

Hexavalent Chromium: Toxic and Genotoxic Effects and its Bioremediation Strategies

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ABSTRACT

A silver-colored heavy metal Chromium (Cr) which exhibits different oxidation states (-2 to +6) has physical properties like being lustrous, brittle, odourless and, tasteless. It has many physicochemical and biochemical properties due to its trivalent chromium (Cr(III)) and chromium (Cr(VI)) electronic structure. Being an extensive metal it has many industrial uses such as in electroplating, leather tanning, pigments, petroleum refining etc. It is a major concern to us as it is non-biodegradable in nature, its chemical treatment results in production of more secondary pollutants. Consequently, when the heavy metals are emitted out of the industries into the environment their concentration should be brought down to an admissible level. The chemical remediation of the Cr(VI) residues is expensive. Hence, bioremediation of this toxic Cr(VI) metal is the focus of this article. Microorganisms or microcosms that can remediate Cr(VI) exhibit distinctive strategies (depends upon the quality, bio-sorbent and further estimating the presence of reductants) such as biosorption, biotransformation and bioreduction. It is very beneficial for Cr(VI) removal in this way, overcoming all the difficulties related between research facilities and modern applications for chromium remediation.

Introduction

Chromium was first discovered by Louis Nicholas Vauquelin as an element ($PbCrO_4$) along with molybdenum and tungsten that belong to the transition group (VI-B) in the modern periodic table [1-5]. This heavy metal is toxic to environment as it contaminates the soils and sediments along with surface and groundwater due to man-made activities. ATSDR (Agency for Toxic Substances and Diseases Registry) states that Cr(VI) is listed under the 17 most toxic chemicals. Chromium occur in several states but the two most stable states are: one being soluble and highly toxic Cr(VI) and the other being comparatively less soluble and toxic Cr(III), which is 10-100 times less bio-available and more stable. Cr(VI), most toxic form of chromium as it is a strong clastogen because it has high oxidation potential, greater solubility in water and further can easily penetrate through biological surfaces. Cr(VI) before being ejected out from the tanneries into the external environment needs to be treated properly before outflow in the environment. Cr(VI) also has an adverse impact on plants such as like alteration in seed germination, reduced growth of roots, stems and leaves. Waste like phenol, chloride, sulphide, tannins and formaldehyde are generated

by tanning of Chromium. Even, less toxic Cr(III) gets converted into highly toxic Cr(VI) upon oxidation during this process. Chromium sulphate [$Cr_2(SO_4)_3$] is used in treating leather. As Cr(VI) is a very stable compound it can sometimes get deposited in the food chain which leads to diseases like stomach irritation, cancer in digestive tract, brain damage, diarrhea, premature death in mammals etc. that can even be even fatal to higher organisms. According to USEPA (United States Environmental Protection Agency) hexavalent chromium is regarded as priority pollutant and listed as class 'A' human carcinogen [6-10].

Some of the methods like reverse osmosis, precipitation, chemical reduction followed by ion exchange and absorption (coal, activated carbon and alum) are generally used to purify the Cr(VI) residues released from the industrial effluents. Besides, being economically expensive the major drawback of these methods is partial removal of metal, high reagent and requires too much energy which leads to the production of toxic material which again need disposal. Most practical methods for reducing Cr(VI) which is toxic in nature to less toxic form Cr(III) is by biological

reduction of Cr(VI) through microorganisms and their enzymes [11,12]. Cr(VI) can also be reduced through wide variety of bacteria which are, *Staphylococcus aureus*, *Stenotrophomonas maltophilia*, *Pediococcus pentosaceus* etc., in presence of air(aerobic) or absence of air(anaerobic) or both. The ability of bacteria to reduce is due to environmental conditions and further, Cr resistant bacteria can undergo bioreduction for long duration. Structural similarity among the molecules, leads to Cr(VI) intake through the sulfate transport channel which occurs in bacterial membranes due to rapid mutation in contaminated environment. Chromium reducing bacteria has different chromate reductase genes such as ChrR, YieF, LpDH and NemA, that are used in catalysing depletion of Cr(VI) to Cr(III). Major source of Chromium contamination is through leather industry, concentration (Between 2000 to 5000 mg/L) which is much more from the acceptable range (2 mg/L) in case of effluents. The aim of this article is to confess the dangerous impact of Cr(VI) including strategies for the use of either naturally occurring or deliberately introduced microorganisms to consume and break down Cr(VI) pollutant in order to remediate the polluted site (bioremediation) [13-15].

