Int. J. Environ. Sci. Technol. (2012) 9:183–193
DOI 10.1007/s13762-011-0013-7

REVIEW

Remediation of chromium contaminants using bacteria

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Received: 6 February 2011 / Revised: 24 April 2011 / Accepted: 25 August 2011 / Published online: 26 November 2011

Abstract Chromium contaminants emanating from industrial activities pose a significant threat to human's well-being. Chromium (III) and Chromium (VI) are the forms in which they are commonly encountered, of which the trivalent form is relatively benign. Hence, biological reduction of hexavalent chromium has been widely explored by researchers, yielding fruitful outcomes, opening up exciting avenues and also throwing up new challenges. This article attempts to review this area of research. Microbes, especially bacteria capable of Chromium (VI) reduction, belonging to a heterogeneous group have been isolated from contaminated sites. They exhibit plasmid-mediated chromate resistance and the reduction is enzymatically mediated. Reduction studies have been carried out with free and immobilized enzymes as well as whole cells. Experiments have been carried out in specifically designed bioreactors operated in batch and continuous modes. Although significant progress has been made, much needs to be done for its successful in situ application as the organism may not withstand the Chromium concentration or may be impeded by the presence of other toxicants. With molecular engineering, it may be possible to derive strains with improved performance even under stressful field conditions.

Keywords Bioreduction · Biosorption · Chromate reductase · Chromium · Resistance · Toxicity

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Introduction

Heavy metals that are frequently discharged in industrial effluents are of grave concern owing to their toxic effects. Chromium compounds find widespread industrial applications in electroplating, wood preservation, leather tanning and steel manufacturing sectors and subsequently end up as environmental contaminants.

Chemistry of chromium

Chromium is a steel-gray, lustrous, hard, brittle metal of Group VI-B of the transition series with atomic number of 24 and atomic weight of 51.996. It exists in a number of valence states ranging from −2 to +6. However, Cr(III) and Cr(VI) are of significance owing to their stability in natural environments, although Cr with valences of I, II, IV and V have also been shown to exist in a number of compounds. The recommended permissible limit in drinking water is 50 μg/L for Cr(VI) (Krishnamurthy and Wilkens 1994).

Transport and toxicity

Cr(III) is an essential nutrient in this diet, aiding in the metabolism of glucose and lipids (Anderson 1997). However, ingesting large amounts of it may cause health problems including lung cancer (Katz and Salem 1994). Cr(VI) on the other hand is a potent, extremely toxic carcinogen and has been estimated to be 10–100 times more toxic than Cr(III) (Katz and Salem 1994). This may be due to the fact that Cr(VI) penetrates mammalian cells more readily than Cr(III). The United States Environmental

Protection Agency (USEPA) determined its reference dose to be 1 mg kg^{-1} day^{-1} (Katz and Salem 1994).

Cr(VI) enters the cell through the membrane sulphate transport channels (Ohtake and Silver 1994; Cervantes et al. 2000; Silver et al. 2001) and reacts with intracellular reductants such as glutathione and ascorbate. This generates short-lived intermediates Cr(V) and/or Cr(IV), free radicals and the end product Cr(III). Cr(V) undergoes a one-electron redox cycle to regenerate Cr(VI) by transferring the electron to oxygen. This process results in the formation of reactive oxygen species (ROS) capable of combining with DNA–protein complexes (Fig. 1). Cr(IV) also binds to cellular materials and impedes their normal physiological functions (Pesti et al. 2000; Cervantes et al. 2000). Skin and nasal irritation, eardrum perforation and lung carcinoma are a few of the adverse effects linked with Cr(VI) (Gibb et al. 2000a, b). Apart from these, it can have teratogenic effects too (Saxena et al. 1990). Environmental effects include alteration in soil microbial communities and reduction in their growth and activity (Turpeinen et al. 2004). Hence, it becomes imperative to remove chromium contaminants, especially Cr(VI) from the environment.

Most of the intracellular Cr(VI) reductants are one electron reducers, which generate Cr(V) and result in the formation of ROS that are predominantly responsible for the adverse effects of Cr(VI) (modified from Cheung and Gu 2007).

Remediation of Cr contaminants: physico-chemical versus biological methods

Conventional chromium removal consists of chemical reduction followed by alkali precipitation or use of ion exchange and adsorption. The former technique generates large quantities of sludge while the latter are expensive and also less specific when other anions of heavy metals are present. Hence, attention has been shifted to biological methods of remediation. Biotransformation and biosorption are commonly used technologies, which utilize the potential of microorganisms or plants to transform or adsorb heavy metals. Of these, biotransformation (i.e. reduction of toxic, mobile Cr(VI) to less toxic, less mobile Cr(III)) may be considered to be the better alternative. In biosorption, the contaminant gets accumulated in the biomass which again has to be disposed off, while in biotransformation, it is converted to an innocuous form. Under aerobic conditions, microbial chromate reduction is brought about by soluble reductases. In the absence of oxygen, both soluble and membrane bound reductases including cytochromes have been implicated. A large array of compounds including carbohydrates, proteins, fats, hydrogen, NAD(P)H and endogenous electron reserves can serve as electron donors in the reduction process (Wang 2000) (Fig. 2).

