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Bacterial Reduction of Cr(VI): Operational Challenges and Feasibility

Wan Azlina Ahmad[1] · Chidambaran Kulandaisamy Venil[2] · Evans M. Nkhalambayausi Chirwa[3] · Yi-Tin Wang[4] · Mohd. Helmi Sani[5] · Abdul Fatah A. Samad[5] · Mohd. Farizal Ahmad Kamaroddin[5] · Edgardo R. Donati[6] · Maria Sofia Urbieta[6] · Zainul Akmar Zakaria[7]

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Abstract

Purpose of Review Hexavalent chromium, Cr(VI), and trivalent chromium, Cr(III), are two chromium compounds with practical importance due to their high occurrence and solubility in the environment. Current Cr(VI) treatment techniques involve chemical reduction of Cr(VI) to Cr(III), which posed serious threat to workers and environment notably from long exposure and toxic fumes.

Recent Findings Numerous reports have demonstrated the feasibility of using biological processes for the treatment of Cr(VI) industrial effluents by either pure culture or a consortium of Cr(VI)-reducing bacteria, with various degrees of success. Among issues to be considered include high cost of nutrient for the bacteria, low Cr resistant-reducing ability of environmental isolates, difficulty in scaling up finding in the laboratory to pilot scale and on-site application as well as the understanding on the dynamic underlying mechanisms for bacterial Cr(VI) reduction.

Summary This review highlights cytotoxicity and genotoxicity properties of Cr(VI), which form the biggest motivation for continuous development in the field of Cr(VI) treatment technologies, latest finding in aerobic and anaerobic bacterial reduction of Cr(VI), operational challenges for bacterial Cr(VI) reduction, and some examples for laboratory-scale and pilot-scale evaluation of free and immobilized (biofilm) cells of Cr(VI) resistant-reducing bacteria.

Keywords Hexavalent chromium · Toxicity · Reduction · Bioremediation · Bacteria · Pilot scale

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✉ Zainul Akmar Zakaria
zainulakmar@utm.my

[1] Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310 Johor Bahru, Malaysia

[2] Department of Biotechnology, Anna University, Coimbatore, Tamil Nadu 641046, India

[3] Water Utilisation and Environmental Engineering Division, Department of Chemical Engineering, University of Pretoria, Pretoria, South Africa

[4] Department of Civil Engineering, University of Kentucky, Lexington, KY 40506, USA

[5] Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia, 81310 Johor Bahru, Malaysia

[6] CINDEFI (CONICET, UNLP), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina

[7] School of Chemical and Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, 81310 Johor Bahru, Malaysia

Introduction

Chromium (Cr), ranked as the 7th most plentiful element, is the 21st rich metal that is widely dispersed in natural accretions like, rocks, soil, water, volcanic dust, and gases [1]. Cr can be generally accepted as a combination of four steady isotopes namely, Cr-52 (83.76%), Cr-53 (9.55%), Cr-50 (4.31%), and Cr-54 (2.38%). Cr is extensively utilised in diversified manufacturing processes that resulted in the generation of huge volumes of Cr-containing wastewater from industries such as electroplating, tanning, metallurgy, dyestuff, and leather tanning and consequently becomes one of the most abundant pollutants in aquatic and terrestrial ecosystems [2].

Having an atomic number of 24 and a mass number of 51.9961, Cr fits into the first succession of changeover metals. Cr exists in numerous oxidation numbers ranging from 0 to VI. Of these, only Cr(III) and Cr(VI) transpire in the environment in a stable state. Cr(IV) and Cr(V) act as transient elements during the reduction of Cr(VI) to Cr(III). In an acidic solution, Cr(VI) exhibits a high-level of decisive redox prospective; Cr(III) demonstrates a robust inclination to develop

hexacoordinate octahedral compounds with diverse ligands like water, ammonia, urea, ethylene diamine, and extra crude ligands having oxygen, nitrogen, or sulphur giver atoms [3]. Nevertheless, once the giver atoms are bounded by a macromolecular structure as humic acids, the Cr(III) compound is relatively static. Cr(III) is less toxic due to its little solubility and its inclination to be adsorbed by organic carbon and mineral surfaces [4].

