

## **Microbial chromium degradation: Biological evolution, mitigation and mechanism**

**Manimita Das\*, Harshita Nigam, Shraddha Chauhan, Poornima Pandey, P. Swati,  
Mahavir Yadav and Archana Tiwari**

*School of Biotechnology (An Autonomous University Teaching Department), Rajiv Gandhi Proudyogiki  
Vishwavidyalaya (State Technological University of Madhya Pradesh), Airport Road, Bhopal*

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### **ABSTRACT**

*Crude shroud/ skin go to the tanners as a by-result of meat industry, which is changed over into worth included calfskin as result of style market. Leather assembling is a concoction methodology of the common organic framework. It utilizes the tremendous amount of water and inorganic and natural chemicals in preparing and in this way releases robust and fluid squanders into the earth. These chemicals found in waste waters are harmful to the environment and their effects on biological system are very hazardous. Microbial metal bioremediation or degradation of CTLs is found to be an efficient strategy due to its low cost, high efficiency and eco-friendly nature. The aim of this paper is to have a deeper review of the problem of processing various types of wastes generated by the leather industry and other textile or chemical industries; its degradation and utilization methodologies.*

**Key words:** bioremediation, chromium, micro-organisms

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### **INTRODUCTION**

Leather industry and the environment are like the two sides of the coins. Due to the repeated processes of soaking raw hides and wringing them out, the tanning process creates large amounts of wastewater that may be contaminated with many different chemicals. Because there is wide variety in the chemicals used during the tanning process, wastewater from this industry can have very different chemical make ups [2].

However, chromium contamination and high chemical oxygen demand are typical problems associated with tannery effluents, both of which can pose serious risks to the environment and human health. From a survey as mentioned in fig 1, it was estimated that Indian population is facing the highest risk of chromium pollution across the country ([http://www.worstpolluted.org/projects\\_reports/display/88](http://www.worstpolluted.org/projects_reports/display/88)).

Chromium is distributed as a potential soil surface and ground water, sediment and air contaminant. Soil chromium levels are generally identified with the chromium level in the guardian material, and aside from in soil got from serpentine soil materials the regular foundation levels of soil chromium are really low [3, 4].

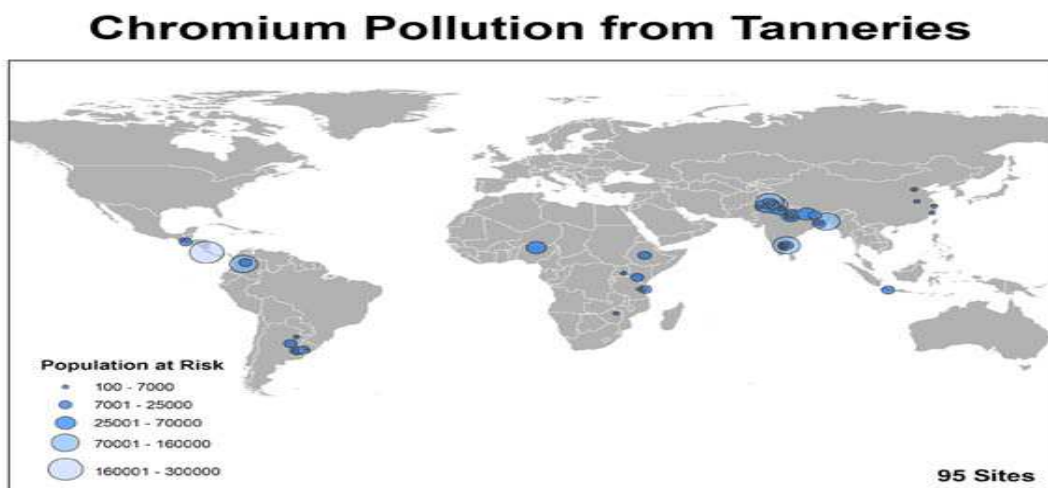


Fig 1: population at risk worldwide due to chromium pollution

Environmental component	Chromium concentration
Continental crust	80-200 mg/kg
Soil	10-150 mg/kg
Fresh water	0.1-6.0 mg/L
Sea water	0.2-50.0 mg/L
Drinking water	0.05 mg/L
Air samples	0.015-0.03 mg/m <sup>3</sup>

