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Bacterial Response to Chromate Exposure - A Review

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Abstract- **Chromium, a heavy metal exists in diverse oxidation states, which determines its toxicity. The most stable oxidation states are Cr VI which is mobile and toxic and Cr III which is stable and nontoxic. Microbial remediation ranks first in comparison to other conventional techniques to minimize the chromium levels in environment. This review summarises the various morphological, metabolic and molecular changes exhibited by different microbes on exposure to chromium. Metabolic pathways reported like efflux mechanism, production of extracellular or intracellular chromium reductases, scavenging of the reactive oxygen species (ROS), DNA adduct formation etc. are adapted by various microbes to combat the chromium oxidative stress. These chromium reducing mechanisms are species dependant and each microbe has its characteristic pathway to resist or detoxify chromium. Among these mechanisms to remediate chromium, microbes that produce extracellular chromium reductases which will detoxify the chromium outside the cell are ideal. Then result would be that the chromate stress to the organism is minimal since scavenging of ROS or removal of DNA adducts will not arise and the problem of bioaccumulation including secondary contamination is avoided. The various changes in microbes induced due to exposure of chromium can be exploited as biomarkers for chromium contamination. This would also provide clues to engineer the microbes by manipulating the concerned genes to facilitate chromium detoxification. The paper helps to identify the microbes in free planktonic form or in consortia to effectively remediate hexavalent chromium toxicity so that the exposure is minimised and environment is protected.**

Keywords- **Hexavalent Chromium Toxicity, Microbial metabolism, Microbial remediation, Removal efficiency.**

I. INTRODUCTION

Chromium is a heavy metal that occurs in nature in its oxide form. It is transition metal of 3d series and hence is positioned in VI-B group of the periodic table. It is reported that chromium occurs in nine oxidation states ranging from Cr II to Cr VI [1]. Of these, Cr III and Cr VI are most stable [2]. Cr (0) is a solid steel grey coloured, used to make steels and alloys. It is produced by the oxide form of chromite ore, $CrFeO_4$. Chromium III occurs naturally in diverse parts of biosphere like rocks, plants, animals, volcanic dusts and gases. Cr III gets heated in presence of atmospheric oxygen and mineral base to produce Chromium VI and this is anthropogenic means generation of Cr VI and release into the environment. Cr VI is an environmental contaminant, at higher levels it is toxic and hazardous to animals and humans necessitating its removal [3].

II.CHROMIUM TOXICITY

Chromium VI is a strong oxidiser having a very broad spectrum of industrial applications like electroplating, tanning, dyeing etc. The industrial effluents carrying Cr VI contaminate the environment (soil, water and air), releasing it into environment. High solubility of Cr VI and Cr VI not being adsorbed by either soil or organic matter, make it highly mobile in soil and groundwater.Chromium VI, once released into the environment is governed by its eco-kinetic properties and reach to several ecosystems. Because of the chemical speciation, chromium enters the terrestrial and aquatic ecosystem and gets accumulated across the successive trophic levels of the food chain [4-5]. The chromates are isostructural with physiological sulphates and phosphates. Since they mimic these physiological molecules, they are easily taken up by the cells

throughsulphate transporters.Chromium VI is a potent carcinogen [6]. Chromium is identified as one of the top 17 chemicals that is hazardous to human health, thereby necessitating its removal to minimise the risk. The principle behindchromium removal or detoxification is conversion of hexavalent chromium to stable oxidation state, Cr III. Cr III is insoluble in water [2] and exhibits membrane impermeability[7].

Environmental Protection Agency (EPA) has notified permissible limits for hexavalent chromium. For inland surface water it is 0.1mg/L, in public sewers, the permissible level is 2.0 mg/L and in marine and coastal areas it is 2.0mg/L. (Table I)

TABLE I

Permissible limits of chromium as per EPA Notification

Sl. No.	Environment component	Chromium levels (ppm)
1.	Primary Drinking Water Standard	0.1
2.	Common Range in Soils ,	1- 1,000;
3.	Livestock Water Quality,	1
4.	Surface Water Quality	0.05
5.	Industrial effluents	2
6.	Land Application of Sewage Sludge	3000

Source-"Pollution Control Acts, Rules, and Notifications" Central Pollution Control Board, IV edition pp358-359.TERI Energy Data Directory & Yearbook, 2005-06.