From a biological viewpoint, Cr(III) is an acknowledged crucial element in carbohydrate and lipid metabolism. Cr(III) acts as a cofactor in glucose tolerance factor to unite insulin to receptor sites on membranes and thus expands the efficiency of insulin. The leftover glucose tolerance factors to be categorized and refined has been proposed to hold two molecules of nicotinic acid and a small oligopeptide such as glutathione, coordinated to trivalent Cr [5]. Alternatively, Cr(VI) can form several species which is dependent on pH and Cr(VI) concentration. Cr(VI) is a tough oxidant which acts as a carcinogen, mutagen, and teratogen in life system; thanks to soaring solubility in water, quick permeability over genetic membranes, and ensuing contacts using intracellular protein and nucleic acids. The structural similarity of the soluble chromate anion with organically important inanimate anions, such as sulphate and phosphate, enables it to voluntarily transverse cell membranes via the sulphate carrying system that can be integrated into cells [6].

Cr contamination is also one of the globally confronting current environmental issues [7]. Cr(III) is swiftly convertible into Cr(VI) as it is less toxic and less soluble than Cr(VI) under diverse oxidation methods. Yet, Cr(III) is reckoned as a trace element crucial for the apt working of living beings. Cr(VI) is a well-known contaminant that can trigger skin hitches and lung cancer, respiratory diseases, kidney damage, and genetic alteration [8].

Hexavalent chromium contamination of the environment is the result of extensive anthropogenic use of chromate and dichromate in industries such as stainless steel production, metal finishing, electroplating, leather tanneries, ink manufacture, pigment fabrication, automobile manufacturing, glass and ceramics, cement, corrosion inhibitors in cooling water, wood preservation, power plants, and nuclear facilities [1]. About 35% of Cr released from all anthropogenic sources is Cr(VI). It is a hazardous contaminant as it readily spreads beyond the site of initial contamination through aquatic systems and groundwater.

In view of this, this work specifically highlights, through two specific sections, firstly on the cytotoxicity and genotoxicity of Cr which can present the fundamental or motivation for continuous study on providing safer treatment techniques such as microbial-based Cr(VI) reduction. Secondly, the inclusion of case studies on author's own experience in the development of a bacterial-based Cr(VI) resistant-reducing system from bench-scale in the laboratory until field application using real Cr(VI)-bearing industrial wastewater.

Cytotoxicity and Genotoxicity of Cr

Cr toxicity is greatly influenced by its oxidation state, solubility, and bioavailability [9]. Regardless of the routes of Cr absorption, Cr compounds may undergo alkylation, changes in their oxidation state, and interaction with other biological molecules. In the comparison of states, Cr(VI) is connected with a series of adverse effects, while Cr(III) has been considered an essential micronutrient that plays a crucial role in insulin-dependent glucose metabolism [10]. Cr(VI) compounds can enter the cell through the sulphate-anion channel and then undergo reduction process by a variety of cellular reductants, such as ascorbate and glutathione (GSH), with the formation of intermediates Cr(V/IV) and finally Cr(III) products [11]. During this process, molecular oxygen is stimulated and reduced to superoxide anion ($\cdot O_2^-$), which would then undergo dismutation process to form hydrogen peroxide (H_2O_2). The resultant intermediates further react with H_2O_2 via the Fenton pathway to produce a wide array of reactive oxygen species (ROS) containing hydroxyl radicals, hydrogen peroxide, singlet oxygen, and superoxide [11, 12]. The interaction between excessive ROS with these intermediates may cause oxidative stress and DNA damage, Cr-DNA binary (mono) adducts, Cr-DNA ternary adducts, DNA protein crosslinks (DPCs), bifunctional (DNA interstrand crosslinks, ICLs) adducts, single-strand breaks (SSBs), and oxidized bases [8]. Due to these adverse effects on genetic material mainly DNA, the International Agency for Research on Cancer (IARC) has classified Cr(VI) as a Group I carcinogen [13]. In addition, exposure to Cr(VI) greatly increases the risk of getting respiratory tract cancer [13]. Furthermore, Cr(VI) may also induce lesions in other internal organs, such as the gastrointestinal tract [14].

