ISSN Online: 2349-7785

Immobilized Chromate Reducing Bacteria and their Enzymes in Bioremediation of Hexavalent Chromium

Satarupa Dey^ and A.K. Paul*
Microbiology Laboratory, Department of Botany, University of Calcutta, Kolkata, India

Received 10 January 2020; accepted in revised form 27 April 2020

Abstract: Environmental contamination by toxic hexavalent chromium [Cr(VI)] due to diverse anthropogenic activities has increased extensively in the recent past, which demands the development of eco-friendly technologies to remediate contaminated sites. Since remediation of toxic chromium by physico-chemical techniques are quite expensive and generates large quantities of solid waste for disposal, bioremediation by chromate reducing bacteria has been recognized as the most cost-effective green technology. Microbial reduction of Cr(VI) not only mitigate toxic hexavalent chromium, but also leads to the physical separation of nontoxic trivalent chromium [Cr(III)] at neutral pH following precipitation. Reductions of chromate during bacterial growth, by whole cells and by cell-free extracts have been reported by a variety of bacterial strains. They have also been exploited under immobilized conditions using several different matrices and also in the form of biofilms. The possible applications of immobilized cells, cell-free extracts, and biofilms in Cr(VI) removal from contaminated effluents using different bioreactor systems have also been assessed successfully under laboratory as well as in *in situ* conditions. The main aim of this review is to provide a thorough overview of the existing systems of Cr(VI) reduction utilizing immobilized cells, crude enzymes, and biofilms in different types of bioreactors and their effectiveness in environmental Cr(VI) bioremediation.

Key words: Hexavalent chromium, bioremediation, chromate reducing bacteria, cell-free extract, chromate reductase, immobilization, biofilms, bioreactor.

Abbreviations

AC = Activated carbon
Ba-alginate = Barium alginate
Ca-alginate = Calcium alginate
CFE = Cell-free extract
CR = Chromate reductase
Cr(VI) = Hexavalent chromium
Cr(III) = Trivalent chromium
CRB = Chromate reducing bacteria
CRM = Chromate reducing microorganisms
CPB = Column packed bioreactors
EDTA = Ethylene diamine tetraacetic acid
MBfR = Membrane biofilm reactor
PU = Polyurethane
PVA = Polyvinyl alcohol
PVC = Polyvinyl chloride
SBR = Sequencing batch reactors
SRB= Sulphate reducing bacteria

Introduction

Management of hexavalent chromium [Cr(VI)] contamination in sites polluted due to mining operations, paint, leather tanning, textile production, electroplating, metallurgy, and petroleum refinery is of major concern because of its carcinogenic properties along with several

^ Present address: Department of Botany, Shyampur Siddeswari Mahavidyalaya, Shyampur, Howrah 711312, India

*Corresponding author (A. K. Paul)
E-mail: < amalk_paul@yahoo.co.in >

other health hazards. Hexavalent chromium is highly toxic as well as mutagenic in nature and has been reported to cause nasal and skin irritation, ulceration, and lung carcinoma [40,41]. Such toxic effects are mainly attributed to the transformation of hexavalent Cr to different lower unstable as well as stable oxidation states, which eventually leads to the production of free radicals generating oxidative stress, causing DNA damage and ultimately leading to an alteration in gene expression.

Methods used for removal of Cr(VI) from the environment involve chemical reduction followed by precipitation, ion exchange, and adsorption. These methods often generate large quantities of solid wastes for disposal and the procedures are expensive and nonspecific. On the other hand, bioreduction of Cr(VI) is an economical as well as an eco-friendly alternative for such treatment. Microbial reduction of Cr(VI) to trivalent chromium [Cr(III)] is of special interest, as it not only detoxifies chromium, but also leads to the precipitation and separation of Cr(III) at neutral pH.

Romnenko and Karenkov [84] were the pioneer researchers who reported for the first time in 1977 about microbes being capable of reducing Cr(VI) and since its discovery, the continuous search for chromate reducing microorganisms (CRM) has lead to the isolation of a huge number of chromate reducing bacteria (CRB) from natural and anthropogenic environments. Pure cultures [4,12] as well as consortium [64] of bacterial strains were reported to be effective in reducing Cr(VI) under both aerobic [63-93] and anaerobic conditions [13]. Such Cr(VI) reducing microbes from diverse extreme ecological niches [2,,49,100] including chromite mining environments have the potential for application in biological mitigation of Cr(VI) polluted wastewater [27,28]. The reducing ability of these microorganisms have been well documented during growth [1,26,32], by use of viable whole cells [31,32,44,74,75,98], cell-free extracts [25,29,86] and cell-free culture filtrates [22].

The use of bacterial whole cells and cell-free extracts (CFE) for chromate reduction has certain disadvantages such as low mechanical strength, difficulties in separation of biomass from effluent, and damage of cells due to toxicity. On the contrary, the immobilized cells and enzymes thereof have higher metabolic activity as well as metabolite production and resistance against toxicants present in the effluent. Moreover, it is easier to regenerate immobilized cells, separate solid wastes, and reuse the immobilized cells with the least possible choking of pipes in continuous systems. Increased plasmid stability compared to free cells is also an added advantage in this process [75,79,97].

Several reviews have described the characteristics of chromate reduction by bacteria obtained from various contaminated sources [53] and elaborated in details the mechanism involved in Cr(VI) reduction by different groups of microorganisms [70] as well as on the future potential of the enzyme chromate reductases in bioremediation of Cr(VI) [95]. This review is an attempt to explore the potential and possible applications of immobilized whole cells, cell-free extracts as well as biofilms of chromium-resistant microorganisms in detoxification and removal of Cr(VI) from contaminated effluents.

Immobilization of chromate reducing bacteria

Since the initial studies of hexavalent chromium reduction by immobilized whole cells of *Desulfovibrio desulfuricans* by Tucker *et. al*., [97], several natural (agar-agar, agarose, alginate, carrageenan, and chitosan) and synthetic polymers (polyacrylamide, polystyrene, polyvinyl alcohol and polyurethane) have been used for microbial encapsulation.

Alginates were found effective and advantageous in encapsulating chromate reducing microorganisms [11,23,24,37]. Permeability, null toxicity, transparency of matrix, and providing gentle environment to immobilized cells were the major advantages of alginate gel entrapment [3]. Both Ba and Ca-alginate beads were effective in chromate reduction forming tough and durable gel beads with greater cell metabolic activities [11,24,37]. Alginate immobilized beads, however, were sensitive to a wide range of chemicals such as phosphates, citrate, and EDTA. The presence of excess sodium and magnesium ions and in solution also results in ion replacement leading

to osmotic swelling [96], an increase in pore size, destabilization, and finally rupture of the gel beads. Alginate immobilized microbes can not be maintained for a longer period as they are susceptible to environmental degradation and being made up of natural polymers are biodegradable [62].

Carrageenan was the choice of immobilization matrix for entrapping cells of *Arthrobacter* sp. SUK 1201 [24], *Desulfovibrio vulgaris* NCIMB 8303 and *Microbacterium* sp. NCIMB 13776 [47] and reduction of chromate. Loss of bead integrity, large pore size, leaching of cells from the matrix, and loss of metabolic activity of cells are the main disadvantages of the carrageenan encapsulation system [24].

