations of $Pb^{2+}$ ions and the cell shape changed from rod-like structure to spindle-like structure after $Pb^{2+}$ biosorption. They reported that these morphological changes of the cells can be attributed to the interactions between heavy metals and the cell surface of *B. cereus*. Li *et al.*[32] investigated Cr (VI) uptake mechanism by living *Ochrobactrum anthropi* both in Tris-HCl buffer and LB medium. Atomic force microscopy observations showed that Cr (III) precipitates were accumulated on bacterial cell surface. AFM roughness analysis exhibited that the surface roughness of bacteria increased when the bacteria-Cr (VI) interaction was in Tris-HCl buffer rather than in LB medium.

Giri[34] investigated the surface morphology of *Bacillus cereus* biomass without and with sorption of arsenic (III) and chromium (VI) with the help of atomic force microscopy. *B. cereus* cells without arsenic (III) and chromium (VI) ions exposure in the control were rod-like with a smooth surface. After arsenic (III) and chromium (VI) ions exposure, the ultrastructures mostly disconnected from the cells adhering to each other randomly. The cell shape changed into a spindle-like structure after arsenic (III) and chromium (VI) sorption. The studies already carried out with AFM on the biosorption of heavy metals by bacteria are listed in Table 2.

## CONCLUSION

Bacterial biomass represents an efficient and potential class of biosorbents for the removal of metal ions from solutions. Though diverse techniques exist for the characterization of biosorbents, a combination of scanning electron microscopy with EDX and atomic force microscopy is commonly required to obtain a complete description of the structure and surface functional groups. The adsorption mechanisms could be the combined action of electrostatic interaction, complexation and ion-exchange between functional groups and metal ions.

## SIGNIFICANCE STATEMENTS

This study reviews heavy metal pollution as one of the major environmental problems that poses serious health risk. It also focuses on biosorption as a beneficial process for the removal of heavy metals from industrial wastewater. This article will serve as a source for researchers to learn the mechanism and chemical nature of heavy metals sequestration by bacteria. It also shows the use of atomic force microscopy to elucidate the morphological changes in bacterial cells following heavy metal binding. Morphological assessment and quantification of heavy metals within the bacterial cells by scanning electron microscopy and energy dispersive X-ray analysis were also appraised. This review would be beneficial for researchers in learning about heavy metal pollution and the various ways bacteria can be utilized to mitigate it.

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