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Review Article
Biosorption of Chromium Using Bacteria: An Overview

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Abstract

Heavy metals present in high concentrations in aquatic systems are posing serious problems to aquatic life. Among the heavy metals, chromium has been used in many industries and hence its removal from waste waters is significant. Biosorption is an economic and ecofriendly method employed in the removal of heavy metals. Use of microbes like bacteria, algae, yeasts and fungi as biosorbents for heavy metal removal has received interest because of high surface to volume ratio, availability, rapid kinetics of adsorption and desorption and low cost. The objective of the present study is to review the removal of chromium from aqueous solutions using materials of biological origin i.e., bacterial biomass. This review also discusses the significance of chromium removal from waste water and the potential of biosorbents and biosorption technology in the removal of chromium.

Key words: Heavy metals, chromium, biosorbents, bacteria, biosorption

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rapid industrialization had a significant impact on the quality of natural water systems due to enormous discharge of heavy metals[1,2]. The release of untreated industrial wastewater into the environment has become the foremost concern in developing countries which is viewed as one of the most important environmental issues. Heavy metal pollution is a most important problem, as it leads to toxicity, risk to human survival and disruption of ecological balance[3]. The occurrence of toxic heavy metals in the environment is either geogenic or anthropogenic.

Natural and anthropogenic sources: The metals are classified into three groups, including toxic metals (such as Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc.), precious metals (such as Pd, Pt, Ag, Au, Ru etc.) and radionuclides (such as U, Th, Ra, Am)[4]. Toxic heavy metals entering into the ecosystem result in geo-accumulation, bioaccumulation and biomagnification processes which have intense ecological and public health implications[5].

Chromium (Cr) is a naturally occurring heavy metal, commonly found in the environment in two valence states: Trivalent Cr (III) and hexavalent Cr (VI). It is widely used in industrial processes, such as leather tanning, electroplating, metal finishing and chromate preparation processes, thereby, contaminating many environmental systems[6].

Toxicity: Hexavalent chromium is water-soluble and it is considered as a toxic pollutant by the United States Environmental Protection Agency (EPA). It has been reported to be mutagenic and carcinogenic to animals and humans[7]. As chromium is non-biodegradable and toxic in nature, removal of Cr from the industrial effluents is mandatory before disposing them in water bodies. Various methods are used to determine the level of heavy metals in industrial effluents.

Chromium removal: Reduction of Cr (VI) to Cr (III) serves as a key process in the removal of Cr (VI) from wastewater. Different technologies are available like chemical precipitation, coagulation, ion exchange, membrane technologies, adsorption and reverse osmosis[8]. The most common conventional method for Cr (VI) removal is reduction to Cr (III) at low pH and precipitation of $Cr(OH)_3$ by increasing pH to 9–10 with lime. The disadvantage of precipitation method is the need to dispose of the solid waste. In addition, the methodologies mentioned above become ineffective with Cr (VI) concentrations below 2 mM[9].

Adsorption technology: Literature suggests the possibility of using adsorption technology with activated charcoal and electrode-based adsorbent for the removal of chromium and other metals[10]. Red mud, sand and fly-ash from fertilizer industries are cheap, freely available and suitable showing good adsorption capacity for heavy metals after little treatment[11,12]. Activated carbon adsorption has been reported to be the most efficient process for Cr (VI) removal from wastewater. Activated sludge is considered as the low cost adsorbent for remediation of heavy metal contaminants[13,14]. The industrial waste materials like carbon slurry have also been reported to be useful in remediation of hexavalent chromium, fluoride and dyes[15,16,12]. Moreover, due to their adsorption properties carbon nanotubes are employed in chromium removal[17].

Microbial biosorption: However, most of these methods have severe drawbacks such as high capital and operational costs and the treatment and disposal of residual metal sludge. Hence, these processes are economically not sustainable on a large scale. Application of microbial technology is ecofriendly as microorganisms are adequate with high growth rate and reflect the suitability to natural environment compared to chemically treated sorbents. They not only adsorb heavy metals but also convert highly toxic metals to less toxic forms through oxido-reduction processes. Bioremediation is the most promising biological process in the treatment of contaminated water and soil to remove heavy metals. The process of heavy metal removal by biological materials is known as biosorption and the biological materials used are called as biosorbents. Various biosorbents like bacteria, fungi, yeasts and agricultural products have been used for biosorption. Some chromate resistant bacteria like *Pseudomonas fluorescens*[18], *Enterobacter cloacae*[19] and *Acinetobacter* sp.[20] have been shown to remove chromium from industrial effluent.