Attempts of Humphries *et. al*.,[47], to immobilize *Desulfovibrio vulgaris* NCIMB 8303 and *Microbacterium* sp. NCIMB 13776 in chitosan and their subsequent use in the removal of toxic Cr(VI) were not successful. This was attributed to the loss of cellular activity and limitations in the diffusion phenomenon. On the contrary, viable cells of *Cellulosimicrobium cellulans* KUCr3 [14] entrapped in agarose were successfully used for chromate reduction. Agarose is a matrix that was advantageous mainly because of its hydrophilic character, ease of derivatization, absence of charged groups, etc.

Apart from natural polymers, several synthetic materials including polyvinyl alcohol (PVA) have been extensively used as immobilization matrix for *Microbacterium* sp., *Streptomyces griseus* and *Bacillus sphaericus* AND 303 cells for chromate reduction [73,75,79]. Recently Hora and Shetty [45] have immobilized *Ochrobactrum* sp. Cr-B4 in PVA-alginate and developed an industrially feasible and economically viable strategy for Cr(VI) bioremediation. Reduced cell leakage and resistance to microbial attack are the main advantages of this synthetic polymer and therefore, could be effectively used in bioreactors for large scale applications. However, as a result of polymerization cell integrity and activity are often impaired leading to restricted diffusion of chromate and electron donor into the beads [75].

Polyacrylamide is non-toxic in the cross-linked form but, cell integrity and activity are often affected during polymerization [39] due to generation of heat and free radicals. Despite this, Tucker *et. al*.[97] and Philip *et. al*.[77] have immobilized intact cells of *Desulfovibrio desulfuricans* and *Bacillus coagulans* respectively in polyacrylamide gel to remove Cr from aqueous solution

The use of both natural as well as synthetic carrier materials in combinations are not exceptional for chromate reduction. Yang *et. al*., [103] immobilized *Intrasporangium* sp. Q5-1 cells in a matrix containing 4.0 % PVA, 3.0 % sodium alginate, 1.5 % active carbon, and 3.0 % diatomite and found promising results. Similarly, *Pannonibacter phragmitetus* LSSE-09 cells encapsulated in liquid-core alginate-carboxy-methyl cellulose capsules under alkaline conditions [102] also showed potential for efficient chromate reduction.

Chromate reduction by immobilized whole cells

Attempt to use polyacrylamide immobilized cells of *Desulfovibrio desulfuricans* for removal of Cr from multi-metal solutions including Mo, Se, and U was for the first time made by Tucker *et. al*.[97]. The enzyme-mediated chromate reduction was facilitated by lactate or H_2 which were used as electron donors and the metal removal efficiencies of 86-96 % were achieved for initial concentrations of 0.5 mM Cr(VI) and 1 mM Mo, Se, and U.

Pattanapipitpaisal *et. al*.[75] and Hora and Shetty [45] immobilized *Microbacterium liquifaciens* MP3 and *Ochrobactrum* sp. Cr-B4 respectively in PVA-alginate beads and used for reduction of hexavalent chromium. However, cells of *Desulfovibrio vulgaris* NCIMB 8303 and *Microbacterium* sp. NCIMB 13776 immobilized in PVA-borate were found to be unstable and dissolved after 24 h, but were capable of reducing some Cr(VI). However, agar, agarose, and k-carrageenan immobilized cells of *D. vulgaris* NCIMB 8303 reduced Cr(VI) in the presence of formate at approximately equal initial rates with those of free cells, which suggested no diffusion constraints. The reduction rates were much lower in *Microbacterium* sp. NCIMB 13776 and the

highest rate was evident in agar immobilized cells. Similarly, *Streptomyces griseus* immobilized in PVA-alginate showed 100 % Cr(VI) removal within 24 h showing reduction rates comparable to the free cells [48]. Immobilized mycelial mass completely reduced 25 mg Cr(VI)/l in 24 h and were recycled for four times without any reduction in efficiency [78]. Likewise, cells of *Bacillus sphaericus* AND 303 entrapped in PVA-alginate beads effectively reduced 87.5% of 20 µM Cr(VI) in 24 h under batch culture and beads were recycled three times without apparent cell leakage and disintegration [73].

A complex matrix containing PVA (4.0 %), sodium alginate (3.0 %), active carbon (1.5 %), and diatomite (3.0 %) when used to immobilize intact cells of *Intrasporangium* sp. Q5-1 [103] was found to be most effective, as it was capable of reducing initial 0.5 mM Cr(VI) to 0.01 mM within 17 h of incubation (97.5 % removal). This Cr(VI) removal efficiency was similar to the free *Intrasporangium* sp. Q5-1 cells.

Viable whole cells of *Cellulosimicrobium cellulans* KUCr3 entrapped in agarose were used in batch culture for Cr(VI) reduction [14]. At 0.5 mM and 2 mM Cr(VI) in peptone-yeast extract-glucose medium, 75 and 52 % of Cr(VI) reduction was evident in 96 h. It could also reduce the Cr(VI) content to 40 % in tannery effluent.

Hexavalent chromium was reduced efficiently to relatively non-toxic Cr(III) by *Pannonibacter phragmitetus* LSSE-09 immobilized in liquid-core alginate-carboxymethyl cellulose capsules under alkaline conditions [102] showing a reduction rate of 4.20 mg/g dry wt/min. Murugavelh and Mohanty [66] tested Ca alginate, acrylamide, and agar as the matrices for immobilization of whole cells of *Halomonas* sp. among which Ca alginate was identified as most suitable.

Dey and Paul [23,24] reported effective Cr(VI) reduction by immobilized whole cells of a potent chromate resistant and reducing actinomycetes, *Arthrobacter* sp. SUK 1201 and SUK 1205, obtained from mine overburden of Sukinda valley chromite mines of Odisha, India. Ba and Ca-alginate immobilized whole cells were most effective as it showed more than 95 % reduction of 100 µM Cr(VI) in 24 h in both the cases without any apparent leakage of immobilized cells and visible disintegration of beads.

Chromate reduction studies so far conducted by immobilized whole cells of different groups of organisms in batch culture have been highlighted in Table 1.

Bacterial biofilms and their application in chromate reduction

It is apparent that more than 99 % of all microorganisms on Earth live as biofilms and are often described as natural metal-immobilizing matrices in aqueous environments [33]. Biofilms tolerate high concentrations of metals in contaminated wastewater [43] and can be effectively used for easy separation of the treated liquid from the contaminants trapped in the biomass. The granular biofilms in particular can settle extremely well and is used for effluent treatment and separation of contaminants by sedimentation. Therefore, biofilm-based reduction of Cr(VI) and its subsequent immobilization appears to be a better option for remediation of chromium pollutants. Biofilms are generally composed of microbial cells and their products, the extracellular polymeric substances (EPS), which are very porous in nature and contain a high amount of water (95 %) [91]. The vital criterion that triggers the process of biofilm formation include i) production of EPS by the bacterial isolate(s) and ii) the presence of suitable substrate for attachment [35].