Initially, Cr(III) was thought to be beneficial without any adverse effect. However, there is a finding that showed Cr(III) can induce oxidative stress damage in culture cells but through different molecular mechanisms compared to Cr(VI). The propidium iodide fluorescence assay in yeast cell revealed that at the same concentration, the percentage of damaged propidium iodide-permeable cells treated with Cr(III) is almost five times greater than those treated with Cr(VI). Meanwhile, the lethal rate indicates that Cr(VI) is more toxic than Cr(III). Additionally, the GSH level in cells treated with Cr(VI) significantly decreases. In contrast, there is an insignificant change in GSH content after Cr(III) treatment, even at a very high concentration, which suggests that the toxicity of Cr(VI) is significantly higher than that of Cr(III). Although both Cr(III) and Cr(VI) can induce cytotoxicity and oxidative stress, serious membrane damage caused by Cr(III) is an indirect consequence of the increase of lipid peroxidation [15].

All compounds that can bind with DNA in cells affect its structure and function, and DNA repair systems are considered genotoxic. This damage can be displayed in different ways, including gene mutation, chromosomal aberration, recombination, and numerical changes. These changes are responsible for heritable effects on reproductive cells which may risk future generations [16•]. The strong redox reaction of Cr(VI) is indicated as a basis of its toxicity. Cr(VI) does not directly interact with DNA, but the genotoxicity of Cr(VI) is rather attributed to its intracellular reduction to Cr(III) via reactive intermediates. The products from the reaction such as ROS, Cr (V), and Cr (IV) are potential sources of oxidative damage induced by Cr(VI) which can lead to two types of DNA damage namely, oxidative damage and Cr(III)–DNA interactions which proceeded via a three-step cross-linking mechanism, i.e., reduction of Cr(VI) to Cr(III), binding of Cr(III) to DNA binding, and protein capture by DNA-bound Cr(III) generating protein-Cr(III)-DNA cross-links [16, 17].

Besides, Cr can also cause chromosomal mutation. A 24 colour M-FISH (multiplex fluorescent in situ hybridization) was used to evaluate the effect of Cr on the structural chromosome in human fibroblast cell. At the lowest physiological dose, both Cr(III) and Cr(VI) resulted in a significant increase in total aberrations. However, Cr(VI) was much more effective than Cr(III) in causing chromosome fragments, which were only induced at the highest doses [18]. Besides, another study explored the molecular mechanism of Cr(VI) and Cr(III) genotoxicity in an intact yeast cell and found that oxidative stress is involved in Cr(VI) genotoxicity at high exposure concentrations. The Cr(III)-DNA interaction appears to be an important genotoxic lesion following Cr(VI) exposure at low-exposure concentrations [19]. Another work had demonstrated that the by-products of Cr(VI) metabolism, Cr(IV) and Cr(V), were able to induce DNA double-strand breaks. However, only Cr(V) can induce DNA damage in mammalian cells. Exposure to Cr(V), but not Cr(IV), results in the initiation of cell cycle checkpoints and activates the ATM kinase, a critical regulator of the DNA damage response [20].

The carcinogenicity of Cr(VI) in humans has been investigated in retrospective ecological mortality studies within Cr-contaminated drinking water area situated at the Oinofyta region of Greece [21]. Proven to be a carcinogen when inhaled and orally consumed, it was classified as a substance which is 'likely to be carcinogenic to humans' when humans are exposed with an estimate of the cancer potency equal to 0.5 $(mg/kg/day)^{-1}$ [22]. It has been reported as a factor that can increase the risk of lung cancer when exposed at a certain level and duration of time, especially in the pigment industry and in chromate production [23]. There is a growing insight that the reaction to stress may be a key player in carcinogenesis. Cr(VI) can stimulate proteotoxic stress and, ultimately, use varying mechanisms to induce stress responses [24].