Under aerobic conditions, ChrR of *Pseudomonas putida* MK1 catalyzes one as well as two electron transfers to

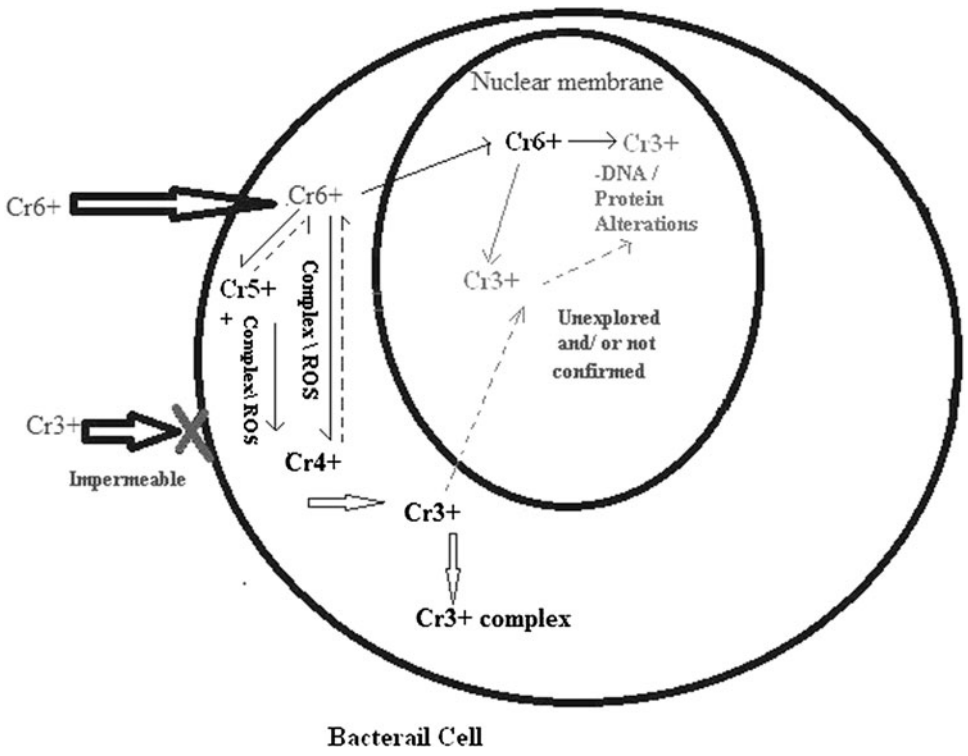

Fig. 1 Schematic diagram of Cr(VI) transport into the cell and its fate

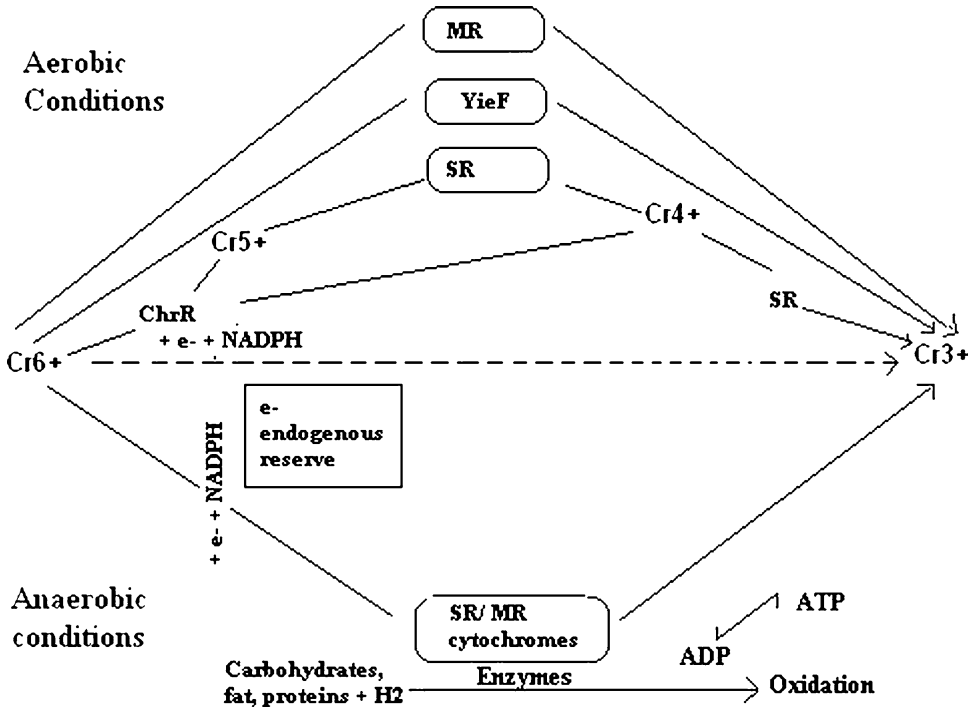

Fig. 2 Mechanisms likely to be involved in enzymatic reduction of Cr(VI) under aerobic and anaerobic conditions

Cr(VI) with the transient formation of Cr(V), whereas YieF of *Escherichia coli* brings about a four electron transfer resulting in the direct reduction of Cr(VI) to Cr(III), with the remaining electron getting transferred to oxygen. A membrane-associated $Cr6^{+}$ reductase isolated from *Bacillus megaterium* TKW3 has not been characterized. Under anaerobic conditions, both soluble and membrane-associated Cr(VI) reducing enzymes, including cytochromes, associated with the electron transfer system are involved (enzymes involved in the reduction of Cr(VI) are given in boxes, SR and MR represent soluble and membrane-associated reductases, respectively) (modified from Cheung and Gu 2007).