Essentiality of Trivalent Chromium

Cr can occur in several oxidation states, depending upon the human exposure. Cr(III) is a part of GTF(Glucose Tolerance Factor), which has an important dietary component that increases the action of insulin and hence regulate the carbohydrate metabolism. Insulin activity is also enhanced by Cr(III), when it attaches to insulin, further boosts its impact by triple times. Thus, Cr deficiency may cause diseases related with Carbohydrates and weight loss [16]. Even nutritional supplements, foods and multivitamins consist of Cr(III) carrying compounds. The supplementation products are as follows: CrPic (Chromium Picolinate), CrHis (Chromium histadinate), CRDC (Chromium dinicocysteinatate), and NBC (Niacin-bound Chromium). As Cr(III) compounds cannot cross the membranes, so they get collected around the cells which leads to the variations in functioning of cells and damage of membrane in cell. It was reported that Cr(III) containing healthy addition for example CrPic can encourage DNA damage outcomes. Within biological media, series of chemical changes can also undergo in Cr(III). In 2016, Cr(III) was the fourth most selling supplement in USA. Behind calcium, magnesium, and iron one can take Cr(III) in their diet within the range of 23-29 µg/day. The dietary intake of Cr(III) has been beneficial for diabetes and in the process of anabolism of muscle and loss of weight according to some studies. But the evidence of it is not very strong and pretty controversial, moreover

some studies even revealed that too much intake of chromium is carcinogenic [17-22].

Physical and Chemical Methods for Remediation of Cr(VI)

Physio-chemical properties of substances for remediation is explained through physical and chemical methods. Physical methods of remediation involves techniques like electro dialysis, adsorption, membrane filtration, capping, photocatalysis, soil washing etc. whereas chemical remediation involves the use of varied variety of chemicals like sodium metabisulfite, sulphur dioxide, sodium sulfite, ferrous sulphate barium sulfite, limestone and lime are used for reducing Cr(VI) to Cr(III). Photocatalysis is a process through which Cr(VI) can be reduced to Cr(III). Cr(VI) reduction can be done through organic acids that are conjugated with the absorption of Cr(III) in soil. Cr(VI) reduction to Cr(III) is highly impacted depending upon soil type, its texture, composition, pH, conductivity, and many different physical properties. However, all the transition metal ions which are found in soil can acts as catalyst during the process of reduction. Manganese found in soil materials plays a key role in Cr(VI) reduction rapidly to Cr(III) [23-26].

The photolytic Cr(VI) reduction through Titanium dioxide (TiO₂) at pH 3 even when the organic compounds are present or absent depending upon the recent studies. Cr(VI) ions in water can also be reduced through highly active photocatalyst which is Lanthanum titanium oxide (La₂Ti₂O₇). Few of the physical and chemical methods are feasible and help metal recuperation, whereas some of the procedures are not that effective and cannot be used daily as it is an expensive procedure to carry out, requires pretty high energy consumption and cannot synthesis the secondary pollutants. Most of these procedures work efficiently when there is a presence of high concentration of metal and its effectiveness depends upon the amount agents with which it can interfere. For the promotion of sustainable and economic method for heavy metal removal the disadvantages of physical and chemical methods are needed to be considered. For typical cleanup technologies bioremediation is a good alternative. Bioremediation is the effective procedure which can eliminate heavy metal pollutants from the living bodies. For metal bioremediation the usage of microbial techniques, comprises of great ability to eliminate heavy metals depends upon its less cost and less secondary waste generating technique. Hence, bioremediation is seen to be an effective methodology for eliminating Cr from polluted environment in an eco-friendly and economical manner (Table 1).