Role of EPS in biofilm production

Production of EPS is an essential step towards biofilm formation. It not only helps in adhesion of cells to the inert surface but also in the accumulation of nutrients from the environment and provides a protective barrier against toxic metal contaminants. Compositionally the bacterial EPS is complex and consists mainly of polysaccharides, proteins, lipids, humic substances, and nucleic acids [34,35]. They contain ionisable carboxyl, phosphoric, amine, and hydroxyl groups, which enable them to absorb minerals, nutrients, and toxic metals [46,92]. The EPS also assists in the bio-mineralization of metal ions by concentrating and precipitating fine-grained

Table 1. List of Cr(VI) reducing bacterial isolates immobilized in different matrices and their applications for chromate reduction under batch mode

Bacterial isolate	Immobilization matrix	Cr(VI) reduction efficiency	Reference
Arthrobacter sp. SUK 1205	Ca alginate	100 % of 100 µMCr(VI) in 24 h	[23]
Arthrobacter sp. SUK 1201	Ba alginate	100 % of 100 µMCr(VI) n 28 h	[24]
Bacillus sphaericus AND 303	Poly vinyl alcohol	87.5 % of 20 µM Cr(VI) in 24 h	[73]
Bacillus sp.	Ca alginate	100 % of 100 mg/l Cr(VI) after 150 h	[54]
Bacillus subtilis	Ca alginate	99.6 % of 570 mg/l Cr(VI) after 192 h	[9]
Cellulosimicrobium cellulans KUCr3	Agarose bead	75 % of 0.5 mM and 52 % of 2 mM Cr(VI) in 96 h	[14]
Halomonas sp.	Ca alginate	98.9 % of 10 mg/l Cr(VI) in 28 h	[66]
Intrasporangium sp. Q5-1	PVA, Na alginate, active carbon and diatomite	97.5 % of 0.5 mM Cr(VI) in 17 h	[103]
Microbacterium liquifaciens MP 30	PVA-borate and alginate	100 % of 100 µM Cr(VI) in 4 days	[75]
Pannonibacter phragmitetus LSSE-09	Alginate-carboxymethyl cellulose	100 % of 50 mg/l Cr(VI) in 120 min	[102]
Pseudomonas aeruginosa	Ca alginate	99.3 % of 570 mg/l Cr(VI)after 192 h	[9]
Pseudomonas sp. S4	Ca alginate	85.1 % of 120 mg/l Cr(VI) in 7 days	[32]
Streptomyces griseus	Poly vinyl alcohol	100 % of 25 mg/l Cr(VI) in 24 h	[79]

minerals within the biofilm mat.

Production of EPS in Cr(VI) resistant and reducing bacteria is well documented in *Methylobacterium mesophilium* MU141 [72] *Enterobacter cloaceae* [51], *Bacillus coagulans* (CECT12), *Streptococcus euisimilis* (CECT926), *Escherichia coli* (CECT 515) [82] and *Pseudomonas aeruginosa* Rb-1 [6], which ranged from 0.059 to 0.063 mg/ml [90]. However, EPS produced by some strains is inefficient for adhesion of cells, and therefore requires the formation of an artificial biofilm by immobilizing the cells. *Pseudomonas aeruginosa* A2Chr, for example, produced EPS but was not sufficient for adhesion and formation of biofilms. Cells carried out chromate reduction when immobilized in agar-agar and agarose matrices [37].

Use of different support material

Inert materials used as support for biofilm formation include polyurethane (PU) foam cubes, coconut coir, glass beads, glass slides, wood husk, granulated activated carbon (AC), and zeolite. Enzymatic reduction of Cr(VI) by different species of *Pseudomonas* has been accomplished by using biofilms formed on membranes layed over iron-deficient solid media [80], films formed on cellulose acetate membrane [56], nylon matrix [37], etc. Biofilms of sulphate-reducing bacteria, have been developed on plastikard sheet [89] and Pozzolana [8] to reduce toxic Cr(VI) to insoluble Cr(III). Use of AC [65], AC and zeolite [59], celite and amberlite [11], along with gravel [20], pumice particles [36], sand, PVC, stone, and rubber tubing [68] as solid supports in the formation of stable biofilm and their evaluation in chromium reduction by variety of chromium-resistant and reducing bacteria are not uncommon. An account of biofilms produced by different bacterial isolates on a variety of support materials and their use in evaluating Cr(VI) reducing efficiency are summarised in Table 2.

Free and immobilized cell-free extracts in chromate reduction

Chromate reductase (CR) was initially isolated and partially purified from *Pseudomonas putida* PRS2000 [50]. Cytoplasmic fractions of *Pannoni-*

Table 2. Biofilms produced by different bacterial isolates on variety of support materials and their use in evaluating Cr(VI) reducing efficiency

Bacterial isolate	Support material	Cr(VI) reduction efficiency	Reference
Acinetobacter haemolyticus	Wood husk	97 % of 15 mg/l Cr(VI) in 3 days	[105]
Arthrobacter sp. CR 47	Granular activated carbon	100 % of 30 mg/l Cr(VI) in 26 h	[20]
Arthrobacter viscosus	Granular activated carbon and zeolite	99 % of 10 mg/l of Cr(VI) in 30 days	[59]
Bacillus sp. ES 29	Celite and amberlite	100 % of 2 mg/l Cr(VI) in 15 h	[10,11]
Cellulosimicrobium sp.	Sand, PVC, stone, and rubber tubing	88.4-96 % of 500 µg/ml Cr(VI) in 96 h	[68]
Halomonas aquamarina TA-04	Sterile pumice particles	94.5 % of the 0.5 mM Cr(VI)	[36]
Mixed culture of sulphate-reducing bacteria	Glass beads	90 % of the 100 µ M/l Cr(VI) in 48 h	[89]

bacter phragmitetus LSSE-09 [102] and *Thermus scotoductus* [71] reduced Cr(VI) in both aerobic as well as in anaerobic conditions. Membrane-bound CR activity was reported in *Enterobacter cloacae* HO1 [99], *Bacillus megaterium*, *Shewanella putrefaciens* MR-1 [67] and *Paracoccus denitrificans* [85]. Periplasmic fractions of *Pseudomonas aeruginosa* A2Chr [38] and *Arthrobacter crystallopoietes* ES 32 [10] also reduced Cr(VI) efficiently.

Despite the advantages of enzyme immobilization such as i) cost-effectiveness, ii) easy separation of reaction mixture, iii) possibility of getting higher activity of enzyme per unit volume in the reactor, and iv) increased activity compared to soluble enzymes [29], very few reports are available regarding usage of an immobilized cell-free extract of CRB in Cr(VI) reduction. The main constrains of CR immobilization are the stability of the enzymes outside the cellular environment, thermolabile nature [94], binding low salt conditions, hydrophobicity of the matrix leading to partial denaturation and low activity yield.

The prerequisite for enzymatic remediation of Cr(VI) is to make the enzyme preparation cheap, production of the enzyme in large quantities, and availability of cost-effective electron donors. Most of the efficient Cr(VI) transforming enzymes reported so far are flavoproteins [42,57,71] and require NAD(P)H as cofactors, which is highly expensive and therefore reduces the economical viability of the process for large scale applications.

Immobilized CFE of *Bacillus* sp. ES 29 was successful in substantial Cr(VI) reduction [11] showing maximum reduction of chromate (k= 0.689 at 3 ml/h) with Ca-alginate immobilized CFE. The flow rate of 3 ml/h and 6 ml/h showed a nearly identical pattern of reduction and nearly all added Cr(VI) was reduced. Likewise, Elangovan *et. al*.[29] immobilized CFE of *Arthrobacter rhombi* RE in Ca-alginate beads and found it to be capable of reducing Cr(VI), but the performance was not very encouraging for a continuous mode of operation.

Chromate bioremediation in *in situ* and bioreactor systems

Common effluent treatment technologies for

removal of toxic hexavalent chromium from industrial waste include ion-exchange, electrodeposition, and chemical reduction with zerovalent iron, ferrous iron [60,101], dissolved sulphides [55], sulphur containing solutions, followed by precipitation at a high pH [5]. Though these methods are effective, but are quite expensive, require high energy input, large quantities of chemical reagents, and generate secondary wastes which could be detrimental to the environment. *In situ* bioremediation process is cost-effective and less environmentally intrusive than the currently employed pump-and-treat method at the polluted sites. The use of indigenous bacterial populations can be advantageous as it is more promising for ensuring durability under various operating conditions. Apart from *in situ* bioremediation, bioreactors with immobilized microorganisms at higher cell densities are being used for wastewater treatment to achieve not only high performance as well as stability along. These bioreactor systems ensure extended biochemical or biotransformation reaction time without any cell washout.