Focus of this review

This review broadly covers various aspects of Cr bioremediation, with the focus on bacteria-mediated Cr(VI) reduction, with more emphasis on recent findings. The genetic basis of Cr(VI) resistance which is plasmid rather than chromosome mediated and is attributed to reduced uptake, regulated efflux mechanisms or reduction along with parameters influencing the rate and extent of reduction by the isolates viz., temperature, pH, availability of oxygen, initial Cr concentration, cell density, exogenous electron donors, presence of nitrates–sulphates, other heavy metals, have been discussed. Reduction potentials of growing cells, resting cells and cell-free supernatants have been compared; purification of chromate reductases and study of enzyme kinetics in case of free and immobilized systems have also been reviewed. Cr(VI) reduction by a few fungi and yeast has been briefly included for comparison sake.

Bacterial Cr(VI) reduction

Since the discovery of the first microbe *P. dechromaticans* capable of reducing Cr(VI) in the 1970s (Romanenko and Korenkov 1977), the search for chromate reducing microorganisms has continued and much information has been gathered on microbial-mediated Cr(VI) reduction, making its practical application feasible. Cr(VI)-reducing bacteria belonging to diversified genera have been isolated from a variety of environments, indicating that such potential is widespread in nature and can be harnessed to facilitate remediation of Cr contaminated sites.

Among tannery isolates, *Microbacterium* sp. MP30 grew aerobically in a medium supplemented with 15 mM chromate but did not reduce it. Under anaerobic conditions, it reduced 100 μM sodium chromate within 30 h at the expense of acetate as electron donor (Pattanapipitpaisal et al. 2001). Moderately halophilic *Nesterenkonia* sp. MF2 was tolerant to a much higher concentration of 600 mM chromate and completely reduced 200 μM Cr(VI) in 24 h under aerobic condition. Initial Cr(VI) concentration up to 400 μM did not have a significant effect on Cr(VI) reduction. The isolate was capable of chromate reduction in the presence of various concentrations of salts (Amoozegar et al. 2007). A strain of *B. circulans* exhibited comparatively far better activity and reduced as much as

190 mM chromate both under anaerobic and aerobic conditions, maximum reduction being observed at 28 h of incubation (Chaturvedi et al. 2007). *Exiguobacterium* sp. ZM-2 showed resistance to 12.37 mM potassium chromate and reduced 500 μM Cr(VI) within 56 h. The presence of metabolic inhibitors sodium azide and sodium cyanide severely affected chromate reduction, while 2, 4-dinitrophenol, and an uncoupling agent, stimulated chromate reduction (Alam and Malik 2008).

In one study using landfill isolates, *Bacillus* sp. XW-4 was tolerant to and reduced 1.9 mM Cr(VI). Chromate reduction by the strain was significantly enhanced by the presence of glucose (Liu et al. 2006). In another study, suspended cultures of *B. cereus* GIDM20, *B. fusiformis* GIDM22 *and B. sphearicus* GIDM64 exhibited more than 85% reduction of 1 mM Cr(VI) within 30 h. The activity was found to be mainly associated with the soluble fraction of cells, expressed constitutively and unaffected by presence of different metal ions except Hg^{2+} and Ag^{+}. Addition of NADH enhanced Cr(VI) reduction (Desai et al. 2008). Another bacterium, *Ochrobactrum* sp. showed Cr(VI) reduction ability under alkaline conditions. This isolate was tolerant to and reduced as much as 15.4 mM Cr(VI). In this case too, the addition of glucose caused a dramatic increase in Cr(VI)-reduction, while the presence of sulphate or nitrate had no influence (He et al. 2009). In another study, filamentous bacterium *Sphaerotilus natans* CSCr-3 isolated from activated sludge systems aerobically reduced up to 1.5 mM Cr(VI) in the presence of a carbon source (Caravelli et al. 2008). This reduction potential can be considered significant as *S. natans* is previously known only for its biosorption ability.

Bacillus sp. KCH2 and KCH3, *Leucobacter* KCH4 and *Exiguobacterium* KCH5 were isolated from Cr contaminated soil. Of these, KCH3 and KCH4 showed higher Cr(VI) tolerance (2 mM) and reduction (1.5 mM) than KCH5 (1.5 and 0.75 mM, respectively). Cr(VI) reduction was inhibited by Hg^{2+} and enhanced by Cu^{2+} (Sarangi and Krishnan 2008). Enriched cultures of indigenous bacteria isolated from a stream sediment contaminated by a pigment manufacturing industry reduced 31% of the 250 μM Cr(VI), which is equivalent to the exchangeable Cr(VI) concentration in the sediment. The removal efficiency was increased with external supply of electron donor in the form of glucose, lactate or acetate (Lee et al. 2008).