Note: India has adopted EPA levels as standard permissible limits of chromium in water.

III. SIGNIFICANCE OF MICROBIAL REMEDIATION OF CHROMIUM

To treat an effluent, a technology is considered to be efficient if it is technically applicable, simple and cost effective [8]. Conventional physicochemical techniques like electrodialysis [9], electrocoagulation [10], limestone treatment [11], ion exchange resins [12], [13] have been reported to be remediating technologies. These are technologically intensive as well as expensive. Also, some adsorption techniques pose the formation of secondary contaminants in environment and its removal is cumbersome. Utilising microbes toremove the chromium from environment appears economical and a feasible technology as chromium could be detoxified effectively, causing least harm to the environment [14]. Bacterial reduction of chromium can happen in aerobic as well as anaerobic conditions [15].To persist in chromium rich environment, the microbes must be resistant to chromium and possess the potential metabolic pathways to combat the negative impact of chromium. Various bacteria occurring in nature like *Bacillus plumulis, Alcaligenesfaecalis* and *Staphylococcus sp*. [16], *Pseudomonas sp* [17].*Bacillus subtilis strainPESA* [18] have the ability to reduce Cr VI ,which is highly toxic into stable nontoxic Cr III. (Fig 1).

IV. CHROMIUM RESISTANCE AND REMOVAL IN MICROBES

The reduction of chromium by microbes is a detoxification mechanism and reduction can be direct or indirect. The direct Cr VI reduction are different in aerobes and anaerobes. In aerobes, the chromium is reduced by soluble chromate reductases consuming NADH or NADPH as cofactors [19]. In anaerobic condition, it is reported that Cr VI becomes terminal electron acceptor in electron transport chain [20].

In some microorganisms which possess sulphate transporters in their cell membrane, chromate is taken up by sulphate transporters as chromate is analogous to sulphates [21]. When chromate gains entry into the microbe, it is detoxified by various mechanisms like efflux through transporters, extracellular or intracellular degradation by chromium reductases, formation of DNA adducts, activation of ROS scavenging enzymes etc.in different bacteria. Ultimately, the chromium resistant microbes either convert it to a safe form to prevent oxidative stress or completely metabolise the hexavalent chromium through various metabolic pathways for nutrition.

In *Bacillus subtilis*, the chromium reduction is mediated by membrane bound enzymes that are constitutively produced. The chromium removal efficiency of 100% is achievable at pH 9 [22]. In *Enterococcus gallinarum*, chromium is reduced by the proteins of the cell that are either membrane bound or soluble [23].chromium reduction of 100% has been reported at concentration of 200mg/l in aerobic conditions at optimum temperature of 37^0C and pH of 10. In *Pseudomonas aeruginosa*, chromium stress is combated by removing the chromium by an effluxmechanism which is attributed to the functioning of a plasmid conferred *chr A* gene [24], [25]. *Pseudomonas aeruginosa*CRM 100 has been reported to remove up to 99.8% of chromium, the initial concentration being 100mg/L [26]. *Alkaligenesfaecalis* is found to reduce chromium up to 97% with an initial concentration of chromium reported to be 100µg/ml [16]. The reduction of chromium is by chromium reductase enzyme produced by *Alkaligenesfaecalis* [27]. *Bacillus subtilis PESA* strain is found to remediate chromium up to 93% at an acidic pH of 3 in a 500mg/L of concentration of media [18]. A tannery effluent isolate *Pseudomonas putida strain K*, is able to reduce 93% of Chromium VI in 24 hours at pH of 7.2. [28]. *Pseudomonas putida (MTCC 102)* is found to remove 88% of chromium after 96 hours at pH 5.2 [29]. In some organisms like *Pseudomonas putida* and *E.coli*, it is reported that reduction of Cr VI to Cr III takes place within the cell followed by salting out of Cr III to exterior of cells. The accumulation of Cr VI inside the cells activates scavenging of the reactive oxygen species (ROS) by various enzymes like superoxide dismutase, catalase, etc. [30]. *Bacillus cereus* is reported to have a removal efficiency of 76% [31].The chromium remediating capacity of *Bacillus cereus* is attributed to Constitutive expression of *chr A* genes [32].In *Arthrobactersp,* chromate efflux is through chrA transport protein.[33]. Extracellular chromium reductases, which are dependent on NADH, convert toxic CrVI to Cr III which is nontoxic [19]. In *Arthrobacterviscosus*, chromium removal efficiency of 72.5% and chromium uptake of 12.6mg Cr/g of biomass takes place at pH 4 [34].The first microbe, *Pseudomonas dechromaticans* reducing chromium was discovered in 1970s [35].

