ijlpr 2020; doi 10.22376/ijpbs/lpr.2020.10.4.L41-49

International Journal of Life science and Pharma Research

Review Article

Microbiology for Health Care

Non-Pathogenic bacteria in Bioremediation of Cr (VI) for decontamination of ground water: An Extensive Review

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Abstract: Cr(VI), one of the most common ground waters heavy metal contaminant due to its indiscriminateuse in different industries has become a matter of major environmental concern. So, it is desirable that remediation methods should be such that brings its level within the permissible limits before effluents are discharged. The methods should be cheap as well as eco-friendly. Nowadays, severalbiological remediation strategies are used by applying microorganisms for its removal involving biosorption and biotransformation. Biosorption is dependent on surface nature of the biosorbentswhereas biotransformations depend on the presence of reductants. The present review includes bioremediation strategies of Cr(VI) based on biosorption or biotransformation or both by non-pathogenic bacteria only.

Keywords: Cr(VI), Heavy metal, Biosorption, Biotransformation, Bioremediation.

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Recieved On 11 February 2020
Revised On 20 March 2020
Accepted On 25 April 2020
Published On 02 October 2020

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Subhadeep Ganguly and Smaranika Pattnaik , Non-Pathogenic bacteria in Bioremediation of Cr (VI) for decontamination of ground water: An Extensive Review.(2020).Int. J. Life Sci. Pharma Res.10(4), L41-49 http://dx.doi.org/10.22376/ijpbs/lpr.2020.10.4.L41-49

1. INTRODUCTION

Chromium is a transition metal and the first member of group VIB in the periodic table with atomic number 24 and is the 21stmost abundant element in the earth crust[1]. The natural source of chromium in the environment includes volcanic eruptions, weathering, forest fire etc. Anthropogenic activities causes maximum deposition of Cr(VI) in the nature. Due to its hardness, high melting point, metallic lustre, odorless nature and anti-corrosiveness, it is largely used by various industries. Rapid industrialization has led to the disposal of various heavy metals into the environment [2]. In today's industrially revolutionized world,contamination of ground water with hexavalent chromium has become a serious public health concern as industrial effluents containing Cr(VI) largely pollute rivers as well as the environment in close vicinity towards the residential areas [3]. Tanneries, electroplating and metal finishing industries, inorganic chemical plants, steel and iron industries, automobile industries, wood treatment industries, pigments used in dyes, paints and ink manufacture industries, plastic manufacturers, defense goods manufacture industries are the major sources of hexavalent chromium toxicants [4,5,6,7].For this reason, Cr(VI) contamination of groundwater has become a serious health issue related to environmental pollution for last few decades in many countries around the globe including India [8]. Chromium exists in nature with nine valence states ranging from -2 to +6, among which Cr(VI) and Cr(III) are the most abundant forms as these two oxidation states are the most stable[2] . It is commonly present as either chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) ions [1, 9].Several conventional strategies, such as filtration, precipitation, membrane separation, ion-exchange chromatography etc have been adopted extensively to remove Cr(VI) from industrial effluents. However,these techniques have appeared as either inefficient or expensive when heavy metals are present in the effluents in minute quantities [10]. They may also yield secondary wastes that are difficult to manage and invite a huge cost as well[11].Currently biological materials have attracted great attention in this regard as they are readily available, cheap as well as show excellent performance [12]. In the present review the authors aim to focus on bioremediations of Cr(VI) including both biosorption and biotransformation by means of non-pathogenic bacteria only to make sure there could not be any further release of toxic substances during the course of its bioremediation by the biosorbents themselves which need further purification.