During fermentative growth on D-xylose, isolates ES6 and WS01 (*Cellulomonas*) from US Department of Energy (DoE) Site decreased Cr(VI) concentrations from 0.04 mM to below the detection limit (0.002 mM) in less than 3 days and retained their ability to reduce Cr(VI) even after 4 months of incubation. Washed ES6 and WS01 cells also reduced Cr(VI) under non-growth conditions for over 4 months (Viamajala et al. 2007). Stimulation of such organisms may lead to effective long-term, in situ passive reactive barriers for Cr(VI) removal.

Staphylococcus epidermidis L-02 isolated from a bacterial consortium used for the remediation of a chromate-contaminated constructed wetland system reduced Cr(VI) by using pyruvate as an electron donor under anaerobic conditions. The presence of nitrate increased the specific reduction rate. Under denitrifying conditions, Cr(VI) reduction was not inhibited by nitrite. The maximum specific reduction rate was as high as 8.8–9.8 μM Cr 10^{10}/cells/h (Vatsouria et al. 2005). *Intrasporangium* sp. Q5-1 isolated from a Mn/Cr contaminated site showed 17 mM/L minimum inhibitory concentration (MIC) for Cr(VI). Resting cells also reduced chromate. When immobilized with compounding beads containing 4% Poly Vinyl Alcohol (PVA), 3% sodium alginate, 1.5% active carbon and 3% diatomite, the reduction rate remained unchanged (Yang et al. 2009). These immobilized cells have the advantage of stability, reusability and less clogging in continuous systems.

In an attempt to bring about Cr(VI) remediation using psychrophiles, core samples obtained from a Cr(VI) contaminated aquifer were enriched in Vogel Bonner medium supplemented with Cr(VI). The isolate *Arthrobacter aurescens* P4 was resistant to 19.2 mM Cr(VI) but reduction was slow or not observed at and above 1.9 mM Cr(VI) (Horton et al. 2006). This species has previously not shown to be capable of low temperature (10°C) Cr(VI) reduction.

Apart from such sites polluted by anthropogenic activities, chromate resistant bacteria have also been isolated from natural environments. *B. sphearicus* from naturally occurring chromium percolated soil of Andaman Islands was tolerant to 15.4 mM Cr(VI) and reduced >80% of it during growth. Soluble fraction of the cell was responsible for aerobic reduction of chromate by this organism (Pal and Paul 2004). *Burkholderia cepacia* MCMB-821 isolated from the alkaline crater lake of Lonar was resistant to 19.2 mM Cr(VI) and reduced 98% of the 1.4 mM Cr(VI) within 36 h in the presence of 2% salt and lactose as the electron donor. Electron paramagnetic resonance (EPR) spectroscopy data suggested the formation of transient Cr(V) intermediate during reduction (Wani et al. 2007) (Table 1).

Other novel findings

In an endeavour to assess the toxic effect of chromate on soil microbial communities, soil microcosms were artificially contaminated with up to 1,000 mg Cr(VI)/kg soil. This affected the structure and diversity of the soil bacterial community. Bacterial strains isolated from the microcosm contaminated with highest concentration of chromate belonged to the genus *Pseudomonas* and no cyanobacterial

Table 1 Chromate reduction potentials of various bacterial isolate

Strain	Chromate conc. the isolate is capable of reducing (mg/L)	References
Microbacterium sp. MP-30	100 μM	Pattanapipitpaisal et al. (2001)
Thermus scotoductus SA-01	500 μM	Opperman and Heerden (2007)
Exiguobacterium sp. KCH-5	0.75 mM	Sarangi and Krishnan (2008)
Bacillus sp. KCH-3, *Leucobacter* KCH-4	1.5 mM	Sarangi and Krishnan (2008)
Sphearotilus natans	1.5 mM	Caravelli et al. (2008)
Arthrobacter aurescens P4	1.9 mM	Horton et al. (2006)
Ochrobactrum sp. CSCr-3	15.3 M	He et al. (2009)

growth was detected in this, indicating their sensitivity. Some enzymes were inhibited, others stimulated and the ATP content in microcosms was strongly affected by chromate (Viti et al. 2006). Thus, the soil microbial community responds to chromate pollution through changes in community structure, in metabolic activity, and in selection for Cr(VI)-resistance.

Cr(VI) is usually known to be reduced to Cr(III). However, as a rare occurrence, *Shewanella oneidensis,* reduced Cr(VI) to Cr(II). Individual cell microanalysis demonstrated that the Cr(II) concentrated near the cytoplasmic membrane, suggesting intracellular localization of the terminal reduction pathway (Daulton et al. 2007).

Acetate was added to aid in the bioremediation of two closed soil-water systems representative of the subsurface environment close to chromium ore processing residue disposal sites; one had a pH of 7.7, the other 9.3. Cr(VI) reduction occurred in both systems as part of a cascade of microbially mediated terminal electron-accepting processes, occurring between nitrate and iron reduction. Cr(VI) and subsequently iron reduction took longer to start and were slower in the more alkaline system. At the point when Cr(VI) reduction was essentially complete, the microbial populations in both systems showed an increase in species closely related to β-proteobacteria that are capable of nitrate reduction (Stewart et al. 2007).