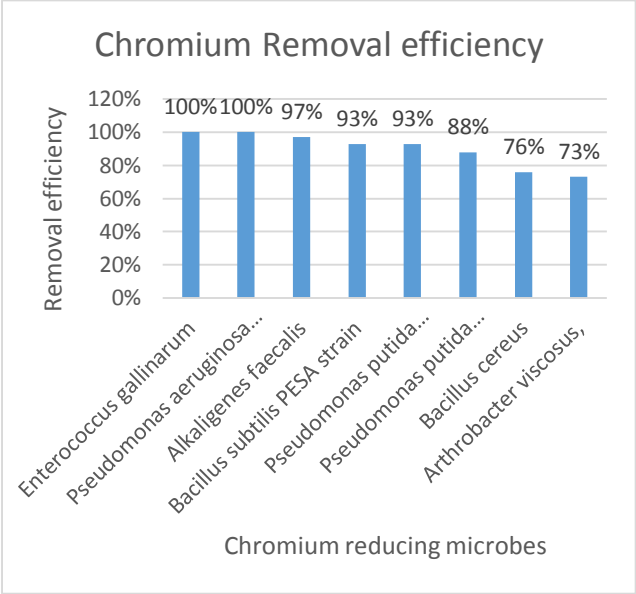

Fig. 1 Graphical representation of chromium removal efficiency of various microorganisms.

V. MICROBIAL METABOLISM AND CHROMIUM EXPOSURE EFFECTS

Many bacteria, tolerant to chromium are able to thrive in chromium rich environments, in spite of the chromate stress. This can be attributed to various metabolic changes that occur in these microbes on exposure to chromium. The rationale behind tolerance for heavy metals in microbes can be either plasmid mediated, [24], or due to genetic mutations, [36], or biotransformation [37] or by bioadsorption [34].Chromium tolerance and reduction are two different abilities of microbes. It is not the single characteristic of the group [38]. *Bacillus cereus* shows a very high minimum inhibitory concentration (MIC) of 750mg/L of chromium. When this organism is exposed to chromium, it shows a lag phase spanning 4 to 8 hours with a maximum growth at 24 hours, while in the control samples, which are not exposed to chromium exhibit a lag phase of 2 to 3 hours with a maximum growth at 20 hours. Because of its high