Leather industry generates various chromium based wastes as chromium-based tanning is being followed predominantly worldwide due to the versatility of chromium. These wastes such as chromium sludge, chrome tanned leather shavings (CTLs) and chrome leather trimmings are unavoidable and possess a serious threat to the environment. It has been estimated that 0.02 million tons of chromium shavings are generated in India per annum. Nearly, 0.8 million tons of CTLs could be generated per year globally [6]. The quantum of solid wastes produced while processing 1 ton of raw hides/skins is given in table 1 [7]. Cowhide industry in India, is one of the best patrons towards the economy as it is one of the most seasoned and most working on assembling businesses. In India, a large number of mechanical tannery units are spread generally crosswise over Tamil Nadu, West Bengal, Uttar Pradesh, Andhra Pradesh, Karnataka, Maharashtra, Rajasthan and Punjab. Over half of India's leather manufacturing units are built around the Gang river basin (<http://www.earthtimes.org/business/india-leather-industry-told-clean-act/1054/>). West Bengal alone 600 tanneries are working utilizing and 20,000 units assembling cowhide items giving vocation to more than 200,000 individuals. Kanpur, which is overall known as the "Leather City of the World" has in excess of 1600 practical calfskin producing units delivering semi-completed, completed and quality included items (<http://en.wikipedia.org/wiki/Kanpur>).

### 1.1. Chromium and its environmental issues

Contamination of the environment with chromium is the major problem caused by the leather industries and most other industries. The hexavalent chromium, Cr(VI) is the most toxic and carcinogenic amongst all the different forms of chromium [4], due to its high solubility in water, rapid permeability through biological membranes and subsequent interactions with intracellular proteins and nucleic acids. The heavy metals in general cannot be biologically transformed to more or less toxic products and hence persists in the environment indefinitely [8]. They are significantly toxic even in very trace amount and can cause diseases in humans and animals as they cause irreversible changes in the body, especially in the Central Nervous System [9]. Other than being carcinogenic and mutagenic, Cr (VI) may lead to liver damage and pulmonary congestion and cause skin irritation resulting in ulcer formation. Chromium accumulation may also cause birth defects and the decrease in reproductive health [10].

The toxicity of chromium in prokaryotes is not fully understood. Under normal physiological conditions, Cr(VI) is readily taken up by the mammalian cells through non specific ion channels [11]. Cr(III) rarely penetrates the cellular membrane. Accumulation of chromium in the cell was justified from the use of <sup>51</sup>Cr for cell lysis and apoptosis [12]. <sup>51</sup>Cr trapped within the cell during the conversion of Cr(VI) to Cr(III), which is released on cell lysis, provides a measure of cell lifetime. Cr(VI) gets readily reduced to Cr(III) in the intracellular level, thereby oxidizing ascorbate, glutathione and cysteine and leading to production of Cr(V), Cr(IV), superoxide, singlet oxygen and hydroxyl

radicals [13]. These superoxide and hydroxyl radicals contributes efficiently to the carcinogenicity and genotoxicity of Cr(VI) as a result of DNA strand breaks, DNA interstrand and DNA-protein crosslinking and inhibition of DNA replication [14].

Chromate predominates at higher Ehs (>500mV at pH6 and >300mV at pH9). At lower Eh values, the Cr(III) species are present at a higher concentration than chromate with the dominant species being dependent on pH. The dissolved chromium concentration in groundwater is strongly dependent on pH and Eh, because Cr(VI) minerals are relatively soluble compared to Cr(III) minerals. Under oxidizing conditions where chromate is stable, total dissolved chromium concentrations can be much greater than 1 mg/L because of the high solubility of chromate minerals. Conversely, under more reducing conditions where Cr(III) species and minerals are stable, the total dissolved chromium concentration is typically much less than 1 mg/L because of the low solubility of Cr(III) minerals such as Cr(OH)<sub>3</sub> and (Fe,Cr)(OH)<sub>3</sub> [HRC Technical Bulletin H-2.7.5].

Disposal of industrial wastes in coastal areas without proper coatings allows the leachate to mix with the ground water, which leads to deterioration of water quality in the vicinity. The presence of chromium also makes the soil unfit for cultivators and for other uses. Due to the presence of chromium content, many countries have restricted the disposal of leather industry wastes via landfill and incineration method [15, 16]. About 90% of the tanneries in India incorporate chrome tanning process [17]. The oxidation of Cr(III) to Cr(VI) in certain conditions by in the presence of air in a wide range of pH is the main problem.

Chromium has been classified as a group “A” human carcinogen by the US Environmental Protection Agency (2004) and is considered as one of the main pollutants as per data available for 976 National Priorities List (NPL) Sites with fiscal year 1982-2003 as shown in Fig 2 [18].