Table 1: List of available methods for Chromium remediation.

Physical Methods	Chemical Methods	Biological Methods
Adsorption	Hydrogen sulfide (H ₂ S)	Biosorption
Ion exchange	Sodium dithionite (Na ₂ S ₂ O ₄)	Bioreduction
Membrane filtration	Sodium metabisulfite (NaHSO ₃)	Bioaccumulation
Electrodialysis	Calcium polysulfide (CaS ₅)	Biomineralization
Reverse Osmosis	Ferrous sulfate (FeSO ₄)	Extracellular precipitation
Electro-kinetics	Calcium metabisulfite (CaHSO ₃)	
Granular activated Carbon	Calcium metabisulfite (CaHSO ₃)	

Molecular Mechanisms involved in Bioremediation of Chromium

Different research workers discovered the molecular mechanism to reduce the toxic Cr(VI) to Cr(III) by biosorption, bioaccumulation and bioreduction. Bacterial cells on coming in contact with increased concentration of chromium starts producing ROS(Reactive Oxygen Species) to tolerate Chromium stress. Different bacteria have different mechanism to tolerate the

high Cr concentration. Chromium can attach to the cell surface of the microorganisms by a chemical bond which is formed between the functional groups that are found on the cell surface structures of microorganisms which has a different glycolipid, proteins, glycoproteins, polysaccharides, and metal ions. Cr(VI) gets reduced into Cr(III) and gets precipitated on the surface of the membrane tissue during adsorption [27-30]. The reduction is catalyzed by a chromate reductase gene or occurs spontaneously.

Through sulfate transport channel present on the membranes of the eukaryotes and prokaryotes the chromate gets transported very actively. With the help of certain specific metal binding proteins, chromium gets transported and finally it gets reduced into less toxic Cr(III), via highly unstable oxidative states. When oxygen is present, any endogenous reserve or NADH, NADPH serve as electron donor but when oxygen is absent Chromium(VI) itself serves as the terminal electron acceptor on the respiratory chain and it often utilizes proteins from cytochrome families like cytochrome b and cytochrome C Efflux of the excess Chromium(VI) from microbial cells usually occurs through a transmembrane protein (efflux pump) and it is encoded by plasmid gene ChrA. Same type of methods has been developed in plants to protect them against Chromium, such as the accumulation of Chromium ions in vacuoles, their binding with organic ligands, scavenging of Chromium generated ROS via antioxidative enzymes (Table 2).

Table 2: Microorganism's genes involved in Cr(VI) biodegradation along with their molecular mechanism.

S.no.	Microorganisms	Genes/Proteins Involved	Mechanisms
1	<i>Candida utilis</i>	Membrane bound chromate reductase	Extracellular reduction of Cr(VI) to Cr(III)
2	<i>Aspergillus tubingensis</i>	Not much is known about the genes/ proteins involved	Decrease in Cr(VI) concentrations in the surrounding by means of metal chelating agents
3	<i>Aspergillus niger</i>	Not much is known about the genes/ proteins involved	Biosorption of Cr(VI) into the cells followed by subsequent reduction
4	<i>Saccharomyces cerevisiae</i>	Protein kinase CK2	Reduction of Cr(VI) toxicity level by decreasing up take of Cr(VI)
5	<i>Paecilomyces lilacinus</i> Strain: MR-1	Not much is known about the genes/ proteins involved	Biotransformation and bioaccumulation of Cr(VI) in the biomass producing highest reduction rate during log phase of growth
6	<i>Shewanella oneidensis</i>	MtrA, MtrB, MtrC and OmcA	Dissimilate Cr(VI) extracellularly by biosorption through biofilms
7	<i>Thermoanaerobacter thermo-hydrosulfuricus</i> Strain BSB-33	NAD(H)nitrite reductase Quinone oxidoreductase Superoxide dismutase	Anaerobic reduction of Cr(VI) Reduction of Cr(VI) preventing formation of ROS and lesser oxidative stress Decrease oxidative stress due to chromate reduction
8	<i>Stenotrophomonas maltophilia</i>	ChrR	Cr(VI) reduction by formation of biofilms
9	<i>Enterobacter cloacae</i> B2-DHA	ChrR and ChrA	Membrane bound chromate reductase enzymes help in transferring electrons to Cr (VI) by NADHdependent cytochromes
10	<i>Klebsiella pneumonia</i> and <i>Raoultella ornithinolytica</i>	FMN reductase genes	Chromate reduction by means of flavodoxin-2 superfamily
11	<i>Bacillus cereus</i> SJ1	Putative chromate transport operon, chrIA1, and two additional chrA genes encoding putative chromate transporters	ChrA is a membrane transporter that causes efflux of chromate ions from the cytoplasmic membrane