Shen and Wang [87] used a two-stage bioreactor system in which aerobically grown *Escherichia coli* cells were pumped into an anaerobic plug-flow reactor to reduce Cr(VI). Under this bioreactor system, almost complete removal of Cr(VI) was achieved under specific operating conditions. The efficiency of the reactor, however, was affected by Cr(VI) concentration and incubation period.

The use of biofilm packed bed reactors for the bioreduction of Cr(VI) has been reported by Chirwa and Wang [17,18]. *Bacillus* sp. was used in a fixed-film bioreactor which was operated in a continuous mode with a high recycle ratio. After 24 days from inoculation, the bioreactor efficiently removed nearly 50 mg/l within a detention time of 24 h with an average reduction rate of approximately 2 mg/l/h.

A fixed-film co-culture bioreactor with glass beads was used for simultaneous Cr(VI) reduction and phenol degradation [16]. *Pseudomonas* sp. was used in the bioreactor system for bioremediation of toxic Cr(VI) contaminated effluent. *Pseudomonas mendocina* reduced 99.7 % of 50 mg Cr(VI)/l from chrome plating effluent of the automobile industry [83], while Ganguli and Tripathi [37] reported 75 % chromate removal from chrome-plating effluent (10 mg Cr(VI)/l) by *Pseudomonas aeruginosa* A2Chr in a dialysis bioreactor. Later Konovalova *et. al*.[56] studied chromate reduction in a membrane bioreactor with bacteria immobilized in agar-agar films on the surface of cellulose acetate membrane which showed an improved cell activity compared with free cells. This study was conducted in a two-chambered reactor separated by a membrane with immobilized bacteria and the membrane bioreactor showed high efficiency by completely reducing 40 mg/l Cr(VI) in 80 h.

Cells of *Bacillus* sp. ES 29 immobilized in celite, amberlite, and calcium alginate and celite showed the highest rate (3 ml/h) of Cr(VI) reduction after 15 h [11] in column packed bioreactors (CPB). PVA immobilized *Microbacterium* cells [75] in a similar CPB completely reduced 50 μM Cr(VI) within 20 days. Later Humphries *et. al*.[47] also used *Microbacterium* sp. NCIMB 13776 immobilized in agar and agarose in cell packed-bed bioreactors and observed 60 % Cr(VI) removal with agar-immobilized *Microbacterium* sp. Columns containing agarose-immobilized *Microbacterium* sp. lost activity after 40 h due to loss of matrix stability and bead integrity. Likewise, agar-immobilized cells of *Desulfovibrio vulgaris* NCIMB 8303 removed 95 % of Cr(VI) in packed-bed bioreactors at a flow rate of 2.4 ml/h, although the reducing activities were lost after 159 h [47].

Acinetobacter sp. has been immobilized by both Dermou *et. al*.[21] and Zakaria *et. al*.[105] and their chromate reduction efficiency was evaluated in a bioreactor. In a pilot-scale aerobic trickling filter bioreactor inoculated with a consortium containing *Acinetobacter* sp. Dermou *et. al*.[21] reported the reduction of 30 mg/l Cr(VI) in the presence of sodium acetate as a carbon source. Under sequencing batch reactors (SBR) mode, trickling filter reached operating cycles as short as 40 min after 50 days of inoculation. Around 97 % of the Cr(VI) in electroplating wastewater [containing 15 mg/l of Cr(VI)] was reduced at a flow rate of 8.0 ml/min in 3 days when *Acineto-*

Table 3. Reduction of hexavalent chromium in different bioreactor systems using chromium resistant and reducing bacteria

Bacterial isolate	Source	Bioreactor system	Cr(VI) reducing efficiency	References
Acinetobacter haemolyticus	Cr(VI)-containing wastewater	Column packed bed bioreactor	97 % of the Cr(VI) in wastewater containing 15 mg/l of Cr(VI), reduced at a flow rate of 8.0 ml/min in 3 days	[105]
Arthrobacter Cr47	Land farming process soil sample	Gravel packed bed reactors	100 % removal of 30 mg/l Cr(VI)	[20]
Arthrobacter viscosus	Spanish Type Culture Collection	Biofilm bioreactor	99.9 % removal of 10 mg/l Cr(VI) during the first 30 days,	[81]
Arthrobacter viscosus	Spanish Type Culture Collection	Zeolite packed column reactor	73 % removal of 100 mg/L	[76]
Arthrobacter rhombi RE	Chromium contaminated soil	Aerobic attached growth reactor	98 % reduction of 36 m/L	[30]
Arthrobacter rhombi RE	Chromium contaminated soil	Anoxic attached growth reactor	98 % reduction of 36 m/L	[30]
Bacillus sp.	Cr(VI) contaminated environment	Re-circulated packed bed batch reactor	100 % reduction of 100-300 mg/l Cr(VI), in 150-200 h	[54]
Cellulosimicrobium cellulans KUCr3	Tannery effluent	Packed bed column	25 % Cr(VI) reduction occurred after 144 h	[14]
Desulfomicrobium norvegicum	Chromium contaminated effluent	Film fixed bioreactor	Feed containin 40 mg/l Cr(VI), 600 mg/l sulfate and H_2, reduction rate (1 mg/l/h)	[7]
Microbial community dominated by *Dechloromonas* spp	Microbial community of denitrifying membrane biofilm reactors	Membrane bioreactor	68 % removal of 250 µg-Cr/l Cr(VI) on the first day	[19]
Escherichia coli 33456	American Type Culture Collection	Fixed film reactor	About 92 % Cr(VI) was reduced under influent Cr(VI) loading rate (11.3 g/[m^3·day])	[104]
Streptomyces griseus	National Collection of Industrial Microorganisms Pune	Glass reactor	100 % reduction of 2 mg/l Cr(VI) in 5.5 h	[78]

table 3. (continued).

Bacterial isolate	Source	Bioreactor system	Cr(VI) reducing efficiency	References
Pseudomonas putida KI	Tannery waste water and sludge	Biochar packed bioreactor	100 % of 10 mg/L Cr(VI) in 24 h	[61]
Mixed sulfate reducers	Electroplating waste water	Sulfidogenic two-stage packed-bed reactor system	About 100 % of initial 225-352.5 mg/L Cr(VI)	[15]
Consortium of Cr(VI) reducing bacteria	Treatment plant at contaminated site in Brits	Microcosm packed-bed reactors	66.26 % of 50 mg/l Cr(VI) in 45 days	[64]
Sulphate reducing bacterial consortium	Digester sludge from sewage treatment plant	Small scale bioreactor	Maximum chromium(VI) removal was found to be 96.0 % at initial concentration of 50 mg/L	[88]
Indigenous consortium	Chromium contaminated sediments	Sand column reactor	Upto 63.6 % of 12 mg/L	[60]
Mixed microbial consortia	Domestic wastewater treatment plant	Granular biofilm bioreactor	Completely reduced 0.2 mM Cr(VI) at 0.15 mM /day/ g in 4 day	[69]
Biofilm containing *Meiothermus* and *Methylosinus*	Cr(VI) reducing biofilm	Membrane biofilm reactor	95 % of 3 mg Cr/L in 90 days	[58]

bacter haemolyticus immobilized on wood husk was used in a packed bed column bioreactor [105].