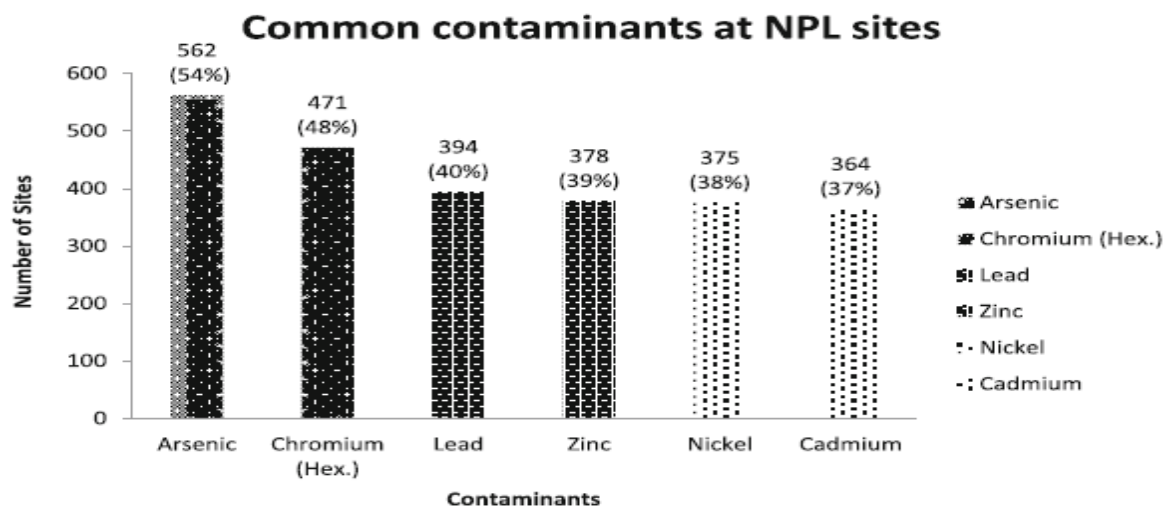


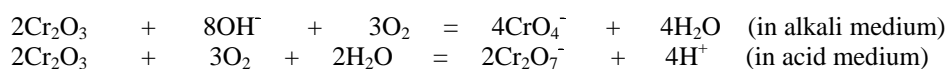
Fig:2 The frequencies of most common contaminants at NPL sites [18],[19]

In fact, there are several positive and negative effects of Cr(III) and Cr(VI) in humans [20]. The word related to occupational safety and health organization (OSHA) has set cut-off points of 500- $\mu$ g water-dissolvable Cr(III) mixes for every cubic meter of working environment air (500  $\mu$ g L<sup>-1</sup>), 1,000  $\mu$ g L<sup>-1</sup> for metallic chromium(0) and insoluble chromium mixes, and 52  $\mu$ g L<sup>-1</sup> for chromium(vi) mixes for 8-h work movements and 40-h weeks [21].

In order to protect the environment from further damages or increases in the accumulated amounts of hazardous waste in the future, most industrial operations should be designed based on “4Rs” (reduction, recycling, reuse and recovery) concept [7].

## 2. SPECIATION AND ITS TRANSFORMATION

The leather industry wastes consists of non-toxic chromium that is in +3 oxidation state, but there is a high chance of conversion of this non-toxic chromium to toxic +6 oxidation state; despite of the fact that it can exist in all the oxidation state from -2 to +6. The possible reaction equation is [22]:



Hence the mobility and the toxicity of chromium depend on its oxidation states.

