



## **A Review on Biosorption of Potentially Toxic Metallic Pollutants from Wastewaters by Bacterial Adsorbents**

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### **Authors' contributions**

*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

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### **ABSTRACT**

Nowadays potentially toxic metal removal from the environment, particularly wastewater, is moving from the use of conventional techniques to the use of biosorption, which utilizes diverse natural materials of biological basis, including bacteria, fungi, algae and yeast, for the binding and concentration of these metal ions or other pollutants. These biomaterials contain functional groups such as hydroxyl, carboxyl, amido and sulphhydryl, which make it feasible for them to attach potentially toxic metal ions from wastewaters. The binding mechanism involved in biosorption process has been found to be quite complex. It consists of several phenomena comprising precipitation, physical adsorption, complexation and ion-exchange. The binding mechanism between the potentially toxic metal ions and these biosorbents will be discussed, including the key functional groups implicated in metal binding process.

*Keywords: Potentially toxic metals; biosorption; bacterial biosorbent; functional groups; binding mechanism.*

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## 1. INTRODUCTION

In recent times, potentially toxic metal ions pollution is a very critical environmental issue, as heavy metal ions have deadly effects on all life forms due to their persistent and non-biodegradable nature and tendency to accumulate within biological system and their concentration are raised along food chain [1-4]. Therefore, the noxious effects of the metal ions are more prominent in the organisms at higher trophic levels [5-6]. Due to the noxious effects of metals, the industries are recommended to treat the wastewater consistently to eliminate/reduce the metal ions concentration in their wastewater. Therefore, elimination of these toxins from industrial wastewater has become a vital concern that is followed in tightening and imposition of environmental set of laws. Economical and valuable treatment is required to treat huge volumes of industrial effluents containing heavy metals [7-8]. As yet, there are various studies taking into consideration the possibility of recovery and removal of potentially toxic metals from diluted solutions [8-11] and comparison of selected techniques used for the purpose are listed in Table 1. The traditional techniques for sequestering potentially toxic metal ions include ion-exchange, precipitation, filtration, oxidation/reduction, membrane separations and electrochemical processes. These techniques have some disadvantages such as incomplete removal, high cost, high energy consumption and low selectivity and some of the techniques (e.g., coagulation and precipitation) produce concentrated and further toxic wastes which are difficult to eliminate, so creating another disposal problem. These methods are unsuitable or become ineffective or too expensive, especially when treating a large amount of wastewater containing potentially toxic metal ions at lower concentration (<100 mg/L), so they cannot be used at large scale [5,8,12-14]. Thus, there is a perpetual urgency to investigate for an optimum technique though taking into account its metal removal efficiency with minimal environmental impacts, cost and materials used. Biological remediation, (or biosorption), is an emerging technology that offers the use of economical natural materials of biological origin, including bacteria, fungi, yeast and algae for treatment of metal polluted effluents. These natural biosorbents possess heavy metal sequestering properties and can sequester rapidly and effectively dissolved metal ions out of diluted solutions. Therefore biosorption is an ideal process for handling the large volume and low

concentration industrial effluents [8,15]. These biosorbents have capacity to tie with metals ions from the metal polluted effluents at their surface or carrying intra cellular for various functions. However, the objective of this study is to obtain an effective biosorption process. For this it is mandatory to understand the binding mechanism between the heavy metal ions and these biosorbents and determine the nature of the chemical ligands which are implicated in metal binding process. This understanding will eventually allow us to design more functional biosorption technology for treatment of heavy metal polluted effluents.

## 2. METAL ION TOXICITY

Heavy metal ions like Ni(II), Cu(II), Fe(II), Zn(II), and Cr(VI), are important for both animals and plants but when they are present in large amounts, they and non-essential metals like Cd(II), Ag(III), Hg(II) and Pb(II), can be very toxic [16]. They are extensively used in many industries including metal plating, petroleum refining, ceramic, storage batteries and Cu (II)-based fertilizers, insecticides and fungicides [10, 11,17-19]. These metals do not degrade naturally, so the control of heavy metals pollution has special significance for both flora and fauna [19-22]. Treatment and toxicity level of certain potentially toxic metal/metalloids ions are reported in Table 2.