tolerance, it is a potential candidate for remediation of chromium [39]A marine bacteria *Bacillus licheniformis*, (deposited at NCBI GenBank;Accession Number-HM194725) is found to exhibit a very high tolerance to chromium in comparison with the terrestrial strains. It is reported to reduce a very high concentration of chromium up to 1500mg/L within 72 hours. This tolerance and efficiency of chromium reduction is attributed to the extracellular surface active agent (bio surfactant) produced by the microbe, that protects the microbe from oxidative stress [40]. The bacterial strains Rb-1 (*Pseudomonas aeruginosa*) and Rb-2 (*Ochrobactrumintermedium*), exhibit a good potential for bioremediation. It is reported that an initial chromate stress of 100 µg/L brings in a cascade of changes in biochemical parameters like high proline content, high nitrate reductase activity [41]. *E.coli k-12* strains, when exposed to chromate, within 3 hours of exposure, the cells assume filamentous morphology. The cellular levels of glutathione and other thiols are depleted, SOS response is activated, and level of proteins which counter oxidative stress ,such as sod B, cyst-K are increased. This is an adaptation by the microbe to combat the chromate stress. Minimizing theoxidative stress during chromate reduction will facilitate bioremediation [42]. The high chromium tolerance ability of the microbe *Ochrobactrumtritici5bvl1*, is attributed to the presence of a transposon *TnOtChr,* whose length is 7,189 base pairs. This belongs to the mixed group of Tn21/Tn3 transposon subfamily. This transposon stretch harbours a cluster of genes, *chrB, chrA, chrC*, and *chrF*. It is also reported that in *O.tritici* that are sensitive to chromium, the *chrB*and *chrA* genes, are crucial for establishment of high resistance, while *chrF* or *chrC*do not play a crucial role in resistance [43]. *Shwenellaodiensis,* when exposed to chromium, exhibits a spectrum of biochemical and molecular changes to combat the chromate stress. Major response to heavy metals like chromium include modulation of two strategies. Firstly, *Oxidative stress protection*, achieved by upregulation of a family of genes referred to as "resistance nodulation cell division protein family genes (RND)",which supports cation efflux and confers heavy metal resistance .Secondly, it is *detoxification*, due to activation of various stress related genes and sigma factor related genes (*RpoS, RpoH, RpoE)*. Perfect coordination of oxidative stress response and detoxification in *shwenella* accounts for its high chromium resistance and chromium reducing ability [44].

IV. MOLECULAR MECHANISMS INVOLVED IN CHROMIUM REMEDIATION

When the bacteria are exposed to chromium, the cells will continuously be in oxidative stress to get rid of chromium. As a result, genes concerned with the production of various chromium countering substances get regulated in those cells accordingly to combat chromium stress. The genes that are regulated vary in different bacteria and thus these genes can serve as markers to indicate chromium stress in a respective bacteria. The *chrA* genes in bacteria ,involved in chromium detoxification, are reported to be located either onchromosomal DNA or plasmid, or on both [45]. These are organised into operons. *Caulobactercresentus* is a ubiquitous microbe that can sustain low nutrient conditions which is a characteristic feature of toxic heavy metal contamination. On exposure to chromate, the principal response is upregulation of manganese dependant superoxide dismutase gene *sod A*. Sulphate transporters are downregulated to prevent the uptake of chromium. Glutathione s transferase (CC2311) is upregulated 6 folds and DNA repair enzymes CC2272 and CC2200 also are upregulated[46]. When *ShwenellaoneidensisMR-1* is chronically challenged with Cr VI for 24 hours, it is revealed by transcriptome profiling at the end of 24 hours exposure that, prophage related genes and other genes concerned with metabolic processes like metabolism of DNA, cell division, biosynthesis of cellular proteins, degradation of peptidoglycan are upregulated. But the genes that regulate transport binding proteins, chemotaxis and motility are repressed to a large extent. Transcriptome analysis by microarray profiling and two