1.1 CR(VI) TOXICITY IN HUMAN

As per Agency for Toxic Substances and Disease Registry (ATSDR) hexavalent chromium is recorded as one of the eighty top toxic metals in the world and World Health Organization (WHO) has declared clearly that it is a potent carcinogenic, genotoxic and mutagenic substance [14-19]. It is a potent irritant to skin. Chronic dermatitis, papules, swelling, erythema, small vesicles in the skin are also very common. Its mutagenic activities in both in vivo and in vitro rat models have already been reported. Among different chromate compounds studied so far, strontium chromate ($SrCrO_4$) appeared to be the most potent carcinogen.[18-20] Lung cancers among different industrial workers dealing with Cr(VI) are very common. Long term exposure may lead to chronic irritation in upper respiratory tract,pharyngitis, chronic rhinitis, and hyperemia, polyps in the upper respiratory tracts,asthma, bronchitis, congestion, tracheobronchitis and ulceration of nasal mucosal membrane with perforation of the septum. Chronic occupational exposure to Cr(VI) causes DNA damage among electroplating workers.[20,21]Mild exposure may also lead to dizziness, weakness, haematological disorders, eye irritations, growth problems, gastrointestinal malfunctions, renal disorders, teeth discoloration and erosion etc[19].

1.2 MECHANISM OF BACTERIAL RESISTANCE TO CR(VI)

ChrA genes, which encode the ChrA proteins, responsible for putative chromate efflux and get controlled by membrane potential, have been well characterized in several bacterial species [22,23,24,25]. Microorganisms bearing ChrA proteins show resistance to Cr(VI) [26]. However, unlike other heavy metals, resistance to Cr(VI) gives only up to sub millimolar range as its efflux is associated with sulfate co-extrusion that may lead to inhibition of growth [27]. ChrA genes may be located either in bacterial plasmid or in bacterial chromosome or in both and constitute operon with other Chr genes [28].Biosorption, bioaccumulation and biotransformation of Cr(VI) by different non pathogenic bacteria. As chromate is chemically and structurally similar to sulfate, it can compete with the latter for cellular uptake and thus gets bioaccumulated via sulfate uptake pathway across the surface membranes [29]. Inside the cell it undergoes chemical alterations via several enzymatic and non-enzymatic reactions and leads to accumulation of different chemical intermediates that can directly alter DNA structure and exert toxicity at the genomic level [7,30,31]. Apart from biosorption, biotransformation of Cr(VI) to Cr(III) is regarded as another important phenomenon involved in bioremediation. A wide range of microorganisms including bacteria can reduce Cr(VI) to Cr(III) either anaerobically and /or aerobically[32]. Bacterial Cr(VI) aerobic reduction was first reported in *Pseudomonas dechromaticans* by Romanenko and KorenKov (1977) [33]. Later on several facultative bacterial strains were studied including Aerococcus, Micrococcus and Aeromonas[34].Aerobic reduction of Cr (VI) by Thermusscotoductus as well as anaerobic reduction by Achromobactersp. were also reported [35,36].Bacteria having the capacity to reduce Cr(VI) are called chromium-reducing bacteria (CRB),which are generally isolated from industrial effluents like tanneries, electroplating manufacturing, textile industries or contaminated soil [37,38,39,40,41]. Since then monocultures of different bacterial strains have been examined for Cr(VI) bioremediation studies [39,42,43]. But Sannasi et al (2006) reported that mixed bacterial culture was more stable in this context [44]. Kader et al (2007) claimed that consortia of cultures were more effective in removal of chromium in the field of its application[45]. Several other studies have supported the involvement of bacterial culture for both biosorption[46, 47].Chromate resistant *Pseudomonas fluorescens* LB300 was isolated from chromium contaminated river sediment. It appeared as a good reductant of Cr(VI) to Cr(III) during anaerobic growth on acetate, where chromate acted as the terminal electron acceptor[48]. Srinath et al (2002) isolated chromate resistant Cr(VI) accumulating bacteria from treated tannery effluent[49]. The effluent contained 0.96 mg/L chromium which was much higher than the statutory limit (0.1 mg/L) for discharge of industrial effluents into the surface water in India. Not only bioaccumulation but biosorption capabilities of both living and dead cells of these strains were also analyzed. It is evident that *Bacillus circulans*and*Bacillus megaterium*could be able to biosorbCr(VI) up to 34.5 and 32.0 mg/g dry cell weight. Another absorbing species of *Bacillus* known as *Bacillus*