## 3. MICROORGANISMS

Microorganisms (eukaryotes and prokaryotes) have been investigated for the sequestration of heavy metal ions from aqueous solutions and they showed promising results without producing toxic intermediates [23]. Both eukaryotic and prokaryotic cells own homeostatic mechanisms (i.e. property of a system to regulates its internal environment and tends to maintain a stable, constant condition) to regulate the concentration of heavy metal ions and reduce the toxic effects which is produced by excessive levels [24]. Different types of microorganisms such as bacteria, algae and fungi found in natural environment play an important role in biosorption process. Table 3 outlines the basic information about the use of various microbial biomasses for heavy metal ions biosorption. On the basic and applied aspects of this process, a large number of studies are present which are reviewed [5,23,25-31]. In the present study the bacterial biosorption process has been described.

### 3.1 Bacterial Biomass as Biosorbents

Bacteria are the most abundant and flexible group of microorganisms and biosorbents derived from its biomass have become admired due to small size, ability to develop under controlled conditions, their flexibility to a broad range of environmental set of conditions and economical nutrient sources are readily available for these microbes. Many researchers studied the use of living and nonliving bacteria biomass in biosorption. The use of living bacteria biomass posses some advantage over nonliving biomass; a) selectivity is governed by specific interactions among analytic species and wall proteins; b) extractant amount is reduced to a minimum; c) it is usually a low-cost process; and d) no waste is generated [32]. Potential metal biosorbents among bacteria comprise genera *Pseudomonas*, *Leptothrix*, *Bacillus*, *Streptomyces*, *Escherichia coli* and *Micrococcus*, etc [33]. In solution, metal ions are adsorbed onto the surface of bacterial biomass through interactions with chemical functional groups present in cell wall. The most common functional groups such as carboxyl, amide, amine, phosphate, imidazole, hydroxyl, thioether are found on the bacterial cell wall [33].

### 3.2 Bacterial Cell-Wall Structure

To understand the mechanisms of interaction involved we have to identify the functional groups which are participating in metal binding. As stated by Brown et al. [34] and Demirbas [35] most of these groups have been found in cell wall, So, a comprehensive study of chemical structures of microbial cells is required [36]. The cell surfaces hosts multiple functionally and structurally diverse proteins and they differ significantly from Gram-negative to Gram-positive bacteria [37]. The cell surface of Gram-negative bacteria is much more complicated than that of Gram-positive bacteria. The Gram-positive bacterial cell surface has two major structures: cell membrane and cell wall. The cell wall of Gram-positive bacteria is consist of multiple layers of peptidoglycan, which is a linear polymer of alternating units of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) [38]. The peptidoglycan layer is present immediately outside the cytoplasmic membrane and it provides structural integrity to the bacterial cell and makes them differing from other groups of organisms. In *Escherichia coli* and most other Gram-negative and many Gram-positive bacteria, peptidoglycan was found to be a powerful binder of the metals ions and

carboxylate groups were the principal components involved in metal binding [39]. The peptidoglycan layer of Gram-negative bacteria is usually a single monolayer. An outer membrane of Gram-negative bacteria is composed of lipopolysaccharides, phospholipids, enzymes, and other proteins, including lipoproteins. The space among inner membrane and outer membrane is known as periplasmic space.

### 3.3 Functional Groups on Bacterial Cell Wall

Earlier we have studied that various functional groups are present on bacterial cell surfaces. The chemical alteration and spectroscopic studies have revealed that cellular components including hydroxyl, carboxyl, sulfhydryl (thiol), sulfate, thioether, amino, imine, amide, phosphate, phenol, imidazole, carbonyl (ketone), phosphodiester and phosphonate have potentials and metal binding properties which act as the functional groups in biomass [29,40]. Due to ionization of the functional groups most microbial cell surfaces are negatively charged [41], and serve as cation-sequestering mechanism.