Chromium reducing actinomycetes, such as *Streptomyces griseus* [79], *Arthrobacter viscosus* [76,81], and *Arthrobacter rhombi-RE* (MTCC7048) [30] are the ideal candidates for chromate reduction. *S. griseus* immobilized in PVA-alginate beads in a glass reactor removed 100 % of 2 mg/l of Cr(VI) in 55 h [79]. *A. viscosus* biofilm on granular AC [81], and in Zeolite packed reactor [76] were evaluated in pilot-scale and an almost 100 % Cr(VI) removal was achieved. The efficacy of *A. rhombi-RE* (MTCC7048) was evaluated in different types of bioreactors under variable operating conditions. Complete reduction of Cr(VI) was achieved under aerobic and anoxic batch [30] experiments.

Sulphate reducing bacteria (SRB) are known to reduce Cr(VI) indirectly by hydrogen sulphide or by using Cr(VI) as a terminal electron acceptor. When *Desulfomicrobium* sp. was used in an anaerobic fixed film bioreactors with a feed containing 40 mg/l Cr(VI), 600 mg/l sulphate, and H_2, a reduction rate of 1 mg/l/h was achieved [7]. Similarly, mixed cultures of SRB have been used in the sulfidogenic fixed-film batch reactor [89] and two-stage packed-bed reactor [15] with high efficacy.

The ability of bacterial consortium immobilized in a bioreactor to reduce toxic Cr(VI) have also been tested by several workers [52,60,64,69]. Jeyasingh and Philip [52] operated the bioreactor at a bacterial concentration of 15 ±1.0 mg/g of soil (wet weight), 50 mg of molasses/g of soil as carbon source, and could reduce entire 5.6 mg Cr(VI)/g of soil in 20 days. Molokwane *et. al*.,[64] set up a microcosm reactor and operated as a packed-bed reactor, which achieved significant removal of Cr(VI) (66.26 %) in 45 days. However, the effectiveness of the bioreactor increased with the addition of sawdust as a carbon source and showed a reduction of a maximum of 93% at a flow rate of 0.304 cm^3/day. Lee *et. al*.[60] and Nancharaiah *et. al*.[69] also evaluated the potential of mixed microbial consortia to reduce chromate in sand column reactor system and granular biofilms respectively under batch mode.

Conclusion

Large-scale Cr contamination of land and water resources due to anthropogenic activities has

demanded for bioremediation of highly toxic and carcinogenic Cr(VI) in an eco-friendly manner. Bioremediation approaches have utilized the Cr(VI)-reducing ability of introduced as well as indigenous microorganisms and found to be more effective for remediation of chromium contaminated water and soils. Immobilization of whole cells and the cell-free extracts as a source of chromate reductase enzymes are useful tools to meet the cost targets and to achieve technological advantages. Immobilization enables the repetitive use of both whole cells and crude enzymes and hence is beneficial and cost-efficient. From the technological viewpoints, immobilized whole cells and enzymes can easily be separated from the reaction liquid and make laborious separation steps unnecessary. Additional benefits arise from stabilization against harsh reaction conditions, which are deleterious to different microorganisms and soluble enzyme preparations. As Cr(VI) poses a threat to humans and the environment, it is also relevant to study a biofilm-based chromium remediation strategy. A simplified flow-through system has been designed for biofilm development on economically feasible substrates for Cr(VI) removal. The use of biofilms developed from indigenous chromate reducing microorganisms and its use as a tool for removal of Cr(VI) gives a perceptiveness into *in situ* remediation of chromium.

References

1. **Alam, M.Z., Ahmad, S. (2011).** Toxic chromate reduction by resistant and sensitive bacteria isolated from tannery effluent contaminated soil. Annals of Microbiology. 62: 113-121.
2. **Amoozegar, M.A., Ghasemi, A., Razavi, M.R., Naddaf, S. (2007).** Short Communication: Evaluation of hexavalent chromium reduction by chromate-resistant moderately halophile, *Nesterenkonia* sp. strain MF2. Process Biochemistry. 42: 1475-1479.
3. **Arau´ jo, A.A., Andrade Santana, M.H. (1996).** Aerobic immobilized cells in alginate gel particles of variable density. Applied Biochemistry and Biotechnology. (57/58): 543-550.
4. **Asianti, N.V., Abuladze, M.K., Kartvelishvili, T.M., Bakradze, N.G., Sapojnikova, N.A., Tsibakhashvili, N.Y., Tabatadze, L.V., Asanishvili, L.L., Holman, H. (2004).** Effect of chromium (VI) action on *Arthrobacter oxydans*. Current Microbiology. 49: 321-326.
5. **Ba-Díaz, C.E., Lugo-Lugo, V., Bilyeu, B. (2012).** A review of chemical, electrochemical and biological methods for aqueous Cr(VI) reduction. Journal of Hazardous Material. 15: 1-12.
6. **Batool, R., Yrjälä, K., Shaukat, K., Jamil, N., Hasnain, S. (2015).** Production of EPS under Cr(VI) challenge in two indigenous bacteria isolated from a tannery effluent. Journal of Basic Microbiology. 55: 1064-1074.
7. **Battaglia-Brunet, F., Michel, C., Joulian, C., Ollivier, B., Ignatiadis, I. (2007).** Relationship between sulphate starvation and chromate reduction in a H_2-fed fixed-film bioreactor. Water Air Soil Pollution. 183: 341-353.
8. **Battaglia-Brunet, F., Foucher, S., Denamur, A., Ignatiadis, I., Michel, C., Morin, D. (2002).** Reduction of chromate by fixed films of sulfate-reducing bacteria using hydrogen as an electron source. Journal of Industrial Microbiology and Biotechnology. 28: 154-159.
9. **Benazir, F.J., Suganthi, R., Rajvel, D., Padmini Pooja, M., Mathithumilan, B. (2010).** Bio-remediation of chromium in tannery effluent by microbial consortia. African Journal of Biotechnology. 9: 3140-3143.
10. **Camargo, F.A.O., Bento, F.M., Okeke, B.C. Frankenberger, W.T. (2004a).** Hexavalent chromium reduction by an actinomycetes, *Arthrobacter crystallopoites* ES 32. Biological Trace Element Research. 97: 183-194.
11. **Camargo, F.A.O., Okeke, B.C., Bento, F.M. Frankenberger, W.T. (2004b).** Hexavalent chromium reduction by immobilized cells and cell-free extract of *Bacillus sp.* ES29. Bioremediation Journal. 8: 23-30.
12. **Camargo, F.A.O., Okeke, B.C., Bento, F.M., Frankenberger, W.T. (2003).** In vitro reduction

of hexavalent chromium by a cell-free extract of *Bacillus* sp. ES 29 stimulated by Cu^{2+}. Applied Microbiology and Biotechnology. 62: 569-573.