*coagulans*was able to biosorb 23.8 mg/g of Cr(VI) in viable state and 39.9 mg/g in dead state respectively. Five isolates of *Bacillus*sp, have been isolated from dichromate contaminated soil and have also been characterized by16SrRNA gene sequencing and subsequently examined for biotransformation abilities of Cr(VI). Among five isolates examined, *Bacillus* sp. ES29 appeared to be the most suitable one which would be able to reduce 90%of Cr(VI) aerobically within six hours of incubation [50]. *Bacillus coagulans*, isolated from tannery waste water, exhibited its Cr(VI) biosorption capacities in both free and immobilized states in different polymeric matrices such as agar, agarose, calcium alginate and polyacrylamide gel[51]. Ilhan et al (2004) isolated *Staphylococcus saprophyticus*from soil and subsequently subject edit for Cr(VI) biosorption by optimizing different culture conditions[52]. This organism appeared to be a good biosorbent for Cr(VI) from wastewater also. Reduction of Cr(VI) by intact cells and cell free extracts of *Actinomyces*and*Arthrobactercrystallopoietes*(strain ES32) isolated from dichromate contaminated soil was reported by Camargo et al (2004)[53]. Both intact cells and cell free extracts exhibited satisfactory reduction above 90% of Cr(VI) within 12 hours of incubation and almost complete reduction was obtained after 24 hours. Faisal and Hasnain (2004) have isolated two Cr(VI) resistant bacterial strains CrT-1 and CrT-13 and identified them as *Ochrobactrumintermedium* and *Brevibacterium* sp. respectively by 16S rRNA gene sequencing. *Brevibacterium*sp. CrT-13 reduced Cr(VI) up to 62% after 96 hours of incubation using initial Cr(VI) concentration of 750µg/ml. [54]Moreover, Cr(VI) resistant *Micrococcus* sp. was isolated from soil contaminated with effluent of electroplating industries waste water. Bioaccumulation of Cr(VI) by that strain was investigated. The results indicated that the bacterial strain could be an effective agent for removal of Cr(VI) from contaminated wastewater [55]. Eleven novel chromium resistant strains had also been isolated (ten from genus *Streptomyces* and one from Amycolatopsis) by Poltietal (2007)[56]. Three different bacterial species (*Streptococcus equisimilis* CECT926, *Bacillus coagulans* CECT12, and *Escherichia coli* CECT515) supported on granular activated carbon were tested for removing Cr (VI) using both batch and column studies. In that study, Gram positive bacteria (*B.coagulans* and *S. equisimilis*) exhibited best metal removal capacities[57]. Srivastava et al (2008) isolated a *Pseudomonas* sp. from tannery effluent in Kanpur, Uttarpradesh, India, which exhibited enough potential to migrate through the contaminated environment on its surroundings and can effectively be applicable for biosorption of hexavalent chromium from aqueous solution[58]. Aerobic reduction of Cr (VI) by *Thermusscotoductus*as well as anaerobic reduction by *Achromobacter*sp.were also reported to be evident [35,36]. Dead *Bacillus subtilis* biomass was examined by Sivaprakash et al (2009) for Cr(VI) biosorption and its effective adsorption onto the surface of the biomass followed by desorption was conducted successfully[59]. Elangovan and Chandraraj (2010) isolated *Arthrobacterrhombi*RE from chromium contaminated sites [60]. Chromium reductase activity of *Arthrobacter rhombi* RE was assessed with cell free extract and then it was immobilized in calcium alginate bead, which proved to be an effective tool for reduction of Cr(VI). Wang et al (2010) used indigenous bacterial flora isolated from Cr(VI) contaminated water and applied it for detoxification of water by reducing Cr(VI) to Cr(III) [61]. The experiment showed that the flora could be