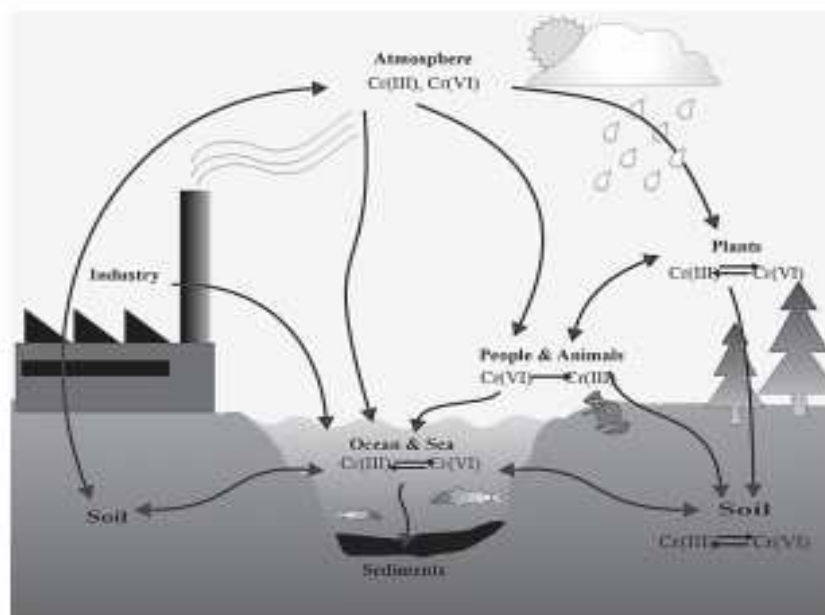


Fig:2 chromium circulation in the polluted environment [20]

### 3. MICROBIAL CONTRIBUTION TO CHROMIUM REMEDIATION

Chromium removal by biosorption process offers an alternative bioremediation of industrial effluents as well as recovery of metal ions from aqueous solution. Microbial reduction of chromate can occur both aerobically [23] and aerobically [24] and due to its cost effectiveness, it is mostly used for bioremediation. Table 2 gives a brief overview of the ability of certain microbes in chromium bioremediation and their efficiency.

Micro-organism	Function	Reference(s)
<i>Pseudomonas fluorescens</i> LB300	Uptake of $\text{CrO}_4^{2-}$ by the strain with plasmid	[25]
<i>Schizosaccharomyces pombe</i>	Lysine and leucine auxotrophic and heterothallic strains of this microbe were used to obtain Cr-sensitive and tolerant mutants by UV radiation-induced and nitrosoguanidine-induced mutagenesis	[26]
<i>Pseudomonas ambigua</i> G-1	Bioreduction of the Cr-concentration from $150\text{-}35\text{mgL}^{-1}$ in 36hr in liquid media	[4]
<i>Bacillus firmus</i>	Capable of absorbing $\text{Cr}^{6+}$ efficiently into their biomass	[27]
<i>Klebsiella pneumoniae</i>	Capable of absorbing $\text{Cr}^{6+}$ efficiently into their biomass	[27]
<i>Mycobacterium</i> sp.	Capable of absorbing $\text{Cr}^{6+}$ efficiently into their biomass	[27]
<i>Bacillus cereus</i> IST105	Absorption of chromate on the bacterial cell wall takes place through surface functional groups like carboxyl, amide, phosphoryl and hydroxyl	[28]
<i>Bacillus megatarium</i> TKW3	Hexavalent chromium reduction associated with membrane cell fraction	[29]
<i>Bacillus circulans</i>	Removal of chromium by bioabsorption	[30]
<i>Bacillus subtilis</i>	Able to reduce chromate at concentrations ranging from 0.1 to 1 mM $\text{K}_2\text{CrO}_4$	[31]
<i>Bacillus methylotrophicus</i>	Chromate reduction activity was found to be 91.3% at 48hrs	[32]

### 4. MECHANISM OF CHROMIUM TOXICITY

$\text{Cr(III)}$  is less toxic than hexavalent chromium due to its impermeable nature through cell membranes. And hence biotransformation of  $\text{Cr(VI)}$  to  $\text{Cr(III)}$  is been found to be an alternative process for the treatment of chromium contaminated wastes and industrial effluents. The sulphate utilizing microbes absorbs hexavalent chromium through the membrane sulphate transport channels present in the cells [25, 33-35]

$\text{Cr(VI)}$  under normal physiological conditions, get reduced in the presence of ascorbate and / glutathione to form  $\text{Cr(V)}$ ,  $\text{Cr(IV)}$ , free radicals and finally  $\text{Cr(III)}$ .  $\text{Cr(V)}$  and other intermediates have a very short life time within the cellular membrane and also  $\text{Cr(V)}$  regenerates  $\text{Cr(VI)}$  by undergoing one electron redox cycle and transferring electron oxygen. This leads to the production of one reactive oxygen species (ROS) that can easily combine with DNA protein complexes.  $\text{Cr(IV)}$  may determine the normal physiological functions by binding to cellular materials [1].

### 5. MECHANISM FOR MICROBIAL DETOXIFICATION OF CR(VI)

The aerobic reduction of  $\text{Cr(VI)}$  via bacterial isolates is a multistep process which includes the initial production of  $\text{Cr(V)}$  and  $\text{Cr(IV)}$  (short life span intermediates) finally leading to the formation of thermodynamically stable end product i.e.  $\text{Cr(III)}$ . This hexavalent chromium reduction process accepts electrons from NADH, NADPH and also

electrons from the endogenous reserves [26, 36]. Fig. 2 depicts the schematic representation of carcinogenicity and mutagenicity of hexavalent chromium.