12	<i>Pseudomonas putida</i>	YieF chrR (enzyme)	NADH dependent reaction transferring two electrons to Cr(VI) to form an unstable intermediate Cr(V) and then transferring one electron to Cr(V) to reduce it to Cr(III)
13	<i>E. coli</i>	Nem A	Cr(VI) reductase reduces Cr(VI) using NADH as a cofactor Carries out an obligatory four electron reduction of chromate by transferring three electrons to chromate to produce Cr(III) and one electron to molecular oxygen
14	<i>Alcaligenes eutrophus</i>	ChrA	chromate resistance plasmids
15	<i>Pseudomonas aeruginosa</i>	ChrA	chromate resistance plasmids
16	<i>Pseudomonas denitrificans</i>	Fer B	Exhibit activity similar to chromate reductase in reducing Cr(VI)
17	<i>Enterobacter cloacae</i> Strain: HO1	Membrane associated enzyme	Electron transfer to Cr(VI) by NADH dependent Cytochrome reducing Cr(VI) to Cr(III)
18	<i>Vibrio harveyi</i>	Nfs A/ Nfs B	Possess chromate reductase activity as a secondary function for reduction of Cr(VI)

Bioremediation using Bacteria

Bacterial remediation in case of Chromium is counted as an easy, economic, and little energy consuming procedure which can be easily accomplished by utilizing native, not harmful strains of bacteria. The transformation of Hexavalent chromium into less toxic Trivalent chromium is usually done by utilizing the microbes. Evacuation of Cr(VI) by bioreduction, biosorption and bioaccumulation could be easily done by microorganisms (both gram positive and gramnegative). The misuse of electroplating of industry diminished Cr(VI) by utilizing a solvent chemical and malate as an outer electron contributor, *Bacillus coagulans* can be isolated. *Bacillus circulans*, *Bacillus megaterium* and *Bacillus coagulans* are known for its interesting capacity of chromium biosorption. Moreover, *Bacillus coagulans* and *Bacillus megaterium* exhibit living and dead cells capacity for biosorption which were contradicted, and dead cells were found to be more powerful for chromium biosorption. For example, *Bacillus coagulans* living cells absorbed 23.8mg Chromium g⁻¹ dry weight, whereas dead cells absorbed 39.9 mg Chromium g⁻¹ dry weight. The comparable outcomes for *B. megaterium* were found 15.7 mg Chromium g⁻¹ for living cells whereas 30.7 mg Chromium g⁻¹ dry weight for dead cells. But the better presentation of latent/dead biomass was observed for living cells because of their defenceless action for poisonous impacts of metal particles which can prompt cell passing during the metal expulsion measure [31].