Bacterial Reduction of Cr(VI)

Biological reduction of highly toxic Cr(VI) to less harmful and less mobile Cr(III) is one of the mechanisms that prokaryotic and eukaryotic cells use to cope with Cr(VI) toxicity that also allows disposal of small amounts of Cr(III), which is a vital micronutrient in humans and mammals in general [25], although the nutritional requirements of Cr in plants or prokaryotes have not been reported [26]. At pH values between 1 and 6, the $HCrO_4^-$ species is dominant especially at low concentrations; this species has a high normal reduction potential (1.33 V) and is therefore easily reducible. However, this potential decreases significantly as the pH increases being approximately 0.7 V at pH 7 where the CrO_4^{2-} species becomes dominant. So, although the reduction is possible, it is significantly simpler to proceed at lower pH values. The biological reduction of Cr(VI) and Cr biosorption constitute the main strategies for Cr(VI) bioremediation [27, 28]. As with other bioremediation processes, its advantages are more evident when the volumes to be treated are very large, and the concentrations of Cr(VI), although above the regulatory limits, are not too high. The bioremediation of Cr through biological reduction can replace equivalent chemical treatments, i.e., using reducing agents, in certain situations. There is a wide variety of microorganisms capable of directly or indirectly reducing Cr(VI); in the first case, Cr(VI) acts as the ultimate electron acceptor while in the second, the microorganisms produce certain metabolites capable of acting as reducing agents. Direct biological Cr(VI) reduction can be produced under aerobic or anaerobic conditions and often carried out by intracellular (associated to the membrane) or extracellular (soluble) chromate reductase enzymes that depend on NADH or NAPH. Under anaerobic conditions, Cr(VI) is the final electron acceptor of the transport chain that includes oxidation of carbohydrates, proteins, fats, hydrogen, and other intra/extracellular compounds. Various Cr reductase enzymes are known to also possess the ability to reduce other compounds such as ferricyanide, azo-dyes, V(V), Mo(VI), and diverse quinones. This actually gave the origin to the very interesting hypothesis that in fact Cr(VI) reduction may not be their primary function but one that developed with the increasing Cr(VI) concentration in many natural environments. In other cases, metabolites generated by the microorganisms provoke the reduction of Cr(VI). Among others, organic acids, amino acids, sugars, sulphide, and iron(II) can all be potential reducing species [27, 29, 30].

Various types of bacterial species have been reported to be able to reduce Cr(VI) to Cr(III) which include *Pseudomonas* sp., *Bacillus* sp., *Microbacterium* sp., and *Shewanella oneidensis* [31]. Most of the reported aerobic Cr(VI) reductases have been found intracellular [32•]. Soluble chromate reductases, which uses NADH or NADPH as cofactors, are most commonly associated with aerobic [33]. Chromate

reductases like ChrR [34], YieF [35], and Tkw3 reduce Cr(VI) by electron transfer to form Cr(III). Other researchers have broadly categorized enzymatic reduction of Cr(VI) into that of chromate reductase (ChrR) in *Pseudomonas putida*, flavoprotein (YieF), oxygen-insensitive NADPH nitroreductase (NfsA) in *Escherichia coli*, OYE, NemA, NfoR, and families [32•]. These enzymes use flavin as the cofactor and NAD(P)H as the electron donor but with different electron transfer mechanisms. Conversely, the different electron transfer mechanisms of these enzymes need further structural and biochemical analysis.