13. **Cervantes, C., Campos-Garcia, J., Devars, S., Corona, F.G., Loza-Tavera, H., Carlos, J., Guzman, T., Sanchez, R.M. (2001).** Interactions of chromium with microorganisms and plants. FEMS Microbiology Reviews. 25: 335-347.
14. **Chatterjee, S., Sau, G.B., Mukherjee, S.K. (2011).** Bioremediation of Cr(VI) from Chromium-Contaminated Wastewater by Free and Immobilized Cells of *Cellulosimicrobium cellulans* KUCr3. Bioremediation Journal. 15: 173-180.
15. **Chang, I.S., Kim, B. (2007).** Effect of sulfate reduction activity on biological treatment of hexavalent chromium [Cr(VI)] contaminated electroplating wastewater under sulfate-rich condition. Chemosphere. 67: 218-226.
16. **Chirwa, E.S., Wang, Y.T. (2000).** Simaltaneous chromium(VI) reduction and phenol degradation in an anaerobic consortium of bacteria. Water Research. 34: 2376-2384
17. **Chirwa, E.M.N., Wang, Y.T. (1997).** Hexavalent chromium reduction by *Bacillus* sp. in a packed-bed bioreactor. Environmental Science and Technology. 31: 1446-1451.
18. **Chirwa, E.M.N., Wang, Y.T. (1997b).** Chromium(VI) reduction by *Pseudomonas fluorescens* LB 300 in a fixed-film bioreactor. Journal of Environmental Engineering. 123: 760-766.
19. **Chung, J., Ryu, H., Abbaszadegan, M., Rittmann, B.E. (2006).** Community structure and function in a H_2-based membrane biofilm reactor capable of bioreduction of selenate and chromate. Applied Microbiology and Biotechnology. 72: 1330-1339.
20. **Cordoba, A., Vargas, P., Dussan, J. (2008).** Chromate reduction by *Arthrobacter* CR47 in biofilm packed bed reactors. Journal of Hazardous Material. 151: 274-279.
21. **Dermou, E., Velissariou, A., Xenos, D., Vayenas, D.V. (2005).** Biological chromium(VI) reduction using a trickling filter. Journal of Hazardous Material B. 126: 78-85.
22. **Dey, S., Paul, A.K. (2016).** Evaluation of chromate reductase activity in the cell-free culture filtrate of *Arthrobacter* sp. SUK 1201 isolated from chromite mine overburden. Chemosphere. 156: 69-75.
23. **Dey, S., Paul, A.K. (2015).** Hexavalent Chromate Reduction During Growth and by Immobilized Cells of *Arthrobacter* sp. SUK 1205. Science, Technology and Development. 34 (3): 158-168.
24. **Dey, S., Paul, A.K. (2014).** Reduction of hexavalent chromium by immobilized viable cells of *Arthrobacter* sp. SUK 1201. Bioremediation Journal. 18(1): 1-11.
25. **Dey, S., Paul, A. K. (2013).** Evaluation of *in vitro* Reduction of Hexavalent Chromium by Cell-Free Extract of *Arthrobacter* sp. SUK 1201. British Microbiology Research Journal. 3(3): 325-338.
26. **Dey, S., Paul, A.K. (2012).** Optimization of cultural conditions for growth associated chromate reduction by *Arthrobacter* sp. SUK 1201 isolated from chromite mine overburden. Journal of Hazardous Material. 213-214: 200-206.
27. **Dey, S., Paul, A.K. (2010).** Occurrence and evaluation of chromium reducing bacteria in seepage water from chromite mine quarries of Orissa, India. Journal of Water Research and Protection. 2: 380-388.
28. **Dhal, B., Thatoi, H., Das, N., Pandey, B.D. (2010).** Reduction of hexavalent chromium by *Bacillus* sp. isolated from chromite mine soils and characterization of reduced product. Journal of Chemical Technology and Biotechnology. 85: 1471-1479.
29. **Elangovan, R., Philip, L., Chandraraj, K. (2010).** Hexavalent chromium reduction by free and immobilized cell-free extract of *Arthrobacter rhombi*-RE. Applied Biochemistry and Biotechnology. 160: 81-97.
30. **Elangovan, R., Philip, L. (2009).** Performance evaluation of various bioreactors for the removal of Cr(VI) and organic matter from industrial effluent, Biochemical Engineering Journal. 44: 174-

186.
31. **Ezaka, E., Anyanwu, C.U. (2011).** Chromium (VI) tolerance of bacterial strains isolated from sewage oxidation ditch. International Journal of Environmental Science. 1: 1725-1734.
32. **Farag, S., Zaki, S. (2010).** Identification of bacterial strains from tannery effluent and reduction of hexavalent chromium. Journal of Environmental Biology. 31: 877-882.
33. **Ferris, F.G., Schultze, S., Witten, T.C., Fyfe, W.S., Beveridge, T.J. (1989).** Metal interactions with microbial biofilms in acidic and neutral pH environments. Applied and Environmental Microbiology. 55: 1249-1257.
34. **Flemming, H.C. (1995).** Sorption sites in biofilms. Water Science and Technology. 32: 27-33.
35. **Flemming, H.C., Wingender, J. (2001).** Relevance of microbial extracellular polymeric substances (EPSs) – Part I: structural and ecological aspects. Water Science and Technology. 43: 1-8.
36. **Focardi, S., Pepi, M., Landi, G., Gasperini, S., Ruta, M., Biasio, P.D., Focardi, S.E. (2012).** Hexavalent chromium reduction by whole cells and cell free extract of the moderate halophilic bacterial strain *Halomonas* sp. TA-04. International Biodeterioration and Biodegradation. 66: 63-70.
37. **Ganguli, A., Tripathi, A.K. (2002).** Bioremediation of toxic chromium from electroplating effluent by chromate-reducing *Pseudomonas aeruginosa* A2Chr in two bioreactors. Applied Microbiology and Biotechnology. 58: 416-420.
38. **Ganguli, A., Tripathi, A.K. (1999).** Survival and chromate reducing ability of *Pseudomonas aeruginosa* A2Chr in industrial effluents. Letter in Applied Microbiology. 60: 1525-1531.
39. **Gianfreda, L., Parascandola, P., Scardi, V. (1980).** A new method of whole microbiol cell immobilization. Applied Microbiology and Biotechnology. 11: 6-7.
40. **Gibb, H.J., Lee, P.S., Pinsky, P.F., Rooney, B.C. (2000a).** Lung cancer among workers in chromium chemical production. American Journal of Industrial Medicine. 38: 115-126.
41. **Gibb, H.J., Lee, P.S., Pinsky, P.F., Rooney, B.C. (2000b).** Clinical findings of irritation among chromium chemical production workers. American Journal of Industrial Medicine. 38: 127-131.
42. **Gonzalez, C.F., Ackerley, D.F., Park, C.H., Matin, A. (2003).** A soluble flavoprotein contributes to chromate reduction and tolerance by *Pseudomonas putida*. Acta Biotechnologica. 23: 233-239.
43. **Harrison, J., Ceri, J., Turner, R.J. (2007).** Multimetal resistance and tolerance in microbial biofilms. Nat. Review Microbiology. 5: 928-938.
44. **He, Z., Gao, F., Sha, T., Hu ,Y., He, C. (2009).** Isolation and characterization of a Cr(VI)-reduction *Ochrobactrum* sp. strain CSCr-3 from chromium landfill. Journal of Hazardous Material. 163: 869-873.
45. **Hora, A., Shetty, V. K. (2015).** Kinetics of bioreduction of hexavalent chromium by polyvinyl alcohol-alginate immobilized cells of *Ochrobactrum* sp. Cr-B4 and comparison with free cells. Desalination and water Treatment. 57: 8981-898
46. **Hullabusch, E.D., Zandvoort, M.H., Lens, P.N.L. (2003).** Metal immobilisation by biofilms: Mechanisms and analytical tools. Reviews in Environmental Science and Biotechnology. 2: 9-33.
47. **Humphries, A.C., Nott, K.P., Hall, L.D., Macaskie, L.E. (2005).** Reduction of Cr(VI) by immobilized cells of *Desulfovibrio vulgaris* NCIMB 8303 and *Microbacterium sp*. NCIMB 13776. Biotechnology and Bioengineering. 90: 589-596.
48. **Humphries, A.C., Nott, K.P., Hall, L.D., Macaskie, L.E. (2005b).** Continuous removal of Cr(VI) from aqueous solution catalysed by palladised biomass of *Desulfovibrio vulgaris*. Biotechnology Letters. 26: 1529-1532.
49. **Ibrahim, A.S.S., El-Tayeb, M.A., Elbadawi, Y.B., Al-Salamah, A.A. (2011).** Isolation and characterization of novel potent Cr(VI) reducing alkaliphilic *Amphibacillus* sp. KSUCr3 from

hypersaline soda lakes. Electronic Journal of Biotechnology. 14: DOI: 10.2225/vol14-issue4-fulltext-4