able to carry out effective reduction of Cr(VI) to Cr(III) under aerobic conditions with unadjusted pH. Bacterial isolates from matchworks industrial wastes containing *Bacillus* spp.M11 and *Micrococcus* spp.M12 immobilized in calcium alginate beads were subjected to Cr(VI) biosorption studies and it revealed that the beads were seemed to be very effective up to 3rd cycle after desorption [62]. Nancharaiah et al (2010) assessed the potential of mixed microbial consortia immobilized in granular biofilms which removed and aerobically reduced Cr(VI) to Cr(III) [63]. Four bacterial strains were isolated from tannery effluents contaminated soil in Jajmau(Kanpur), India among which two were Cr(VI) resistant and the rest two were sensitive to Cr(VI). 16S rDNA sequencing revealed that they were *Stenotrophomonasmaltophilia*, *Exiguobacterium*sp.,*Pantoea*sp. and*Aeromonas*sp. respectively. Cr(VI) biosorption was studied in all species using both dead and living cells. Both the living and dried biomass of *Exiguobacterium*sp. absorbed maximum amount of Cr(VI) from aqueous solution[64]. Furthermore indigenous chromium reducing bacterial strain, Ochrobactrumintermedium RB-2 was isolated from tannery waste samples and was examined for its potential to reduce Cr(VI) to Cr(III). Its cell free extract contained reductase activity and transmission electron microscopy revealed the outer as well as inner distribution of Cr(III) [65]. A Cr(VI) resistant bacterium *Ochrobactrumintermedium*SDCr-5 was studied and optimized for Cr(VI) reduction to Cr(III) and maximum Cr(VI) reduction was obtained with 96 hours of incubation at 37^0C at pH 7[66]. Sugiyama et al. isolated an actinobacterial strain *Flexivirga alba* ST13(T) reported to execute Cr(VI) reducing activity that could be further enhanced by molasses[67]. On the other hand,Cr(VI) biosorption by four resistant autochthonous bacterial strains was examined by Oyetibo et al. to assess their potential for use in marine water pollution control[68]. The bacterial strains exhibited their high chromium removal efficiency by removing 70%-90.5% Cr(VI) from the aqueous solution. Among four strains examined (*Rhodococcus*sp. ALO3Ni, *Burkholderiacepacia*AL96, *Corynebacteriumkutscheri*FL108Hg and *Pseudomonas aeruginosa*CA207Ni) to execute maximum biosorption of Cr(VI) were obtained with *Rhodococcus*sp LO3Ni with a maximum uptake of 107.6 mg/g dry cell weight. Srivastava and Thakur(2014) also isolated a bacterium from soil and sediment of a leather tanning mill's effluent and subsequently enlisted as *Serratia*sp. by 16S rDNA analysis[69]. They examined its potency for chromium biosorption in shake flask culture containing chromium and also in tannery waste water by examining scanning electron microscopy-energy dispersive X-ray analysis (SEMEDX) and transmission electron microscopy (TEM). The SEM EDX analysis confirmed the interaction of Cr(VI) with the surface molecule of the bacterium. The TEM analysis depicted the accumulation of Cr(VI) throughout the bacterial cells. Abioye et al (2015) isolated Bacillus subtilis and Pseudomonas aeruginosa from the waste dump site and tested for Cr(VI) biosorption[70]. They optimized different parameters like pH, biomass concentration, metal concentration, temperature and contact time to improve the efficiency of biosorption. *Bacillus subtilis* exhibited higher biosorption(86.7%) capacity than *Pseudomonas aeruginosa*(83.0%). Pun et al (2013) used non-living biomass of *Bacillus* sp. isolated from the soil of Sisol Landfill site and obtained excellent effectiveness with 99% removal of Cr(VI) from the leachate[71]. Figure. 1 depicted the Overview of the mechanism of bacterial Cr(VI)transport, toxicity, reduction and efflux.