Cr(III) produced as a result of Cr(VI) reduction is mostly considered to be insoluble $Cr(OH)_3$. However, Cr(VI) reduction in the presence of cellular organic metabolites formed both soluble and insoluble organo-Cr(III) end-products (Puzon et al. 2005). These organo-Cr(III) complexes may account for the mobile form of Cr(III) detected in the environment. Such soluble complexes have been demonstrated with four bacterial cultures (*S. oneidensis* MR1, *Cellulomonas* sp. ES6, *Rhodococcus* sp. and *Desulfovibrio vulgaris var Hildenborough*). Structural characterization of the complexes indicated that they were composed of multiple chromium ions bound together by the cellular metabolites (Puzon et al. 2008). A better understanding of the organo-Cr(III) pathway will be useful in designing remediation strategies for Cr-impacted soils.

The shape of *Leucobacter* Ch1 cells before and after chromate reduction was observed using scanning and transmission electron microscopy. The composition of reduction products was determined using Energy Dispersive X-ray Analysis and EPR Spectroscopy and was found to contain 28.2% Cr in the form of $Cr(OH)_3$ precipitate (Zhu et al. 2008).

Cr(VI) migrating downwards from a chromium ore processing residue was of concern because groundwater emerging from the site was alkaline, visibly yellow and had an elevated Cr(VI) concentration. Sandy clay from immediately beneath the waste contained up to 3,000 mg/kg of Cr, and around 60% of 0.5 N HCl extractable iron in the form of reduced Fe(II). Very recent microcosm experiments showed that the soil contained a viable bacterial population capable of iron-reduction. This sandy clay layer, despite a pH value of 10.5, acted as a natural reactive zone beneath the waste as it accumulated chromium (Stewart et al. 2010). The mechanism of Cr(VI) reduction is believed, most likely to be an abiotic reaction with the Fe(II) present in the soil, and the Fe(II) being replenished by microbial iron-reduction.

Purification and characterization of bacterial chromate reductases

Chromate reductases have been purified and characterized by several researchers. Cloning the genes that encode chromate reductases can result in further advancements in bioremediation. Characterization of chromate reduction by a flavin reductase from *E. coli* revealed faster reduction when compared to chemical reduction by NADH and glutathione suggesting that unlike eukaryotes, enzymatic reduction is the dominant mechanism in bacterial cells (Puzon et al. 2002). Chromate reductase encoding genes vary according to the microorganism and also occurs in

plasmids, few literatures are available describing the gene sequence (Barak et al. 2006; Gonzalez et al. 2005).

A soluble Cr(VI) reductase purified from the cytoplasm of *E. coli* ATCC 33456 was a dimmer with a molecular mass of 84 kDa. The pI was 4.66, and optimal enzyme activity was obtained at pH 6.5 and 37°C. The purified enzyme used both NADPH and NADH as electron donors for Cr(VI) reduction, while NADPH was the better, conferring 61% higher activity than NADH. The K_m values for NADPH and NADH were 47.5 and 17.2 μmol, and the V_{max} values 322.2 and 130.7 μmol Cr(VI) min/mg protein, respectively. The activity was strongly inhibited by *N*-ethylmalemide, Ag^{2+}, Cd^{2+}, Hg^{2+} and Zn^{2+} (Bae et al. 2005).

Chromate reductase from *P. putida* was purified by $[NH_4]_2SO_4$ precipitation, anion exchange (DEAE sepharose CL-6B) and gel filtration (Sephadex-G-25) chromatography. The enzyme activity was dependent on NADH or NADPH, the temperature and pH optima were 75°C and 8.5, respectively, and the K_m was 260 μM with a V_{max} of 8.8 μmol/min/mg of protein. It had a molecular weight of 20 KDa (Renuga et al. 2008).

Thermus scotoductus SA-01, a South African gold mine isolate reduced Cr(VI) when grown aerobically in an organic medium containing 0.5 mM Cr(VI) (Opperman and Heerden 2007). Cell suspensions also reduced Cr(VI) under non-growth conditions. The reduction was found to be catalysed by a cytoplasmic, constitutively expressed enzyme. This enzyme was purified to homogeneity and was found to be a homodimeric protein, with a monomer molecular mass of 36 kDa, containing a non-covalently bound Flavin Mononucleotide cofactor. Enzyme activity was dependent on NADH or NADPH, with a preference for NADPH. The catalytic efficiency of chromate reduction was 50-fold greater than that of the quinone reductases and 180-fold more efficient than that of the nitroreductases. It was encoded by an open reading frame (ORF) of 1,050 bp, and was under the regulation of an *E. coli* σ^{70}-like promoter (Opperman and Heerden 2008). *T. scotoductus* also has a second strictly anaerobic membrane bound enzyme capable of chromate reduction. This enzyme showed a preference towards NADH. It was also a homodimer, but with a monomeric molecular weight of 48 kDa and a non-covalently bound FAD coenzyme. The catalytic efficiency of this enzyme was comparable to that of quinone reductases but more efficient than the nitroreductases. N-terminal sequencing and subsequent screening of a genomic library of *T. scotoductus* revealed an ORF of 1,386 bp, homologous (84%) to the dihydrolipoamide dehydrogenase gene of *T. thermophilus* HB8 (Opperman et al. 2008). These results extend the knowledge of chromate reductases mediating Cr(VI) reduction via noncovalently bound or free redox-active flavin groups and the activity of dihydrolipoamide dehydrogenases towards physiologically unrelated substrates.