50. **Ishibashi, Y., Cervantes, C., Silver, S. (1990).** Chromium reduction in *Pseudomonas putida*. Applied and Environmental Microbiology. 56: 2268-70.
51. **Iyer, A., Mody, K., Jha, B. (2004).** Accumulation of hexavalent chromium by an exopolysaccharide producing marine *Enterobacter cloaceae*. Marine Pollution Bulletin. 49: 974-977.
52. **Jeyasingh, J., Philip, L. (2005).** Bio-remediation of chromium contaminated soil: Optimization of operating parameters under laboratory conditions, Journal of Hazardous Material. 118: 113-120.
53. **Kamaludeen, S.P.B., Megharaj, M., Juhasz, A.L., Sethunathan, N., Naidu, R. (2003).** Chromium-Microorganism Interactions in Soils: Remediation Implications. Reviews in Environmental Contamination and Toxicology. 178: 93-164.
54. **Kathiravan, M.N., Karthiga Rani, R., Karthick, R., and Muthukumar, K. (2010b).** Mass transfer studies on the reduction of Cr(VI) using calcium alginate immobilized *Bacillus* sp. in packed bed reactor. Bioresource Technology. 101: 853-858.
55. **Kim, C., Zhou, Q., Deng, B., Thornton, E.C., Xu, H. (2001).** Chromium(VI) Reduction by Hydrogen Sulfide in Aqueous Media: Stoichiometry and Kinetic. Environment Science and Technology. 35: 2219-2225.
56. **Konovalova, V.V., Dmytrenko, G.M., Nigmatullin, R.R., Bryk, M.T., Govzdyak, P.I. (2003).** Chromium (VI) reduction in a membrane bioreactor with immobilized *Pseudomonas* cells. Enzyme Microbial Technology. 33: 899-907.
57. **Kwak, Y.H., Lee, D.S., Kim, H.B. (2003).** *Vibrio harveyi* nitroreductase is also a chromate reductase. Applied and Environmental Microbiology. 69: 4390-4395.
58. **Lai, C., Zhong, L., Zhang, Y., Chen, J., Wen, L., Shi, L., Sun, Y., Ma, F., Rittmann, B.E., Zhou, C., Youneng Tang, C., Zheng, P., Zhao, H. (2016).** Bioreduction of Chromate in a Methane-Based Membrane Biofilm Reactor. Environment Science and. Technology. 50(11): 5832-5839.
59. **Lameiras, S., Quintelas, C., Tavares, T. (2008).** Biosorption of Cr (VI) using a bacterial biofilm supported on granular activated carbon and on zeolite. Bioresource Technology. 99: 801-806.
60. **Lee, S.E., Lee, J.U., Chon, H.T., Lee, J.S. (2008).** Microbiological reduction of hexavalent chromium by indigenous chromium-resistant bacteria in sand column experiments, Environmental Geochemistry and Health. 30: 141-145.
61. **Mahmood, S., Khalid, A., Mahmood, T., Arshad, M., Loyola-Licea, J.C., Crowley, D.E. (2015).** Biotreatment of simulated tannery wastewater containing Reactive Black 5, aniline and Cr(VI) using a biochar packed bioreactor. RSC Advances. 5: 106272-106279
62. **Martins, S.C.S., Martins, C.M., Fluza, L.M.C.G., Santaella, S.T. (2015).** Immobilization of microbial cells: A promising tool for treatment of toxic pollutants in industrial wastewater. African Journal of Biotechnology. 12: 4412-4418.
63. **Megharaj, M., Avudainayagam, S., Naidu, R. (2003).** Toxicity of hexavalent chromium and its reduction by bacteria isolated from soil contaminated with tannery waste. Current Microbiology. 47: 51-54.
64. **Molokwane, P.E., Meli, C.K., Chirwa Evans, M.N. (2008).** Chromium (VI) reduction in activated sludge bacteria exposed to high chromium loading: Brits culture (South Africa). Water Research. 42: 4538-4548.
65. **Mondaca, M.A., Campos, V., Moraga, R., Zaror, C.A. (2002).** Chromate Reduction in *Serratia marcescens* isolated from Tannery Effluent and Potential Application for Bioremediation of Chromate Pollution. The International Conference on Environmental Concerns and Emerging Abatement Technologies 2001: Collection of Short Communications The Scientific World Journal. 2: 972-977.

66. **Murugavelh, S., Mohanty, K. (2013).** Bioreduction of chromate by immobilized cells of *Halomonas sp.* International Journal of Energy and Environment. 4(2): 349-356.
67. **Myers, C.R., Carstens, B.P., Antholine, W.E., Myers, J.M. (2000).** Chromium(VI) reductase activity is associated with the cytoplasmic membrane of anaerobically grown *Shewanella putrefaciens* MR-1. Journal of Applied Microbiology. 88: 98-106.
68. **Naeem, A., Batool, R., Jamil, N. (2013).** Cr(VI) reduction by *Cellulosimicrobium* sp. isolated from tannery effluent. Turkish Journal of Biology. 37: 315-322.
69. **Nancharaiah, Y.V., Dodge, C., Venugopalan, V.P., Narasimhan, S.V., Francis, A.J. (2010).** Immobilization of Cr(VI) and Its Reduction to Cr(III) Phosphate by Granular Biofilms Comprising a Mixture of Microbes. Applied Microbiology and Biotechnology. 2433-2438.
70. **Narayani, M., Shetty, K.V. (2013).** Chromium-Resistant Bacteria and Their Environmental Condition for Hexavalent Chromium Removal: A Review. Critical Reviews in Environmental Science and Technology. 43: 955-1009.
71. **Opperman, D.J., Piater, L.A., van Heerden, E. (2008).** A novel chromate reductase from *Thermus scotoductus* SA- 01 related to old yellow enzyme. Journal of Bacteriology. 190: 3076-3082.
72. **Ozturk, S., Aslim, B., Ugur, A. (2008).** Chromium(VI) resistance and extracellular polysaccharide (EPS) synthesis by *Pseudomonas*, *Stenotrophomonas* and *Methylobacterium* strains. ISIJ International. 48 (11): 1654-1658.
73. **Pal, A., Datta, S., Paul, A.K. (2013).** Hexavalent Chromium Reduction by Immobilized Cells of *Bacillus sphaericus* AND 303. Brazilian Archives of Biology and Technology. 56(3): 505-512.
74. **Pal, A., Paul, A.K. (2004).** Aerobic, chromate reduction by chromium-resistant bacteria isolated from serpentine soil. Microbiology Research. 159: 347-354.
75. **Pattanapipitpaisal, P., Brown, N.L., Macaskie, L.E. (2001).** Chromate reduction by *Microbacterium liquefaciens* immobilized in polyvinyl alcohol. Biotechnology Letters. 23: 61-65.
76. **Pazos, M., Branco, M., Neves, I.C., Sanroman, M.A., Tavares, T. (2010).** Removal of Cr(VI) from aqueous solutions by a bacterial biofilm supported on zeolite: optimization of the operational conditions and scale-up of the bioreactor. Chemical Engineering and Technology. 33: 2008-2014.
77. **Philip, L., Iyengar, L., Venkobachar, C. (1999).** Immobilized microbial reactor for the biotransformation of hexavalent chromium. International Journal of Environmental Pollution. 11: 202-210.
78. **Poopal, A.C., Laxman, R.S. (2009).** Chromate reduction by PVA-alginate immobilized *Streptomyces griseus* in a bioreactor. Biotechnology Letters. 31: 71-76.
79. **Poopal, A.C., Laxman, R.S. (2008).** Hexavalent Cr(VI) reduction by immobilized *S. griseus*. Biotechnology Letters. 30(6): 1005-1010.
80. **Priester, J.H., Olson, S.G., Webb, S.M., Neu, M.P., Hersman, L.E., Holden, P.A. (2006).** Enhanced exopolymer production and chromium stabilization in *Pseudomonas putida* unsaturated biofilms. Applied and Environmental Microbiology. 72: 1988-1996.
81. **Quintelas, C., Fonseca, B., Silva, B., Figueiredo, H., Tavares, T. (2009).** Treatment of chromium(VI) solutions in a pilot-scale bioreactor through a biofilm of *Arthrobacter viscosus* supported on GAC. Bioresource Technology. 100: 220-226.
82. **Quintelas, C., Fernandes, B., Castro, J., Figueiredo, H., Tavares, T. (2007).** Biosorption of Cr(VI) by three different bacterial species supported on granular activated carbon-A comparative study. Journal of Hazardous Material. 153: 799-809.
83. **Rajwade, J.M., Paknikar, K.M. (1997).** Microbiological detoxification of chromate from chromate-plating effluents. In: Proceedings, International Biohydrometallurgy Symposium IBS97. Australian Mineral Foundation, Glenside, Australia. pp E-ROM4.1-EROM4.10.
84. **Romanenko,V.I., Korenkov, V.N. (1975).** Bacterial reduction of ions. Inform Byul In-ta Biol Vnutr Vod Akad Nauk SSR. 25: 8.