There is some indication that confirms the direct participation of O-, N-, S-, or P-containing groups in binding certain metals ions. Various active sites which are involved in the metal uptake have been recognized by using diverse techniques [42]. Kumar et al. [9] studied the functional groups involved in the biosorption of Cr(VI), Ni(II) and Zn(II) with fungal biomass of *Trichoderma viride* and *Aspergillus niger* and bacterial biomass of *Bacillus brevis* and *Oligella urolytica*. The Major functional groups involved in metal binding with fungal biomass are bonded amino groups (-NH), hydroxyl groups (OH), carboxylate anions (COO<sup>-</sup>) and carboxyl groups (-CO) and with bacterial biomass are bonded hydroxyl groups (OH), amino groups (-NH),-CH stretching vibration of -CH<sub>2</sub> and -CH<sub>3</sub> groups. Loukidou et al. [43] observed the Fourier transform infra-red (FTIR) spectra of Cd(II) loaded and unloaded biomass of *Aeromonas caviae*. He reported the potential participation of amino, carboxyl, carbonyl, and phosphate groups in the biosorption of Cd(II). Cayllahua et al. [44] also studied the FTIR spectra to verify the existence of carboxyl, amide and phosphate groups in *Rhodococcus sp.* biomass. Carboxyl groups are negatively charged, available in large quantities and actively participate in binding of metal cations. Mishra and Doble pointed out that

carboxyl and amino groups were responsible for the binding of chromate [45]. Kang et al. [46] concluded that amine groups are protonated at pH 3 and attracted negatively charged chromate ions by electrostatic interaction. A study by Tan and Xiao [47] shows the contribution of carboxyl groups in the sorption of cadmium. When carboxyl groups were esterified, there was a decrease in the metal biosorption capacity. This was due to minimization of the number of carboxyl groups. After the material was hydrolyzed again, an increase in the biosorption

was observed. The structural changes were also studied using spectroscopic techniques like Fourier transform infra-red (FTIR), X-ray Photoelectron Spectroscopy (XPS) etc. The role of different groups can be illustrated using conventional techniques such as titration [48] or more advanced instrumental analyses such as FTIR, Raman microscopy, Energy dispersive X-ray spectroscopy (EDS), XPS, X-ray diffraction (XRD) etc. [49]. Each one can reveal certain information and thus can contribute to explain the actual mechanism of biosorption.