The kinetics of enzymatic reduction has also been studied using immobilized enzyme systems. In a recent work, chromate reductases associated with cell-free extracts of *A. rhombi*-RE were immobilized with different matrices and calcium alginate beads proved to be most effective (Elangovan et al. 2010). However, the performance was not encouraging under continuous mode of operation (Table 2).

Bioreactor studies for bacterial Cr(VI) remediation

Membrane bioreactors have been used in order to minimize the toxic effects of Cr(VI) on microorganisms or to facilitate the isolation of chromium that is reduced. Immobilized cells have also been used in such membrane reactors. One example is the use of *Pseudomonas* immobilized on agar–agar films for chromate reduction (Konovalova et al. 2003).

The relationship between sulphate starvation and chromate reduction was studied in a H_2-fed fixed-film bioreactor under continuous feed conditions. The bioreactor was inoculated with a bacterial consortium containing *Desulfomicrobium norvegicum*. With 500 mg/L of sulphate in the feed solution, total chromate-reduction was observed in the effluent whereas sulphate-reduction was strongly decreased. In the absence of sulphate, chromate-reduction activity was still observed but was lower than in the presence of sulphate.

In another study, columns packed with quartz sand alone and hydrous ferric oxide (HFO)-coated quartz sand were inoculated with *Cellulomonas* sp. ES6, fed nutrients to stimulate growth and then nutrient-free Cr(VI) solutions were injected into the column. In HFO-containing columns, Cr(VI) reduction was significantly prolonged and effluent Cr(VI) concentrations remained below detectable levels for periods of up to 66 residence times after nutrient injection was discontinued as against 5.7 residence times for columns packed with quartz sand alone. Fe was continuously detected in the effluent indicating that the insoluble Fe(III) bearing solids were being reduced to form soluble Fe(II) resulting in prolonged abiotic Cr(VI) reduction (Viamajala et al. 2008). Thus, growth of Cellulomonas resulted in formation of permeable reactive barriers that could reduce Cr(VI) and Fe(III) for extended periods even in the absence of external electron donors. This may be a viable technology for in situ remediation of Cr(VI)-contaminated groundwater.

Chromate reduction by *Arthrobacter* CR47 isolated from a landfarming soil sample was studied in batch operated packed bed biofilm reactors and in recirculating packed bed biofilm reactors. The reduction was evaluated

Table 2 Characterization of chromate reductases from a few bacteria

Bacteria from which the chromate reductase is purified	Mol. Wt. (kDa)	Temp. optima (°C)	pH optima	K_M	V_{max} (Cr(VI) reduced/ min/mg protein)	References
Bacillus sp. QC1-2	44	37	7	0.35 mM	50 nM	Garcia et al. (1997)
E. coli ATCC 33456	84	37	6.5	47.5 μM	322 μM	Bae et al. (2005)
Pseudomonas putida	20	75	8.5	260 μM	8.8 μM	Renuga et al. (2008)
Thermus scotoductus SA-01 (soluble reductases)	72	65	6.3	8.4 μM	16 μM	Opperman and Heerden (2008)
Thermus scotoductus SA-01 (membrane bound reductase)	96	65	6.5	55.5 μM	23 μM	Opperman et al. (2008)
Exiguobacterium sp. ZM-2	–	–	–	106.1 μM	1.24 μM	Alam and Malik (2008)
Bacillus sp. KCH-3,	–	35	5.5	45–55 μM	–	Sarangi and Krishnan (2008)
Leucobacter KCH-4	–	30	5.5	45–55 μM	–	Sarangi and Krishnan (2008)
Exiguobacterium sp. KCH-5	–	35	6	200 μM	–	Sarangi and Krishnan (2008)
Bacillus sp. GIDM-22	–	–	–	200 μM	5.5 μM	Desai et al. (2008)
Arthrobacter rhombi-RE	–	30	5.5	48 μM	4.09 nM	Elangovan et al. (2010)

with laboratory solutions and industrial effluents containing 30 mg/L Cr(VI). Under batch mode, the reduction reaction by the biofilm fit an exponential-decay model with a first order kinetic parameter of 0.071 mg (L/h) Cr(VI). In the recirculating reactor fed with laboratory solutions, the removal rate was 0.79 mg/L/h. In the reactor fed with the industrial model solutions, the maximum Cr(VI) removal rate attained was 0.49 mg/L/h (Cordoba et al. 2008).