Generally, the enzymatic reduction mechanism under anaerobic conditions are different from the aerobic conditions. In an anaerobic condition, bacteria can use Cr(VI) as an electron acceptor in the respiratory chain for electron donors like carbohydrates, proteins, fats, hydrogen, NAD(P)H, and endogenous electron reserves [33]. The reduction of Cr(VI) to Cr(III) during an anaerobic process was due to soluble and membrane-associated enzymes which form insoluble precipitate. Up to 74% of Cr(VI) reduction was reported for an anaerobic granular consortium that combined Cr(VI) reduction and adsorption mechanism when dealing with 50–500 mg Cr(VI)/L [36]. The biosorption mechanism is the predominant process of removal with 297 mg of Cr VI/L or 31.39 mg of Cr VI/g biomass at the highest concentration. The biosorption principles of Cr(VI) by microorganisms may include chelation, precipitation, and electrostatic interaction. Nevertheless, Cr(VI) at 100 mg/L have been reported to inhibit the growth rate and morphology of *Pseudomonas aeruginosa* [37]. It has also been reported that anaerobic Cr(VI) reductions involved the extracellular electron transport (EET) pathway linkages [32•]. In the linkages, Cr(VI) acts as the terminal electron acceptor, and three EET pathways have been recognized. The first combination of Cr(VI) and EET pathway is the cytochrome c, the electron transfer chain in *Shewanella oneidensis* MR-1 which transfers the electron from NADH to ubiquinone by dehydrogenase. The second combination pathway is the electron shuttles (ECs) which can be oxidized and reduced reversibly during the electron transfer from microbes to Cr(VI). The third reduction pathway is through the microbial nanowire which transfers electron produced by bacteria via electrically conductive proteins to electron acceptors [32•].

Aerobic reduction is considered to be a detoxification mechanism where normally the reduction of Cr(VI) by the soluble protein fraction takes place either internal or external to the plasma membrane. Anaerobic reduction of Cr(VI) has been reported to proceed through simultaneous Cr(VI) reduction and methane oxidation in a membrane-based biofilm reactor. However, it was postulated that energy generated in the anaerobic respiration process is insufficient to sustain cell growth because fermentable organic compounds generated is utilised for cell metabolism [31, 38]. Initial pH of Cr(VI) in wastewater, i.e., 0.50–3.75 ± 0.05, was reported not to interfere with the survival of Cr(VI)-reducing bacteria as the final pH of the mixture between Cr(VI) and the growth medium (nutrient broth) would be between 6.80 to 7.65 ± 0.05. The presence of oxidizable organic materials in the growth medium also assisted to reduce the Cr(VI) concentration, which is the primary characteristic for its toxicity. Time of Cr(VI) addition to the culture broth is also another significant factor that would affect the survival and Cr(VI) reduction capability of the Cr(VI)-reducing bacteria [39]. It was suggested that the addition of Cr(VI) at the early stationery growth stage (12 h) of *Acinetobacter haemolyticus* (*A. haemolyticus*) EF369508 was successful to reduce 75% of 70 mg/L Cr(VI) compared to less than 50% reduction when Cr(VI) was added at the start of culture growth. Cr(VI) reduction by *A. haemolyticus* was unaltered in the presence of 10 mM of phosphate (PO_4^{3-}), sulphate (SO_4^{2-}), sulphite (SO_3^{2-}), and nitrate (NO_3^-), indicating Cr(VI) as a better electron acceptor than these oxyanions. The insignificant impact of sulphate (a competitive inhibitor of bacterial chromate transport) on the reduction of Cr(VI) suggested that the reduction process does not involved chromate transportation into the bacterial cytoplasmic region, rather, the reduction would probably occur in the cytosol. Also, this would suggest that the Cr(VI) reduction process by *A. haemolyticus* was solely aerobic. This can be substantiated by the fact that one of the features characterizing anaerobic chromate reduction systems is sensitivity to nitrate and sulphate ions, a condition that was not encountered with Cr(VI) reduction by *A. haemolyticus*.

Operational Challenges for Bacterial Cr(VI) Reduction

Bacteria is the domain with the highest number of reports on the ability to reduce Cr. Hundreds of bacterial species are capable of direct Cr(VI) reduction under anaerobic and/or aerobic conditions; many of those species belong to the genera *Pseudomonas*, *Bacillus*, *Paenibacillus*, *Arthrobacter*, and *Corynebacterium*, among others. This ability has been demonstrated for mesophilic, moderate thermophilic, and extreme thermophilic bacteria [40]. One of the limitations for on-site application of bacterial Cr(VI) reduction is the low Cr(VI) reduction rate. Nevertheless, more and more findings were reported on the faster Cr(VI) reduction as the one demonstrated for high-density cell cultures of *Geobacter sulfureducens* where complete reduction of 100 mg/L Cr(VI) was achieved within 20 mins of contact time [41]. Genetic approaches have also provided significant advances in the topic of Cr(VI) reduction. It is also very important to simulate the conditions to apply the technology in the field. Thorough laboratory assays are of paramount importance to ensure smooth transition of findings at lab-scale to ex situ and in situ demonstration of this