**Table 1. Merits and demerits of conventional technologies for metal ions removal**

Technologies	Merits	Demerits
Oxidation and reduction	<ul style="list-style-type: none"> <li>• Simple</li> <li>• Small installation costs</li> <li>• Easily applied to large water volumes</li> </ul>	
Chemical Precipitation	<ul style="list-style-type: none"> <li>• Solid obtained can be removed through sedimentation and filtration</li> <li>• Simple</li> <li>• Most of metals can be removed</li> </ul>	<ul style="list-style-type: none"> <li>• Large amounts of sludge produced</li> <li>• Disposal problems</li> </ul>
Chemical Coagulation	<ul style="list-style-type: none"> <li>• Simple.</li> <li>• Easily applied to large water volumes Low capital and operative costs</li> </ul>	<ul style="list-style-type: none"> <li>• High cost</li> <li>• Large consumption of chemicals</li> <li>• Low removal efficiency</li> <li>• Disposal of the arsenic-contaminated coagulation sludge may be a concern</li> </ul>
Adsorption (activated alumina, iron oxides/hydroxides, TiO <sub>2</sub> , cerium oxide, metals).	<ul style="list-style-type: none"> <li>• Simple</li> <li>• Not other chemicals required</li> <li>• Effective with water with high TDS</li> <li>• Useful at community or household level</li> <li>• Most of metals can be removed</li> <li>• High efficiency (&gt;99%)</li> </ul>	<ul style="list-style-type: none"> <li>• Cost of activated carbon</li> <li>• No regeneration</li> <li>• Performance depends upon adsorbent</li> <li>• Moderate efficiency</li> <li>• Interferences: Se, F<sup>-</sup>, Cl<sup>-</sup> and SO<sub>4</sub><sup>2-</sup> Application of point-of-use treatment devices needs regeneration and replacement</li> </ul>
Membrane process and ultrafiltration	<ul style="list-style-type: none"> <li>• Minimal membrane operation and maintenance</li> <li>• Less solid waste produced</li> <li>• Less chemical consumption</li> <li>• High efficiency (&gt;95% for single metal)</li> </ul>	<ul style="list-style-type: none"> <li>• High initial and running cost</li> <li>• Low flow rates</li> <li>• Removal (%) decreases with the presence of other metals</li> </ul>
Electrodialysis, electro dialysis with reversion of polarity of the electrodes.	<ul style="list-style-type: none"> <li>• Efficiency similar to reverse osmosis, effective in treating water with high TDS</li> <li>• Minimize scaling by periodically reversing the flows of dilute and concentrate and polarity of the electrodes.</li> </ul>	<ul style="list-style-type: none"> <li>• Very high costs</li> </ul>
Ion exchange	<ul style="list-style-type: none"> <li>• Effective removal</li> <li>• High regeneration of materials</li> <li>• Metal selective</li> </ul>	<ul style="list-style-type: none"> <li>• High cost</li> <li>• Less number of metal ions removed</li> </ul>

Source: [63-64]

**Table 2. Heavy metal/metalloids toxicity and their human health effects**

Metals	Effects		*Toxic concentration	*Treatment	References
	Acute	Chronic			
Ni	Dermatitis; nickel carbonyl: myocarditis, ALI, encephalopathy	Occupational (inhaled): pulmonary fibrosis, reduced sperm count, nasopharyngeal tumors	Excessive exposure: $\geq 8 \mu\text{g/L}$ (blood) Severe poisoning: $\geq 500 \mu\text{g/L}$ (8-h urine)		[65]
Cr	Gastrointestinal hemorrhage, hemolysis, acute renal failure ( $\text{Cr}^{6+}$ ingestion)	Pulmonary fibrosis, lung cancer (inhalation)	No clear reference standard	NAC	[66-67]
Cd	Pneumonitis (oxide fumes)	Proteinuria, lung cancer, osteomalacia	Proteinuria and/or $\geq 15 \mu\text{g/g}$ creatinine		[66-67]
As	Nausea, vomiting, "rice-water" Diarrhea, encephalopathy, MODS, LoQTS, painful neuropathy	Diabetes, hypopigmentation/hyperkeratosis, cancer: lung, bladder, skin, encephalopathy	24-h urine: $\geq 50 \mu\text{g/L}$ urine, or $100 \mu\text{g/g}$ creatinine	BAL (acute, symptomatic) Succimer DMPS	[66-67]
Zn	MFF (oxide fumes); vomiting, diarrhea, abdominal pain (ingestion)	Copper deficiency: anemia, neurologic degeneration, osteoporosis	Normal range: $0.6-1.1 \text{ mg/L}$ (plasma) $10-14 \text{ mg/L}$ (red cells)		[59,68]
Pb	Nausea, vomiting, encephalopathy (headache, seizures, ataxia, obtundation)	Encephalopathy, anemia, abdominal pain, nephropathy, foot-drop/ wrist-drop	Pediatric: symptoms or $[\text{Pb}] \geq 45 \mu\text{dL}$ (blood); Adult: symptoms or $[\text{Pb}] \geq 70 \mu\text{dL}$	BAL $\text{CaNa}_2 \text{EDTA}$ Succimer	[66-67,69-70]
Cu	Blue vomitus, Gastro-Intestinal irritation/ hemorrhage, hemolysis, MODS (ingested); MFF (inhaled)	vineyard sprayer's lung (inhaled); Wilson disease (hepatic and basal ganglia degeneration)	Normal excretion: $25 \mu\text{g}/24 \text{ h}$ (urine)	BAL D- Penicillamine Succimer	[70-71]