In some of the experiments performed with intact cells and cell free extracts of *Arthrobacter* species and *Bacillus* species, it was found that *Bacillus* species could be substandard when compared with *Arthrobacter* species as far as their resistance to hexavalent chromium and its decreasing capacity. Reduction of Cr(VI) due to the cell free extract of *Arthrobacter* species, demonstrated the inclusion of certain solvent proteins in the reduction process. It has been more broadly elucidated the contribution of a dissolvable protein during reduction of Cr(VI) to Cr(III) by *Pseudomonas putida* and this protein can use NADH or NADPH as an electron donor. How *Pseudomonas* species can evacuate the cancer-causing Cr(VI) by the

usage of a blended populace has also been reported. This report got much more highlight due to the decrease of chromate through a sulfur lessening bacterium which was detached from metal sullied marine residue of Tokwanan, Hong Kong in absence of air. There was huge decrease of chromate brought in in 168hours by the improvement of bacterial consortium (98.5%).

This microbial consortia were developed by immobilized cells of *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Saccharomyces cerevisiae* on a strong help which developed a huge ability in expulsion of Cr(VI) from tannery effluent. The microbial consortium which was created by using native microscopic organisms from tannery squander defiled biotopes can lessen Cr(VI) at different focuses going from 100 to 300 mg Chromium L⁻¹ of a wide scope of pH, utilizing an assortment of electron contributors. Initially *Pseudomonas* species was found to obstruct towards Cr(VI), later on, this mechanism was found in *Cupriavidus metallidurans*, *Ochrobactrum tritici* and so on Chromium safe microorganisms with high remediation productivity under both high impact and in absence of air have been dissociated from highly polluted premises of TCCL(Tamil Nadu Chromates and Chemicals Limited), 0.15mg of bacterial cells g⁻¹ of soil and molasses have been used to fill greatest chromate decrease as a carbon source. Gram positive bacterium *Corynebacterium paurometabolum* which has properties of biosorption, just as biotransformation, also contributes to limit the poisonousness of chromium mixes can also be utilized (Table 3).

Fungi Being used in Bioremediation

Fungi has also been reported and introduced for remediating the sites which are contaminated by Chromium. In case of fungi biosorption has been found to be productive than bioreduction. In *Aspergillus oryzae*, *Aspergillus niger*, *Trichoderma* species and *Fusarium oxysporum* the biosorption of Chromium has been discovered. Both metabolism dependent and independent approaches are utilized by fungi for Chromium biosorption. During adsorption of Chromium(VI) on the fungal cell surface it usually forms a chemical bond with the functional groups which are usually present on the cell surface proteins. The system which helps to

depict the functional groups Carboxyl, carbonyl and hydroxyl helps to bind the Cr(VI) on the fungal cell wall surface. With the help of fungal strains many biosorptive products had been evolved and they have been studied how to remove chromium in an effective way. *Carica papaya* plant dry stems were used for the development of a biosorbent matrix in which cells of *Fusarium oxysporum* were collected. At the 5th day of incubation, the highest level of Chromium biosorption was achieved (90%). When *Aspergillus flavus*

biomaterial was charged with Fe²⁺ ions, it promotes the removal Chromium and the stickiness involved with the biomaterial which was developed. Even strains of marine seaweed like *Aspergillus flavus* and *Aspergillus niger* were tested for its capability of its capacity and accumulation of Cr(VI). The results were found to be more than 25% of the supplied Cr(VI). Some fungi like *Hypocrea tawa* and *Trichoderma inhamatum* can reduce Cr(VI) to Cr(III) [32].

Table 3: Remediation Chromium(VI) using bacteria.