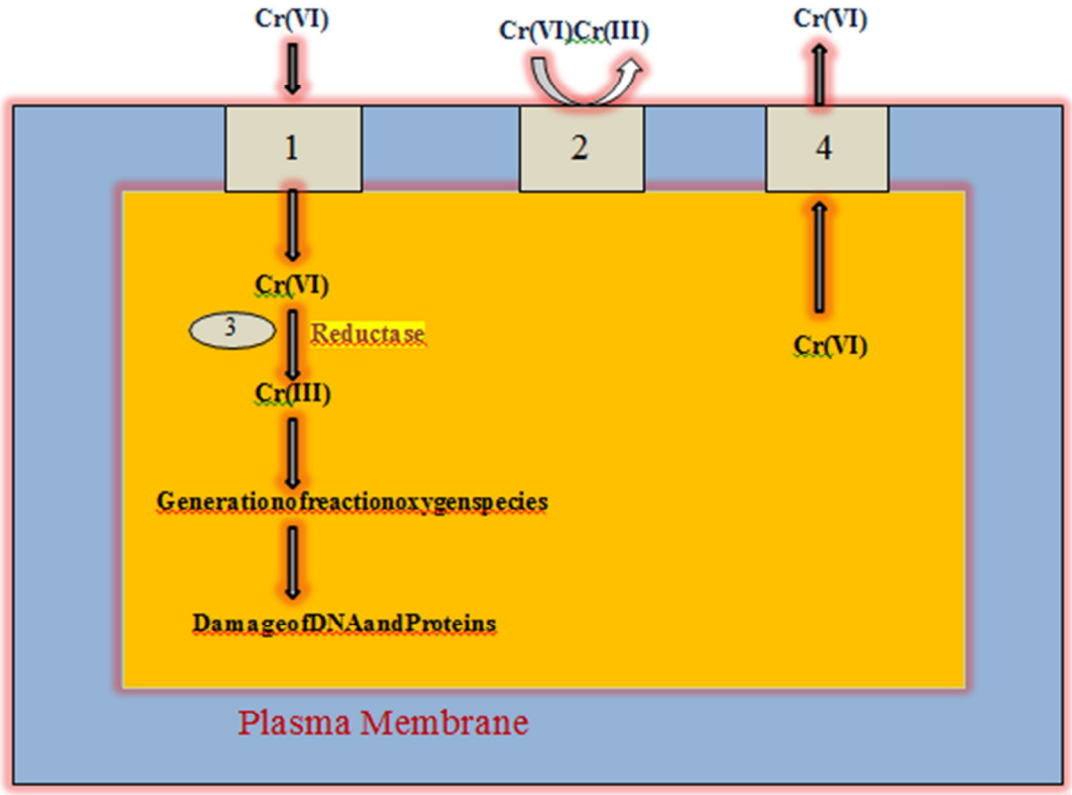

Figure 1: OverviewofthemechanismofbacterialCr(VI)transport,toxicity,reduction and efflux

(1) Cr(VI) entry via sulfate uptake pathway; (2)Membrane associated chromate reductase; (3) Intracellular reduction of Cr(VI) to Cr(III); (4) Active efflux of intracellular Cr(VI) by membrane bound ChrA protein.