85. **Sedla´c¡ek, V., Kuc¡era, I. (2010).** Chromate reductase activity of the *Paracoccus denitrificans* ferric reductase B (FerB) protein and its physiological relevance. Archives of Microbiology. 192: 919-926.
86. **Shen, H., Wang, Y.T. (1993).** Characterization of enzymatic reduction of hexavalent chromium by *Escherichia coli* ATCC 33456. Applied and Environmental Microbiology. 59: 3771-3777.
87. **Shen, H., Wang, Y.T. (1995).** Modelling simultaneous hexavalent chromium reduction and phenol degradation by a defined coculture of bacteria. Biotechnology and Bioengineering. 6: 606-613.
88. **Singh, R., Kumar, A., Kirrolia, A., Kumar, R., Yadav, N., Bishnoi, N.R., Lohchab, R.K. (2011).** Removal of sulphate, COD and Cr(VI) in simulated and real wastewater by sulphate reducing bacteria enrichment in small bioreactor and FTIR study. Bioresource Technology. 102(2): 677-682.
89. **Smith, W.L., Gadd, G.M. (2000).** Reduction and precipitation of chromate by mixed culture sulphate-reducing bacterial biofilms. Journal of Applied Microbiology. 88: 983-991
90. **Sundar, K., Mukherjee, A., Sadiq, M., Chandrasekaran, N. (2011).** Cr (III) bioremoval capacities of indigenous and adapted bacterial strains from Palar river basin. Journal of Hazardous Material. 187: 553-561.
91. **Sutherland, I. W. (2001).** The biofilm matrix-an immobilized but dynamic microbial environment. Trends in Microbiology. 9: 222-227.
92. **Teitzel, G.M., Parsek, M.R. (2003).** Heavy metal resistance of biofilm and planktonic *Pseudomonas aeruginosa*. Applied and Environment Microbiology. 69: 2313-2320.
93. **Thacker, U., Parikh, R., Shouche, Y., Madamwar, D. (2007).** Reduction of chromate by cell-free Extract of *Brucella* sp. isolated from Cr(VI) contaminated sites. Bioresource Technology. 98: 1541-1547.
94. **Thacker, U., Parikh, R., Shouche, Y., Madamwar, D. (2006).** Hexavalent chromium reduction by *Providencia* sp. Process Biochemistry. 41: 1332-1337.
95. **Thatoi, H., Das, S., Mishra, J., Rath, B.P., Das, N. (2014).** Bacterial chromate reductase, a potential enzyme for bioremediation of hexavalent chromium: A review. Journal of Environmental Management. 146: 383-399.
96. **Thu, B., Bruheim, P., Espevik, T., Smidsrød, O., Soon-Shiong, P., Skjåk-Bræk, G. (1996).** Alginate polycation microcapsules: I. Interaction between alginate and polycation. Biomaterials. 17: 1031-1040.
97. **Tucker, M.D., Barton, L.L., Thomson, B.M. (1998).** Reduction of Cr, Mo, Se and U by *Desulfovibrio desulfuricans* immobilized in polyacrylamide gels. Journal of Industrial Microbiology and Biotechnology. 20: 13-19.
98. **Wang, Y.T., Xiao, C. (1995).** Factors affecting hexavalent chromium reduction in pure cultures of bacteria. Water Research. 29: 2467-2474.
99. **Wang, P.C., Mori, T., Toda, K., Ohtake, H. (1990).** Membrane-associated chromate reductase activity from *Enterobacter cloacae*. Journal of Bacteriology. 172: 1670-1672.
100. **Wani, R., Kodam, K.M., Gawai, K.R., Dhakephalkar, P.K. (2007).** Chromate reduction by *Burkholderia cepacia* MCMB-821 isolated from the pristine habitat of alkaline crater lake. Applied Microbiology and Biotechnology. 75: 627-632.
101. **Wielinga, B., Mizuba, M. M., Hansel, C. M., Fendorf, S. (2001).** Iron Promoted Reduction of Chromate by Dissimilatory Iron-Reducing Bacteria. Environment Science and Technology. 35: 522-527.
102. **Xu, L., Luo, M., Jiang, C., Wei, X., Kong, P., Lang, X., Zhao, J., Yang, L., Liu, H. (2012).** *In vitro* reduction of hexavalent chromium by cytoplasmic fraction of *Pannonibacter phragmitetus* LSSE-09 under aerobic and anaerobic condition. Applied Microbiology and Biotechnology. 166: 933-941.

103. **Yang, J., He, M., Wang, G. (2009).** Removal of toxic chromate using free and immobilized Cr(VI)-reducing bacterial cells of *Intrasporangium* sp. Q5-1. World Journal of Microbiology and Biotechnology. 25: 1579-1587.

104. **Yen-Hui, L., Yu-Chien, T., Guan-Lun, C. (2015).** Kinetics of Chromium(VI) Reduction with Acetate Biodegradation by *Escherichia coli* 33456 in a Fixed Biofilm Reactor. Environmental Engineering Science. 32(9): 761-772.

105. **Zakaria, Z.A., Zakaria, Z., Surif S., Ahmad, W.A. (2007).** Biological detoxification of Cr(VI) using wood-husk immobilized *Acinetobacter haemolyticus*. Journal of Hazardous Material. 148: 164-171.