In yet another attempt, chromate reduction was carried out in a pilot-scale trickling filter packed with plastic media under continuous mode of operation with recirculation. Mixed bacterial cultures originated from industrial sludge were used for this study. The effect of the organic carbon concentration was examined for constant Cr(VI) influent concentration at about 5.5 mg/L and volumetric flow rates ranging from 60 to 900 mL/min. The highest reduction rate achieved was 1,117 g Cr(VI)/m^2/day for a volumetric flow rate of 900 mL/min (Dermou and Vayenas 2008). Even though the system mandated frequent backwashing of the filter, the high reduction rates combined with low operating cost make this an option worth considering for the treatment of industrial chromate effluents.

Recently, activated sludge process was used for continuous Cr(VI) removal and the highest specific Cr(VI) removal rate was attained with cheese whey or lactose as electron donors. Batch assays with different nitrogen to carbon source ratios demonstrated that Cr(VI) reduction was associated with the cell multiplication phase; as a result, maximum removal occurred when there was no substrate limitation (Orozco et al. 2010).

Bacterial Cr(VI) resistance

Cr(VI) resistant bacteria have been isolated from a number of environments and the genetic basis of resistance has been studied. Such studies have abundantly been carried out with tannery isolates. In one such work, among several isolates, all of which harboured plasmid DNA, *Corynebacterium hoagie* showed maximal resistance to 22 mM Cr(VI) (Viti et al. 2003). Thermotolerant strains resistant to Cr too have been isolated from tannery sources. These plasmid bearing isolates showed resistance to other heavy metals and antibiotics as well, which make them particularly useful. Three of the isolates belonged to uropathogenic *E. coli* serotype 04 (Verma et al. 2004). In another study, *B. brevis* showed good plasmid-mediated resistance and reduction potential as established by transfer and curing studies (Verma et al. 2009).

Enterobacter cloacae CYS-25, isolated from a chromate plant showed a strong tendency for chromate resistance due to reduced uptake and not reduction. Atomic force microscopic investigation showed that the average length of the bacterium in the stationary phase is about 2.3 ± 0.6 μM, while under the stimulation of 400 mg/L CrO_4^{2-}, the length increased to 3.2 ± 0.7 μM. Moreover, the smooth surface of the bacterium changed into one with discontinuous features. These compact convex patches are organic components stimulated by CrO_4^{2-} (Yang et al. 2007).

Hence, an over expression of extracellular biological components for preventing the permeability of CrO_4^{2-} into the cell helps the bacteria to survive chromate stress.

DNA helicases are involved in chromate resistance by *P. aeruginosa* PAO1. Chromate-hypersensitive mutants were generated using transposon insertion mutagenesis. Comparison of the nucleotide sequences of the regions interrupted within the PAO1 genome showed that mutant strains were affected in ORF PA0967 and PA5345, which correspond to the ruvB and recG genes, respectively. These genes encode helicases RuvB and RecG involved in DNA replication, recombination and repair. The chromate resistance phenotype was restored by cosmids bearing wild type ruvB or recG genes (Miranda et al. 2009). *P. aeruginosa* RuvB and RecG helicases are thus involved in repairing DNA damage caused by chromate.

The genome of *Arthrobacter* sp. FB24 is known to contain a chromate resistance determinant (CRD), consisting of a cluster of eight genes located on a 10.6 kb fragment of a 96 kb plasmid. The CRD includes chrA, which encodes a putative chromate efflux protein, and three genes with amino acid similarities to the amino and carboxyl termini of ChrB, a putative regulatory protein. There are also three novel genes that have not been previously associated with chromate resistance; they encode an oxidoreductase (most similar to malate:quinone oxidoreductase), a functionally unknown protein with a WD40 repeat domain and a lipoprotein. In one study, chromate-sensitive mutant (strain D11) of this bacterium was generated by curing FB24 of its 96-kb plasmid. The mutants accumulated three times more chromium than wild type cells. Introduction of the CRD into strain D11 conferred chromate resistance comparable to wild-type levels, whereas deletion of specific regions of the CRD led to decreased resistance. Using real-time reverse transcriptase PCR, the expression of each gene within the CRD was shown to be specifically induced in response to chromate. Higher levels of chrA expression were achieved when the chrB orthologs and the WD40 repeat domain genes were present, suggesting their possible regulatory roles (Henne et al. 2009). These findings indicate that chromate resistance in Arthrobacter FB24 is due to chromate efflux through the ChrA transport protein (Table 3).

Table 3 Chromate resistance of bacterial isolates

Strain	Chromate conc. to which the isolate is resistant	References
Bacillus brevis	180 μM	Verma et al. (2009)
Exiguobacterium sp. KCH-5	1.5 mM	Sarangi and Krishnan (2008)
Bacillus sphearicus	15.3 mM	Pal and Paul (2004)
Ochrobactrum sp. CSCr-3	15.3 mM	He et al. (2009)
Arthrobacter aurescens P4	19.2 mM	Horton et al. (2006)
Bacillus circulans MNI	19.2 mM	Chaturvedi et al. (2007)
Burkholderia cepacia sp. MCMB-821	19.2 mM	Wani et al. (2007)
Nesterenkonia sp. MF-2	600 mM	Amoozegar et al. (2007)