technology. Phytoremediation using plants and their symbiotic microorganisms is another powerful tool for bioremediation of chromate-contaminated soils and aquifers [42]. In phytoremediation, microorganisms associated with the roots can contribute to the immobilisation or mobilisation of Cr(VI) followed by its reduction to Cr(III). Some metallophytes [43] and ornamental plants [44] have been used for Cr(VI) decontamination. Table 1 summarizes some of the Cr(VI) reducing capabilities of Cr(VI)-resistant-reducing environmental isolates which have the potential to be further developed for industrial applications.

Cr(VI) contamination in groundwater systems is conventionally treated using the pump-and-treat methods which involve the extraction of contaminated water from the aquifer, treatment above ground, and injection of the treated water back into the aquifer [64, 65]. Even though a number of successful reports are available on the success of using the pump-and-treat technique to remediate Cr-contaminated groundwater, the issue in removing residual Cr persists until the present day [66]. Both chemical and biological processes have recently been used to treat Cr(VI) in groundwater using the pump and treat methodology [67, 68]. Chemical treatment processes employed in the remediation of Cr(VI) are efficient but at the same time produce unwanted chemical by-products which result in the production of toxic sludge [69]. Therefore, the chemical processes are viewed as costly and environmentally intrusive. Alternative biological treatment methods using aquatic biomass and/or Cr(VI)-reducing bacteria have long been investigated and proposed by a number of researchers [64, 68]. These methods may be applied ex situ [70] or in situ in biological barriers [71]. When applied in situ, it is desirable to use bacteria from the same or nearby environments to avoid the issue of introducing "new" strains across country or regional boundaries [70].

In situ biological permeable reactive barriers (BPRBs) have been used mainly for the removal of toxic organic compounds. This was achieved by the introduction of organisms or by enhancing the activity of the portion of the indigenous community possessing inherent capability to degrade recalcitrant organic compounds [72, 73]. Specific application of BPRB systems for the removal of Cr(VI) in groundwater has been extensively studied at laboratory-scale with various degrees of success/feasibility [73–76]. However, demonstration on the feasibility of such system at pilot-scale level is still limited with earlier notable studies include the use of agricultural discharges as nutrient for the bacteria [77], minimal nutrient conditions and inoculated microbial barrier [78, 79] or the application of thick bio-barrier and reactive zone technologies [80]. More recently, evaluation at the pilot-scale level involves the use of combination of biological and physico-chemical approaches [65, 81–83] or solely physico-chemical techniques [84–86]. The slow progress towards full implementation of biological barriers for remediation of Cr(VI) and toxic metals has been both due to the unavailability of microorganisms capable of growing under nutrient stressed conditions and lack of information on the speciation and mobility of the reduced metal ion species in the soil.

Example of Case Study for Lab-Scale and Pilot-Scale Evaluation

One example of a lab-scale study on bacterial Cr(VI) reduction is the ability of one environmental isolate, *Bacillus thuringiensis* (*B. thuringiensis*) [79]. *B. thuringiensis* was isolated from sand drying beds at a wastewater treatment plant that received high periodic loadings of Cr(VI) contaminated effluent from an abandoned chrome processing foundry in Brits (North West Province, South Africa). From the 16S rRNA analysis, *B. thuringiensis* was identified as the main species present in the dried sludge cultures of the sand drying beds, which showed the ability to completely reduce 200 mg/L Cr(VI) after contact for 96 h. Within similar contact time, cultures present in the mixed liquor samples (activated sludge) displayed much lower Cr(VI) reducing capability with Cr(VI) reduction of 67.6% while no Cr(VI) reduction was observed for sewage cultures (influent to sewage treatment plant) and soil cultures (soil from surrounding area). Higher Cr(VI) reducing ability by cultures present in sand drying beds was attributed to better acclimation and selection for Cr(VI) reducing species in the sludge due to exposure to higher Cr(VI) concentrations and longer exposure in the sludge zone than in the mixed liquor.