Mechanisms of biosorption: Elucidation of the mechanisms of metal removal is essential to develop technologies related to metal recovery[21,22].

- Metal cations may bind on cell surfaces (biosorption) within the cell wall (bioaccumulation) and in turn, metal uptake is augmented through microprecipitation
- Metal ions may be actively translocated inside the cell through metal binding proteins
- Metal precipitation may occur when heavy metals react with extracellular polymers or with anions (e.g., sulphide or phosphate) produced by microbes

- Metal volatilization through enzyme mediated biotransformation

Cell wall of Gram-positive bacterium contains relatively higher percentage of cross-linked peptide-based peptidoglycan. The cross linking of peptides is usually not found in Gram-negative bacterium cell wall. The cross linking of peptides in peptidoglycan matrices result in peptidoglycan sacs. These sacs are flexible and hence molecules can easily fit in these sacs. The peptidoglycan of Gram-positive bacterium contains polymers of glycerol and ribitol joined by phosphate groups. These polymers have unique characteristics and are termed as teichoic and teichuronic acids, which bind to the peptidoglycan with covalent bond or attach with lipids of plasma membrane. Teichoic acids extend to the outer surface of the peptidoglycan, which creates a negative charge on bacterial cell surface. Various studies proved teichoic and teichuronic acids having significant role in metal ion binding. The cell wall of Gram-negative bacterium contains lipopolysaccharides (LPS), proteins and phospholipids. The presence of LPS on the cell wall of Gram-negative bacterium renders net negative charge on its outer surface. Phosphate and carboxyl groups present in LPS and phospholipids are the primary sites of metal ion binding[23,24].

Removal of chromium by bacteria: Bacteria detoxify chromium mainly by reducing Cr (VI) to Cr (III), through Cr (V) and Cr (IV) intermediates[25] and it is a potentially useful process in the remediation of Cr (VI)-affected environments. Reduction of Cr (VI) to Cr (III) can be performed by a wide range of bacteria including., *Pseudomonas aeruginosa*[26], *P. synxantha*[27], *P. putida*[28], *P. ambigua*[29], *P. fluorescens*[30], *P. dechromaticans* and *P. chromatophila*[31]. Bacteria from other genera that have been shown to reduce Cr (VI) include *Acinetobacter lwoffii*[32], *Bacillus megaterium*[31], *Aeromonas dechromatica*[30] and *Escherichia coli* ATCC 33456[33,34]. Sulfate-reducing bacteria like *Desulfovibrio desulfuricans* and *D. vulgaris* have also been reported to reduce Cr (VI)[30]. Some extremophiles have been found to reduce Cr (VI) like the radiation-resistant *Deinococcus radiodurans*[35] and *Thermoanaerobacter ethanolicus* isolated from subsurface sediments[36] and *Pyrobaculum islandicum*, which is capable of reducing Cr (VI) at high temperatures[36]. Resistance to Cr (VI) has been investigated in *Pseudomonas aeruginosa*, which has been attributed to the decreased uptake and/or enhanced efflux of Cr (VI) by the cell membrane[25]. A similar resistance mechanism has been reported in *Cupriavidus metallidurans* CH 34 which is resistant to eight metals including CrO_4^{-2}[37,38].

Hexavalent chromium is toxic to bacteria present in contaminated soil or waste water. The bacterial strains growing in toxic conditions are assumed to be tolerant/resistant to chromium[39]. *Pseudomonas* sp. was the first hexavalent chromium resistant strain isolated from waste water[40]. Resistance is the ability of the microorganism to survive toxic effects of metal exposure by means of detoxification mechanisms produced in direct response to the metal concerned. Tolerance is the ability of the microorganism to survive metal toxicity by means of intrinsic properties and or environmental modification of toxicity[41]. The reduction of Cr (VI) can be identified by using diphenyl carbazide and read at 560 nm. Bacterial strains isolated from electroplating industry showed higher reduction rate[42].