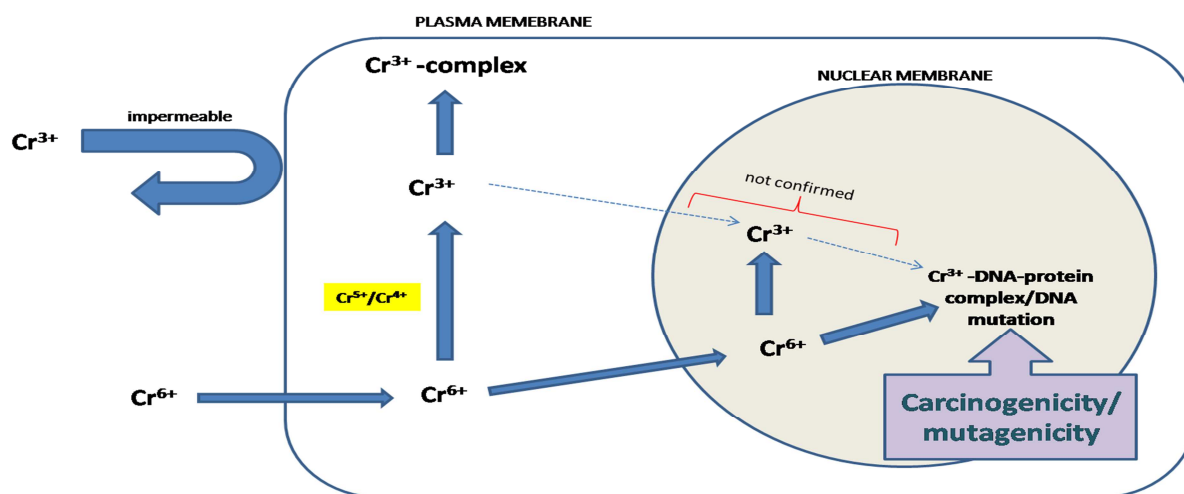


Fig.2: Schematic representation of carcinogenicity and mutagenicity of Cr<sup>6+</sup> (modified from [1])

Cr<sup>6+</sup> reductase ChrR, which is isolated from *Pseudomonas putida* MK1, is capable of reducing hexavalent chromium by catalysing a combination of one- and two- electron transfer to Cr(V) with the momentary formation of Cr(V). The chrR, which is the ChrR-coding gene, was identified from the genomic sequence of *Pseudomonas putida* MK1, on the basis of the known amino acid sequences of the N- terminal and internal amino acid segments of the pure enzyme [37, 38]. ChrR is described as a dimeric flavoprotein which catalyses the hexavalent chromium reduction at 70°C as optimum. Despite the fact that an extent of the Cr(V) intermediate is spontaneously re oxidized to produce ROS, its decrease through two electron exchange catalyzed by ChrR diminishes the chance to create unsafe radicals [39].

The gene *chrR* has been found to have a similarity with an open reading frame (ORF), *yieF*, present on the *Escherichia coli* with no particular assigned function. The *yieF* gene encodes for the protein YieF on cloning and showed higher rate of hexavalent chromium reduction at 35°C [38]. YieF catalyses direct hexavalent chromium reduction to Cr(III) via four electron transfer, in which three electrons are utilised in the reduction of Cr(VI) and the rest are transferred to oxygen. The ROS generated during this process is less and hence is regarded as the more effective reductase than ChrR [38].

Several earlier approaches have come up with the purification of soluble hexavalent chromium reductase from *Pseudomonas* sp., for instance, *Pseudomonas putida* PRS2000, *Pseudomonas ambigua* G-1 [40-42]. In a later examination, the quality encoding this reductase was found to display a high nucleotide grouping homology (58%) to a nitro-reductase of *Vibrio harveyi* KCTC 2720 that was likewise enriched with Cr(VI)-decreasing activity [42]. A membrane associated Cr(VI) reductase isolated from *Bacillus megaterium* TKW3 used NADH as an electron donor, however the kinetics of Cr(VI) diminishment is so far uncharacterized [29]. Fig. 3 represents the plausible mechanism for the aerobic reduction of hexavalent chromium.