1.3 MECHANISM OF BACTERIAL BIOSORPTION, BIOACCUMULATION AND BIOTRANSFORMATION OF CR(VI)

Prokaryotic organisms being ubiquitous occupy and acclimatize with all the niches in our environment. They appear in a large variety of sizes and shapes and the most familiar eubacterial forms are named as *coccus, bacillus, vibrio, spirilla* and *spirochete* as well as the filamentous mold resembling actinomycetes; even coccus can appear in different forms depending on arrangement and plane of division and bacilli also differs in terms of arrangement. The eubacterial cell walls unlike plant cell walls contain a saccular peptidoglycan or murein meshwork which contribute to cellular shape and rigidity, protect the cell from osmotic and toxic stress and in pathogens enhance pathogenicity. The peptidoglycan layer alone can bring about significant diversity in the prokaryotes. If we concentrate only on the eubacteria domain, we will find noteworthy differences in the murein structure and its accessory components in both gram positive and negative bacteria; grossly at the firmness of the cross linkage and thickness of the murein meshwork and ultrastructurally at the absence of peptide interbridge in most gram negative bacteria. Gram positive cell walls also have anionic polymers such as teichoic and lipoteichoic acids as well as teichuronic acids extended from the murein meshwork which is completely absent in the gram negative ones. The peptide interbridge also has significant variation in the amino acid sequence from organisms to organisms. These anionic polymers contribute significantly to the negative charge of the gram positive cell wall and provide a very good site for interaction with metal ions. On the other hand, gram negative bacteria have lipopolysaccharides in their outer membrane contributing negative charge to the bacterial cell surface. The eubacteria glycocalyx such as capsule and slime layer also contribute additional negative charge to the bacterial cell surface. Eventually, we find bacterial cell surfaces owing to their high negative charge density have a very high potential to interact with metal ions and even they do so. Bacterial outer cell surface is the principal component that first gets exposed to metal ions. Solute interaction with dead cells is extracellular and therefore the chemical functional groups of cell surface play vital roles in the biosorption process. Depending on species variations, several functional groups such as carboxyl, phosphate, amine, hydroxyl, sulfate etc are present on the bacterial cell surface. Among all the negatively charged functional groups, carboxyl group plays a major role in metal biosorption[72-74]. Cr(VI) upon adsorption, either gets precipitated over the surface of the bacterial cells or is transformed into Cr(III)[8]. The biotransformation of Cr(VI) to Cr(III) is either spontaneous or mediated via chromate reductase enzyme[75]. In bacterial cells, chromate is transported via active transport through sulfate transporters. Within the cells, it is translocated via chromium binding proteins and finally converted to Cr(III) through several unstable oxidation states, either aerobically or anaerobically. In the presence of oxygen, either NADH or NADPH acts as an electron donor,but in anaerobic condition,Cr(VI) itself acts as a terminal electron acceptor and several respiratory chain complexes are involved in this process [8,75,76]. Excess Cr(VI) is pumped out off the bacterial cells via plasma membrane associated transmembrane transporter proteins (encoded by plasmid gene Chr A).[29]For biosorption study, proper characterization of the surfaces of bacterial biomass is very important. Conventional methods used for such characterization include:Potentiometric titrations, Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), Scanning electron microscopy (SEM) etc.[73,77-85]Potentiometric titration helps to find the nature and the

number of binding sites[73]. FTIR-spectroscopy indicates the nature of binding sites and their involvement during biosorption[84]. X-ray diffraction study confirms the involvement of cellular carboxyl and phosphate groups in the biosorption process[81]. The morphological characteristics of the cell surface can be studied by scanning electron microscope[82].Ohtake et al (1987) had postulated that CrO_4^{2-} resistance in *Pseudomonas fluorescens* LB300(PLHB1) demonstrated reduced uptake of CrO_4^{2-} compared to the plasmid less strain LB303[86].$^{51}CrO_4^{2-}$ was transported via SO_4^{2-} active transport system. So, the cells grown in a medium containing repressor (such as cysteine) of SO_4^{2-} transport system appeared to be much more resistant to CrO_4^{2-} than the cell grown in the medium containing djenkolic acid (a derepressor of $^{35}SO_4^{2-}$ transporter system)[87]. Kinetic studies for $^{51}CrO_4^{2-}$ uptake by *Pseudomonas fluorescens* with and without plasmid revealed that the V_{max} for $^{51}CrO_4^{2-}$ uptake with the resistant strain was 2.2 times less than that sensitive strain but K_m remained the same in both cases the reductase activities from different bacterial species and ultimately adopted immobilized NADH-dependent reductase for *E.coli*NemA from Cr(VI) reduction instead of using whole cells [88,89]. However, till date chromate reductase activity was best studied in NADP-dependent ChrR isolated from *Pseudomonas putida*[90]. During the course of Cr(VI) reduction, reactive oxygen species are generated which reduce quinones that protect the bacterial cells from oxidative damage [29]. Barak et al (2006) reported a similar enzyme, ChrR in *E.coli*that shares sequence homology with ChrR in *P.putida*[91]. From several proteomic studies, it has been revealed that ChrR in *P.putida*F1 contains a ChrR with 100% structural homology with *Pseudomonas putida*2404 which is down regulated in response to acute chromate exposure [91,92]. On the contrary, genomic and proteomic studies of *S.oneidensis*MQ-1 showed that a NADPH-dependent FMN-reductase enzyme exhibited 28% structural homology with ChrR of *P.putida*, which is upregulated at high Cr(VI) concentration[93]. Mugerfeld et al (2009) demonstrated that deletion of the SO3585 gene was not critical for the survival of bacterial cells in presence of Cr(VI)[94]. Fein et al (2002) demonstrated non-metabolic bacterial reduction of Cr(VI) to Cr(III) [95,96]but, Nancharaiah et al (2010) clearly stated that reduction of Cr(VI) to Cr(III) is highly dependent on nutrient supply in aerobic bacteria[63]. However, in recent study it has been postulated that in some cases dead microbial cells exhibited better Cr (VI) biosorption capacity than living counterpart[96]. Figure. 2 showed an outline of the reduction of Cr(VI) to Cr(III) in bacteria.