dimensional liquid chromatography and mass spectrophotometry it is found that gene products of 14 open reading frames (ORFs) with annotations that correspond to mobile and extrachromosomal elements are upregulated. This indicates that chronic exposure to chromium and its derivatives lead to induction of lytic cycle [47]. In *Ochrobacteriumtritci,* resistance to Chromium VI and superoxide is conferred by chr BACF, an operon which is located on a transposable element [43]. There are chromium resistant determinants in Archea, Bacteria and Eukarya which are coded by genes of chromate ion superfamily [7]. In *Arthrobacter sp. Strain FB24*, chromium resistance determinants, a cluster of 8 genes are on a plasmid, that code for *chr A , chr B* and oxidoreductase coding genes [33]. *Pseudomonas corrugate28* is a chromate resistant bacterium. By phenotype microarray, it is found that gene *osc A* is involved in utilization of organosulpher compounds. It is located upstream of ABC transportergene and codes for a protein that binds with transcriptional unit of *sbp* which is overexpressed during chromate exposure. Sulphate uptake is modulated during chromate stress as chromates and sulphates, which are analogues, compete for the same sulphur binding proteins. As a result, *oscA-sbp* is overexpressed. The mutant strains in which the *osc A* is inactivated by insertional inactivation, gives rise to chromate sensitive strains. This indicates that functional *osc A* gene, principally accounts for chromate resistance in *P. Corrugate 28*[48]. In *Bacillus cereus sj 1*, which is a rapid chromium reducing organism is an aerobe. On chromium exposure, a chromate transport operon *chr l A* and two additional *chr A* genes are activated and upregulated. *chr A1* and *chr l* are upregulated on chromium exposure while the other two chromium transporter gene of the operon *chr2* and *chr 3* are constitutive [32]. *Pseudomonas putida*, *strain F1*, when exposed to chromate, in different media, LB and M9L with varied carbon sources, upregulation of proteins of different functions such as transcription, inorganic ion transport ,metabolism, amino acid metabolism are triggered. These proteins possess the potential to serve as the indicators or markers for chromate reduction in microbial communities [49]. In *Bacillus subtilis, nfrA* gene is upregulated 5.3 folds upon chromium treatment. So this strain is identified as a prospective agent for bioremediation [50]. In *Bacillus subtilis*, an error prevention oxidised Guanine system is involved in prevention of the mutagenesis occurring due to ROS scavenging. In mutants, that lack this system, 7,8, dihydroxy,8-oxodeoxyguanosine lesions have been reported to have formed in chromosomes of *Bacillus subtilis* exposed to chromium, thereby confirming the mutagenesis in the mutant strains but not in the wild type [51]. Transcriptome profiling and qRT-PCR of *Staphylococcus aureus-LZ-01* has confirmed thioredoxinreductase genes and genes that code for main subunits of cytochrome c oxidase complex, ABC-type metal/multidrug transporters and efflux pumps are up-regulated, upon Cr(VI) treatment [52]. Four different strains of *Microbacterium* sp"*Cr-K1W, Cr-K20, Cr-K29, and Cr-K32*" isolated from contaminated sediment of Seymore, (Indiana) reveal varying chromate responses despite their phylogenetic similarity of harbouring identical 16S rRNA gene sequences. Cr-K-29 and Cr K32 are fast reducers of chromium while the other two are slow reducers of chromium. The fast reduction of chromium in these strains may be attributed to presence of additional iron transport regulating genes like ABC-type iron transporter, component of synthase permease, and siderophoresynthetase [53]. Engineering genes to develop novel preferred traits in chromate reducing bacteria by to enhance chromiumicrobes [54]. So, the organisms can be screened for genes that can be engineered, to remediate chromium in vitro in the effluents. In nature, usually, single species are unable to endure a composite environment. Pure cultures under laboratory controlled conditions may not mimic actual environmental conditions in areas that are highly contaminated with a mixture of metals. Bacteria in a mixed culture are more stable and have high survival rates. Consortia of cultures have been reported to be superior metabolically for removing metals and apt for

field applications, since they are more competitive and have high survival rates [55]. To remediate the chromium *invitro* using a microbial consortia, immobilized cells are preferred over the free floating planktonic cells as they can withstand higher chromium toxicity [56], stable and easier to re-use [57], can be easily separated from the biomass, have better particle size, better capability of regeneration [58] and minimum depletion of nutrient source.

VI. CONCLUSION

Chromium metal exhibits toxicity in its hexavalent form. Chromium VI is the principal toxicant released to the environmental media by anthropogenic activity.Chromium III is nontoxic and several microorganisms have the intrinsic capability of this detoxification process. Hence, microbial remediation outweighs its usefulness in several ways like ease and efficiency of remediation. This review facilitates engineering for optimal remediation to achieve feasibility and enhancibility of chromium remediation by exploring theutility of reported microbes and remediation by formulating a consortia.

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