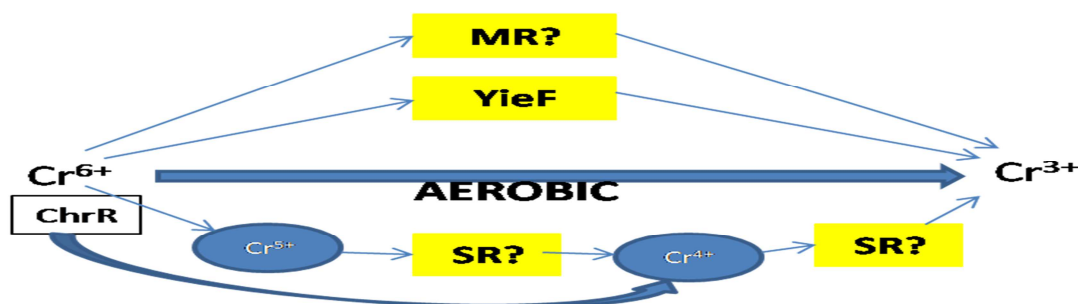


Fig.3: Possible mechanism for the aerobic reduction of Cr<sup>6+</sup> (modified from [43])

Under the oxygen depleted conditions (as shown in fig. 4), both the soluble and membrane associated hexavalent chromium reductase, including cytochromes, accepts terminal electrons from carbohydrates, proteins, fats, hydrogen, NAD(P)H and endogenous electron reserves [44].

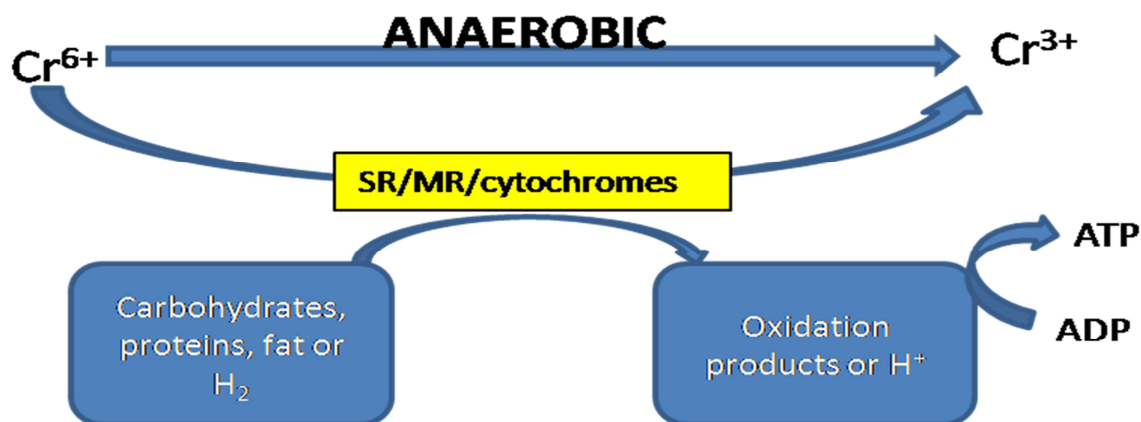


Fig 4: Possible mechanism for anaerobic reduction of Cr<sup>6+</sup> (modified from [1])

An uncharacterised membrane associated hexavalent chromium reductase was isolated from *Bacillus megaterium* TKW3, as its kinetics for hexavalent chromium reduction is not known [29]. The process of oxygen depleted hexavalent chromium reduction does not provide energy for microbial growth [45].

### CONCLUSION

Microorganisms are the most easily available and cost effective mode of bioremediation or biodegradation and can be efficiently used for the removal of toxic heavy metals from the industrial effluents and heavy metal contaminated soil. The microorganisms utilise these toxic metals as nutrients and absorb the metals or reduce them to non-toxic forms. The microbial reduction of hexavalent chromate by ChrR, which is a 4 electron transfer chromate reductase reduces chromium directly to its trivalent form; nitrosoreductase reduces chromate by both mixed di- and semi-electron transportation and the reduction of cytochrome c is due to the redox potential of its heme. Late studies have proposed that it might be conceivable to expand the uptake and the specificity of biosorbents utilizing the apparatuses of molecular biology by focusing on designed metal binding proteins to the cell surface. Expanded comprehension of metabolic pathways in the microorganism in charge of metal solubilisation, and enhancing their survival rates and soundness opens the way to the control of parameters, for example, energy and metal selectivity, with the point of upgrading the evacuation and/or recuperation of chromium.

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