Cr(VI) reduction by fungi, yeast and actinomycetes

Although a wide variety of bacterial cultures have been reported for their capability to reduce Cr(VI), little information exists on filamentous fungi (Pal. 1997) and yeasts (Muter et al. 2001; Ramirez-Ramirez et al. 2004). One such study was the Cr(VI) reduction by *Trichoderma viride* in stirred tank and concentric tube airlift bioreactors. The airlift reactor performed better because the agitation in stirred tank bioreactor inflicted physical damage on the mycelia, causing their defragmentation (Morales-Barrera and Cristiani-Urbina 2005). *Lecythophora sp.* NGV-1, *Candida sp.* NGV-9 and *Aureobasidium pullulans* VR-8 isolated from tannery effluents and from a nickel-copper mine in Argentina showed tolerance to multiple heavy metals including Cr(VI). Growth in YNB-glucose medium supplemented with 0.5 and 1 mM Cr(VI) revealed that resistance was mainly due to reduction rather than bioaccumulation (Villegas et al. 2008).

Actinomycetes have also been screened for chromate reduction. *Streptomyces griseus* NCIM 2020 reduced Cr(VI) at the expense of a variety of electron donors. Sulphate, nitrate, chloride and carbonate had no effect on chromate reduction while cations such as Cd, Ni, Co and Cu were inhibitory. Cell-free extracts also reduced chromate and addition of NAD(P)H resulted in two- to three-fold increase in activity. Sonication was the best method to release the chromate reductase expressed constitutively. The enzyme showed optimum activity at 28°C and pH 7 (Poopal and Laxman 2009a). Different matrices were tested for whole cell immobilization of this chromate-reducing organism and PVA-alginate was found to be the most effective and was used in a bioreactor. Cr(VI) reduction efficiency decreased as Cr(VI) was increased from 2 to 12 mg/L but increased with an increase in biomass concentration. However, increasing the flow rate from 2 to 8 ml/h did not significantly affect Cr(VI) reduction (Poopal and Laxman 2009b). Such immobilized *S. griseus* cells could be applied for large-scale bioremediation of chromate-containing wastewaters.

Chromium biosorption

Several biosorption studies have also been carried out for Cr. Although voluminous research has been carried out on this front, this paper takes only a fleeting glance at it, confining itself largely to biological reduction. Microorganisms such as bacteria (Srinath et al. 2002), fungi (Bai and Abraham 2003) and algae (Aravindhan et al. 2004) have been used in biosorption studies. The bacterium *P. aeruginosa* (Kang et al. 2006) fungus *Mucor hiemalis* (Tewari et al. 2005), yeast *Pichia guilliermondi* (Kaszycki et al. 2004) and microalgae *Scenedesmus incrassatulus* (Pena-Castro et al. 2004) are a few examples of organisms whose metal-binding capacities have been demonstrated.

As biosorption of metals using living biomass is time-consuming and it is expensive to find appropriate biomass, research interest turned to the use of dead microbial biomass and other biomaterials which are of low cost and are abundant. The removal of heavy metals by biomass by-products from agricultural, industrial or pharmaceutical industry has proved to be highly efficient. These biomaterials are of little commercial value and serve as substitutes for more expensive sources. They possess large quantity of adsorption sites (Law et al. 2003; Tarley and Arruda 2004) and functional groups for ion-exchange such as hydroxyl, carboxyl and phosphate groups as revealed by Fourier transform infrared spectrum (Law et al. 2003; Tarley and Arruda 2004; Chen et al. 2005). Cone biomass of *Pinus sylvestris* showed biosorption ability for Cr(VI) (Ucun et al. 2002). The agricultural biomass by-product *Lentinus edodes* also exhibited Cr(VI) biosorption potential. Nitrogen oxides and carboxyl groups were increased after adsorption and cation exchange was the underlying mechanism (Chen et al. 2006).

In another interesting revelation, chromate-tolerant bacteria isolated from the rhizosphere of aquatic plant *Eichhornia crassipes* enhanced Cr uptake by the plant. Plants inoculated with RA1 (*P. diminuta*), RA2 (*Brevundimonas diminuta*), RA3 (*Nitrobacteria irancium*), RA5 (*Ochrobactrum anthropi*), RA7 and RA8 (*B. cereus*) had 7-, 11-, 24-, 29-, 35- and 21-fold, respectively, higher Cr concentrations in roots compared to the control (Abou-Shanab et al. 2007). These bacterial isolates can play a supportive role in phytoremediation of Cr contaminants.

Conclusion

Bacterial reduction of hexavalent Cr to its trivalent form is an option worth exploring for the remediation of diverse chromium contaminated sites. Its advantages over physical-chemical treatments are innumerable and research in this direction has come out with promising findings. Yet, large scale field application of this technology warrants an improved understanding of the mechanisms involved in resistance and reduction of Cr(VI). Application of genetic and protein engineering tools to broaden enzyme activity should also be further researched. Enzyme purification, characterization and cloning of genes encoding chromate reductases are prerequisites for this, areas in which work has progressed well. Furthermore, successful implementation of a bioremediation program necessitates a thorough assessment of the contaminated site and choice of an appropriate technology as well as microbial strain that is best suited for the prevailing conditions.

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