S.no.	Microorganism	Isolation Site	Mechanism of Removal Cr
1	<i>Acinetobacter baumannii</i> L2	Crude oil samples	Biosorption, Bioreduction
2	<i>Pseudomonas stutzeri</i> L1	Crude oil samples	Biosorption, Bioreduction
3	<i>Ochrobactrum</i> sp.	Soil around electroplating Industry	Reduction
4	<i>Cellulosimicro-bium funkei</i> strain AR6	Leather industry effluent contaminated soil	Biosorption, Bioaccumulation & Reduction
5	<i>Corynebacterium Paurometabolum</i>	Procured from culture, collection bank	Biosorption, Bioreduction
6	<i>Acinetobacter junii</i>	Chromite mine site	Reduction
7	<i>Enterococcus casseliflavus</i>	Tannery effluent	Biosorption
8	<i>Pannonibacter phragmitetus</i>	Steel-alloy industry site	Reduction
9	<i>Acinetobacter</i> sp.	Tannery effluent	Reduction
10	<i>Providencia</i> sp.	Chromium contaminated chemical industrial site	Reduction
11	<i>Arthrobacter oxydans</i>	Basalt rock from contaminated site	Reduction
12	<i>Bravibacterium</i> sp. CrT-12 (live cells)	Tannery effluent	Reduction
13	<i>B. coagulans</i> (dead cells)	Treated tannery effluent	Biosorption
14	<i>B. coagulans</i> (live cells)	Treated tannery effluent	Biosorption
15	<i>B. megaterium</i>	Treated tannery effluent	Bioaccumulation, Biosorption
16	<i>B. circulans</i>	Treated tannery effluent	Bioaccumulation, Biosorption
17	<i>B. coagulans</i>	Electroplating waste discharge contaminated soil	Biotransformation

According to some studies even yeast can reduce to Cr(VI). Two yeast strains i.e., *Pichia anomala* M10 and *Pichia jadinii* M9 are isolated from the effluent of textile dry industry by them. Yeast with the help of chromate reductase (ChrR) enzyme can help to convert Cr(VI) into Cr(III), which is less toxic. According to some investigations biosorption of Cr(VI) is possible from aqueous solution and even sometimes from sewage of tannery industry with the help of Biomass /Polymer Matrices Beads (BPMB). By using immobilizing baker's yeast in alginate extract(3%) these beads were designed and Chromium concentration was reduced under favourable conditions at 200ppm of initial Chromium concentration. Even administration of fungal bacterial biofilms can remove toxic Chromium(VI). From the contaminated sites of Chromium: *Aspergillus*, *Fusarium* and *Penicillium*, are usually isolated. *Aspergillus* has the tolerance at 200g/mL, 600g/mL, 650g/mL with 5000g/mL for total chromium. Other fungus like *Aspergillus flavus* have had the tolerance of Chromium(VI) 600g/mL with 800g/mL for total chromium, *Aspergillus niger* has 600g/mL and 1000g/mL for total Chromium and *Avicularia versicolor* has the tolerance of 1000g/mL for Chromium(VI).

Conclusion

Uncontrollable and uneven excretion of heavy metals contaminated industrial effluent into the environment has led to a major concern for the environment of 21st century. Some techniques like biosorption and bioreduction has become effective for the remediation of highly toxic and carcinogenic Cr(VI). Biomass of microorganisms, fungi, plants, algae has been reported effective for the compelling of highly toxic hexavalent chromium reduction. The molecular remediation system also gave a better vision about Cr(VI) removal. These all helped in building up the current advances of chromium remediation to be progressively proficient. There is a wide gap between lab discoveries and efficient usage of bioremediation modification for elimination of Cr(VI). Many, important trials are practiced involving artificial solutions of chromium to investigate proficiency of the made framework of remediation in case of chromium. The ability in case of natural frameworks for the cure of Hexavalent Chromium polluted soil and mechanical effluent can be found. Moreover the descriptive discoveries are from the cluster method of the treatment with small

quantity of effluent. If we all are looking into all these issues, then the invention of integrated method for the better and continuous elimination of Hexavalent chromium from industries are not much far enough.

Till now we all are working on the culture dependent techniques which involves the culturing of organisms in the artificial laboratory conditions but this technique is not totally reliable for the remediation of toxic heavy metals as we know that very low number of microbes can be grown or cultured in artificial media in laboratories so we need to switch our work towards the culture independent techniques which involves metagenomics etc so that we can work in a broader way and on more microorganisms. By using culture independent technique basically we can explore more on an environmental community of microorganisms and not only on a single organism. This technique may reduce the gap between the laboratory work and its application on the remediation of Cr(VI) on the actual site in environment.

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