*\*<http://emedicine.medscape.com/article/814960-overview>*

*ALI, acute lung injury; MODS, multi-organ dysfunction syndrome; LoQTS, long QT syndrome; ATN, acute tubular necrosis; DMPS, 2,3-dimercapto-1-propane-sulfonic acid;  $\text{CaNa}_2 \text{EDTA}$ , edetate calcium disodium; NAC, N -acetylcysteine. BAL British Anti-Lewisite; MFF metal fume fever.*

**Table 3. Use of various Microorganisms for heavy metal (Ni, Zn and Cr) ions biosorption**

Microorganisms	Heavy metals	References
<b>Bacterial species</b>		
<i>Pseudomonas aeruginosa</i>	Cr	[10,32]
<i>Bacillus brevis</i>	Cr, Ni, Zn	[72]
<i>Bacillus sphaericus</i>	Cr, Ni	[73]
<i>Bacillus cereus</i>	Cr	[74]
<i>Bacillus pumilis</i>	Cr	[74]
<i>Thiobacillus ferrooxidans</i>	Zn, Cr	[75-76]
<i>Escherichia coli</i>	Cr, Ni, Zn	[77]
<b>Fungal species</b>		
<i>Penicillium Chrysogenum</i>	Zn, Ni	[78]
<i>Aspergillus niger</i>	Ni, Zn, Cr	[9]
<i>Aspergillus sydoni</i>	Ni, Zn, Cr	[9]
<i>Penicillium janthinellum</i>	Ni, Zn, Cr	[9]
<i>Termitomyces clypeatus</i>	Cr	[79]
<i>Mucor rouxi</i>	Zn, Ni	[41]
<i>Penicillium citrinum</i>	Ni	[11]
<i>Trichoderma viride</i>	Cr, Zn, Ni	[17,80]
<i>Streptomyces ciscaucasicus</i>	Zn	[81]
<i>Fusarium spp.</i>	Zn	[82]
<b>Algal species</b>		
<i>Lyngbya taylorii</i>	Ni, Zn	[83]
<i>Spirogyra spp.</i>	Cr	[18]
<i>Oscillatoria anguistissima</i>	Zn	[84]
<i>Apanothece halophutica</i>	Zn	[85]
<i>Sargassum spp.</i>	Zn	[86]
<i>Laminaria japonica</i>	Ni	[87]
<i>Oedogonium hatei</i>	Ni	[88]
<i>Cystoseira indica</i>	Ni	[89]
<i>Sargassum glaucescens</i>	Ni	[89]
<i>Scenedesmus obliquus</i>	Cr, Ni	[90]

#### 4. BINDING MECHANISM FOR BIOSORPTION OF HEAVY METAL

The binding mechanism of metal biosorption is a complex process. A number of factors such as type of biomass (i.e. living or non-living), property and chemistry of metal solutions, types of biomaterials, environmental factors such as temperature, pH, biosorbent dose etc influence the binding mechanism of metal biosorption. The process of metal biosorption involves a solid

phase (biomass i.e. biosorbent) and a liquid phase containing metal ion concentration (metal ions solution/wastewater). Due to biosorbent's higher affinity for the metal ions, the later is attracted and sequestered by various mechanisms [25]. The process continues till equilibrium is reached among the amount of solid-bound metal ion species and its fraction left behind in the solution. The complicated structure of microorganisms suggests that there are various ways of metal ions sorption by microbial cell [50]. The biosorption mechanisms are different and are not fully understood. They can be classified according to various criteria [51]. According to the reliance on the cell's metabolism, biosorption mechanisms can be:

1. Metabolism dependent
2. Metabolism independent/Non -metabolism dependent

According to the location where the metal ion sequestered from solution is found biosorption can be:

1. Extra cellular accumulation/precipitation
2. Cell surface sorption/precipitation and
3. Intracellular accumulation.

During non-metabolism dependent biosorption, metal ions are sorbed to the surfaces of microbial cells by physico-chemical interaction between the metal and the functional groups. These physicochemical processes comprises of a number of mechanism such as physical adsorption, ion exchange and precipitation [25]. As discussed earlier the cell surfaces of microbial biomass, mainly consist of polysaccharides, lipids and proteins have abundant metal binding groups such as carboxyl, phosphate sulphate, and amino groups [51]. This type of biosorption, i.e., non-metabolism dependent is relatively rapid and can be reversible [50]. In metabolism dependent biosorption the heavy metal sequestration from solution is associated with microorganism's active defense system [25]. When heavy metal are present, microorganisms can respond by producing specific compounds (like metallothionein, phytochelatins) inducing the precipitation or the chelation of the heavy metal ions [52-55]. Metal ion transport across the cell membrane produces intracellular accumulation, which depends on the cell's metabolism. It means that this type of biosorption may take place only with living cells. In the presence of toxic metal these living cells are mainly linked with an active defence system of microbes [15].

## 5. PARAMETERS INFLUENCING BACTERIAL BIOSORPTION

### 5.1 The Effect of Temperature

Metal biosorption by live biomass is considerably affected by the temperature as the metabolism of living cells depends upon temperature, and so change in temperature will strongly affect the biosorption processes. Adsorption reactions are exothermic; hence the rate of biosorption enhanced with decline in the temperature [56]. It is very significantly noticed from the experimental studies, that the rate of metal ion removal can be attained at the ambient temperature.

### 5.2 The Effect of pH

The pH of solution has been found to be the most important in biosorption among all other parameters, [17]. The chemistry of metal ions and biosorbents is influenced by initial pH of aqueous solution. It not only affects the solubility of metals but also affects charges on the sorption sites of biosorbents [57-58]. So, it is essential to know the ionisation states of the functional groups (hydroxyl, carboxyl, phosphate and amino groups) of the biosorbent [59] and the metal solution chemistry as well at varying pH values.

### 5.3 The Effect of Initial Concentration of Metal Ions

The rate of biosorption depends upon the charge and mobility of the metal ions present in the aqueous solutions. The metal ions favour to be biosorbed at oppositely charged centers on the cell surfaces of the biosorbents. Consistently it has been observed that rate of biosorption process decreases with the increase in the initial biosorbent concentration in the aqueous solution [60].

### 5.4 The Effect of Adsorbent Dose

The biosorbent dose strongly influences the level of biosorption process. An increase in biosorbent dose normally increases the amount of solute biosorbed, because of increased surface area of the biosorbent, which consecutively increases the number of binding sites [61-62]. A significant factor at higher biosorbent dosages is that the available solute is inadequate to absolutely cover the available exchangeable sites on the biosorbent, and ultimately results in low solute uptake [33].

## 6. CONCLUSION

Biosorption puts forward a cost-effective feasible technique for economic/productive removal and recovery of metals from waste streams/aqueous solution. The natural biosorbents such as bacteria, algae, fungi etc possess heavy metal sequestering properties and can sequester rapidly and effectively dissolved metal ions out of diluted solutions as they contains a variety of functional groups on their cell surfaces. Therefore biosorption is ideal process for handling the large volume and low concentration industrial effluents. Binding mechanism involved in the biosorption study includes physical adsorption, transport across cell membrane, precipitation and ion exchange. On the way to provide an efficient treatment technology, the appropriate selection of biomass and proper operational conditions must be documented. Biosorption necessitate exploration in multi-metal studies, structural studies of biosorbents, mechanistic modelling and development of biosorption capacity through alteration of biosorbents.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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