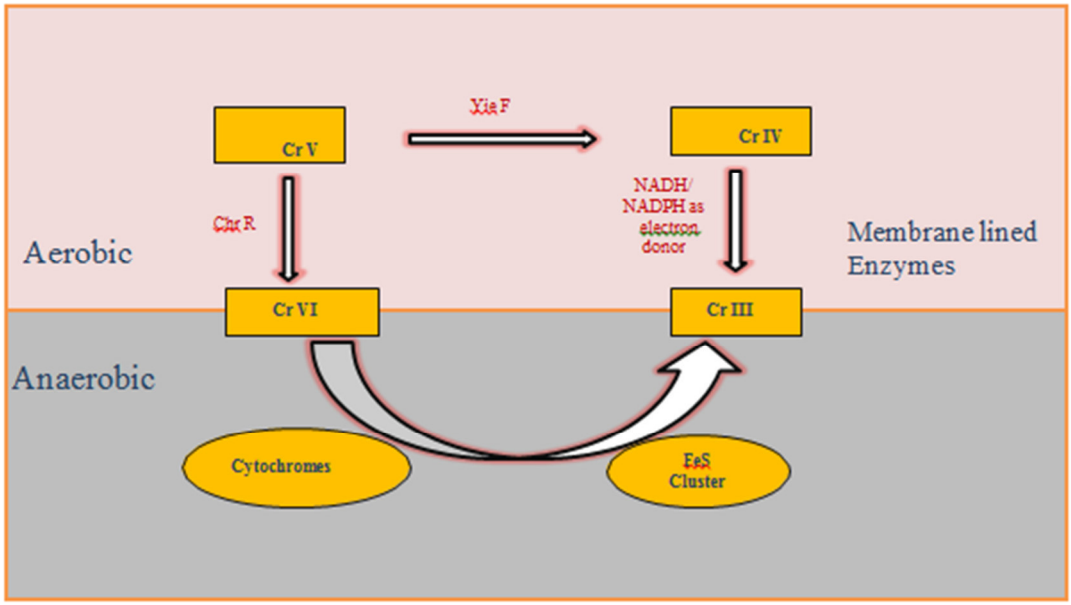

Fig 2. Schematic diagram of Cr(VI) reduction to Cr(III) by bacteria. Under aerobic condition, NADH/NADPH serves as electron donor, where as cytochromes and iron -sulfur (FeS) clusters promote the conversion of Cr(VI) to Cr(III)

2. CONCLUSION

Bacterial biomass provides a potential biosorbent for removal of toxic Cr(VI) by biosorption, bioaccumulation and biotransformation from aqueous solution. Several researchers have identified superior bacterial strains for remediation of Cr(VI) contaminated groundwater, but poor selectivity and lack of reusability of the strains hinder their applications under real conditions. However, these limitations can be easily overcome by immobilization techniques with the continuing advanced research, especially on pilot and full scale biosorption processes the situation is likely to change in near future and gradually novel bioremediation technologies such as biosorption strategy will conquer over all other conventional remediation technology still now used for removal of chromium from contaminated groundwater.

3. AUTHORS CONTRIBUTION STATEMENT

Dr. Subhadeep Ganguly conceived this idea and Dr.Smaranika Pattnayak guided him intellectually to make the review in a proper shape.

4. CONFLICT OF INTEREST

Conflict of interest declared none.

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