B. thuringiensis was further evaluated for Cr(VI) reduction capacity in continuous flow-mode. Using the known Cr(VI) reducer, *Escherichia coli* ATCC 33456 as positive control, *B. thuringiensis* and *Escherichia coli* ATCC 33456 were each immobilised in a packed-bed reactors with the following properties; 4.8 cm (i.d.), 11.5 cm (height) glass column packed with approximately 760 (6 mm diameter) glass beads and hydraulic loading rate of 40 mL/h (across a pore volume of 60 mL) to achieve a hydraulic retention time of 1.5 h. Reactors operated under optimal loading conditions towards steady-state operation were challenged by NADH inhibitors, cytochrome-b inhibitors and ATPase uncouplers at different times of operation to evaluate the electron pathway mechanisms in the organisms. Continuation of Cr(VI) reduction activity after blocking cytochrome-b activity would indicate Cr(VI) reduction via NADH-dehydrogenase [87] or Cr(VI) reduction connected to sulphate shuttle mechanisms that can also transport chromate (CrO_4^{2-}) into the cytoplasm of the cell [30, 87, 88]. Cr(VI) reduction in the presence of uncouplers could imply membrane associated Cr(VI) reduction pathway via the membrane electron phosphorylation transport system [87]. The following parameters were determined for the experiment; Cr(VI), total Cr, Cr(III)-OH_3, cell surface reactions,

Table 1 Summary on the Cr(VI) reducing capabilities of some Cr(VI)-resistant-reducing environmental isolates

Species/consortia	Isolated from	Growth condition	Cr(VI) reduction capabilities	Related information	Reference
Cellulosimicrobium funkei AR8	Tannery industrial effluent	Aerobic, neutrophilic, mesophilic, halophilic	• Intra and extracellular reduction of up to 0.1–0.2 mg/L Cr(VI) • Tolerance up to 1.1 mg/mL Cr(VI) • pH range of 6–8 • In the presence of 2% NaCl	• From the FTIR, SEM-EDX, XRD, TEM analysis, Cr(VI) reduction occurred both extracellular and intracellularly • Cr(III) immobilisation and accumulation in the cytosolic region	[45]
Klebsiella pneumoniae, *Mangrovibacter yixingensis*	Tannery industrial effuent	Aerobic, neutrophilic, mesophilic	• *Klebsiella pneumoniae* - up to 80 mg/L Cr(VI) • *Mangrovibacter yixingensis* – up to 100 mg/L of Cr(VI)	Proceed in the presence of *chrR* gene	[46]
Bacillus strain BYCr-1	Rare-earth ore contaminated with heavy metals	Aerobic, neutrophilic, mesophilic	Reduction of 0.2 mM Cr(VI)	• Intracellular and extracellular precipitation of Cr(III) • Presence of the NfrA nitro/ flavin reductase – high similarity with other chromate reductases • Upregulation of NfrA 5.3 times in the presence of Cr(VI) • Enhancement of Cr(VI) reduction by NfrA	[47]
Escherichia coli	Cr(VI) contaminated environment	• Aerobic, neutrophilic, mesophilic • *Escherichia coli* transformed with the *chrA*, *chrB* and *chrAB* genes from the pathogenic *Serratia* sp. S2	• *chrAB* transformed *E. coli* showed highest Cr(VI) metabolic capacity (resistance, reduction, efflux) • Cr-absorption and Cr-efflux of *chrA* and *chrAB* engineered strains were significantly stronger than the control strain.	• The Cr-transporter, encoded by *chrA* gene, confers the ability to remove intracellular Cr on *chrA* and *chrAB* engineered strains. • The Cr metabolism ability of the *chrAB* engineered strain is stronger than the *chrA* engineered strain. • Cr-resistance in *chrA* and *chrAB* genes may involve several mechanisms, such as sulphate ion channel and respiratory chain electron transfer	[48]
Morganella morganii 1Ab1	Tannery effluent contaminated area	Aerobic, neutrophilic, mesophilic	Reduction of 4600 mg/L of Cr(VI) with 90% efficiency in 48 h in raw tannery effluent at pH 7 and 37 °C	Interaction of Cr with bacterial cell wall successfully documented	[49]
Aeribacillus pallidus BK1	Volcanic geothermal region	Aerobic, neutrophilic, thermophilic (60 °C)	• High tolerance to Cr(VI) (CIM 400 mg/L) • High reduction of 100 mg/L Cr(VI) after 36 h of contact time	• Cells remained viable even after 2 h of heat treatment at 155 °C • Cytoplasmic Cr(VI) reduction • Detection of intracellular amorphous precipitates of Cr(III)	[50]
Enterococcus avium BY7	Culture collection	Anaerobic, neutrophilic, mesophilic • Sulphate-reducer • Free cells as well as immobilised on graphene oxide (GO) particles	• Sulphate and Cr(VI) reduction by free and immobilised cells enhanced by EPS addition • GO capable of abiotic Cr(III) reduction to Cr^0	• Increased production of EPS (indicating its resistance pathway) in both free and immobilised cells when exposed with Cr(VI) • Structural and compositional changes in EPS were also recorded • Increased EPS production was also responsible for enhanced Cr((VI) reduction	[51•]
Microbial community dominated by	Cr(VI)-contaminated aquifer associated	Anaerobic, neutrophilic, mesophilic	• Complete reduction of 19 mg/L Cr(VI) present in the groundwater after 80 days • Cane molasses was used as C source	• Specific role of cane molasses (relative to other complex organic substrates) in	[52]