Bacillus coagulans isolated from electroplating industry was capable of reducing Cr (VI) with soluble enzyme and utilizing malate as external electron donor. The biological reduction of Cr (VI) is similar to sulphate reduction process. The ability of sulphate reducing bacterial bioflims[43] to reduce Cr (VI) to insoluble Cr (III) using lactose as carbon source was reported. Almost 88% of 500 µmol L^{-1} was removed by bacteria within 48 h and 80% of Cr was precipitated by biofilm and this study proved sulphate reducing bacteria exhibiting the ability to reduce chromium in soil. Sulphate reducing bacteria reducing Cr (VI) under anaerobic conditions were isolated from metal contaminated marine sediments of Tokwawan, Hong Kong SAR[44]. The enrichment consortium almost completely (98.5%) reduced 0.6 mM Cr (VI) in 168 h. *Bravibacterium* sp. CrT-13, CrT-11, CrT-12, CrT-14 isolated from industrial wastewater exhibited ability to reduce Cr (VI) at various concentrations.

The bacterium *Providencia* sp., isolated from the contaminated site of chemical industry was found to remove Cr (VI) to 100% at concentrations ranging from 100-300 mg L^{-1} and 99.31% at 400 mg L^{-1}, pH 7 and temperature 37°C. It also exhibited cross metal resistance to Pb, Co, Hg and Zn. Water soluble Cr (VI) decreased from the initial concentration of 383.8-1.7 mg kg^{-1}. Exchangeable Cr (VI) and carbonates-bound Cr (VI) were removed by 92.6 and 82.4%, respectively. Bacterial consortia also help in chromium removal from soil and wastewater[45].

Many bacterial species like *Bacillus circulans*, *B. megaterium*, *B. coagulans*, *Agrobacterium radiobacter* EPS-916, *Bacillus* spp., *Pseudomonas fluorescens, Bacillus* sp. QC1-2, *Microbacterium liquuefaciens*, *Zoogloea ramigera* and *Pseudomonas aeruginosa* have been tested for chromium removal[46-55] (Table 1). The highest percent removal of chromium was observed for *Agrobacterium radiobacter* EPS-916, *Bacillus* spp., *Pseudomonas fluorescens* and *Bacillus* sp. QC1-2[50,52,53,55], while the lowest removal was noticed for *Zoogloea ramigera*[51].

Table 1: Biosorption of Cr (VI) using various bacterial strains

Bacterial strain	Cr (VI) removal (%)	References
Bacillus *circulans*	48	Khanafari *et al.*[46]
Bacillus circulans	34.5	Sarah *et al.*[47]
Bacillus megaterium	32	
Bacillus coaglans	23.8	
Bacillus megaterium	30.7	Srinath *et al.*[48]
Bacillus coagulans	39.9	
Microbacterium liquuefaciens	90	Pattanapipitpaisal *et al.*[49]
Bacillus sp. QC1-2	99	Campos *et al.*[50]
Zoogloea ramigera	3.40	Nourbaksh *et al.*[51]
Agrobacterium radiobacter EPS-916	99	Llovera *et al.*[52]
Bacillus spp.	99	Pun *et al.*[53]

Table 2: Chromium (VI) removal experiments carried out in our laboratory

Bacterial strain	Cr (VI) removal (%)	References
Pseudomonas aeruginosa strain1	86	Pandian *et al.*[54]
Pseudomonas fluorescens	99	Devi *et al.*[55]
Pseudomonas aeruginosa strain2	80	Unpublished

Laboratory findings: Physiological and genetic features of Pseudomonas make it a promising agent for utilization in biotechnology, agriculture and environmental bioremediation applications. Some strains of *Pseudomonas* spp. were shown to have a significant role in the bioremediation of heavy metals and pesticides[56]. Table 2 shows the chromium (VI) removal experiments carried out in our laboratory. The highest chromium (VI) removal was observed in 99% using *Pseudomonas fluorescens.* But the *Pseudomonas aeruginosa* indicated the lowest chromium (VI) removal at 80%.

When different concentrations of hexavalent chromium were used with nutrient agar, *Pseudomonas fluorescens* was able to resist up to 1000 ppm of hexavalent chromium. Highest hexavalent chromium uptake (99% removal) was found to be at 800 ppm concentration from 5-8 min. Bacteria express a wide range of complex molecules on their cell wall, which confer anionic net charge to the cell surface at acidic pH values. When the cell wall is in direct contact with environment, negatively charged groups are able to attract and bind to metallic cations based on electrostatic forces, without cellular energy consumption which is favored by high surface volume ratio in bacteria[57].

CONCLUSION

The sources, toxicity and removal methods for chromium have been reviewed. Among the methods available for Cr removal, biosorption using bacteria had several advantages and they are discussed with the reports available from the literature and also from present laboratory findings.

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