Table 1 (continued)

Species/consortia	Isolated from	Growth condition	Cr(VI) reduction capabilities	Related information	Reference
Sporolactobacillus, *Clostridium*, and *Ensifer*	with electroplating factory		• Cr(III) precipitation on the soil particles	enhancing Cr(VI) reducing capabilities of indigenous bacterial species	
Geobacter sulfurreducens	Culture collection	Anaerobic, neutrophilic, mesophilic	• Almost complete (99%) reduction of 100 mg/L Cr(VI) in 20 min • Time significantly increased to 120 min at 200 mg/L Cr(VI)	• Extracellular proteins highly involved in Cr(VI) reduction • Detection of intracellular Cr(III) deposition	[41]
Microbial community dominated by *Pannonibacter*	Anaerobic sludge	• Aerobic, alkalophilic, mesophilic • Simultaneous removal of nitrate and chromate	• Complete co-removal of 50 mg/L nitrate and 10 mg/L Cr(VI) between pH 10.0 to 11.0 • At any other pH values, Cr(VI) reduction between 10 to 30% only	Significant up-regulation of functional genes *narG* and *azoR* at initial pH of 10.0 and 11.0	[53]
Pseudomonas fluorescens LB 300 and *Shewanella oneidensis* MR-1	Culture collection	• Anaerobic, neutrophilic, mesophilic • Cr(VI) reduction in the presence of geothite, haematite and aqueous Fe(III)	• *P. fluorescens* LB300 – Cr(VI) reduction inhibited in the presence of goethite or haematite; higher Cr isotopic fractionation with haematite • *S. oneidensis* MR-1 - goethite or haematite increased Cr(VI) reduction rates and have no effect on Cr isotopic fractionation factors due to different attachment and reduction mechanisms	• Cr isotope fractionation allows distinction between biotic and abiotic Cr(VI) reduction • Different attachment to minerals and Cr(VI) reduction mechanisms in both strains • *P. fluorescens* LB300 showed tight attachment to Fe minerals - Cr(VI) reduction governed by membrane bound enzymes. • Production of nanowires by *S. oneidensis* MR-1	[54•]
Enterobacter sp. Z1	Sediment in a pesticide industry	• Anaerobic, neutrophilic, mesophilic • Simultaneous chromate reduction and arsenite oxidation	• Almost complete (98%) Cr(VI) reduction and As(III) oxidation by immobilised sodium alginate-based bacterial beads • Posterior precipitation of Cr(III) using $Ca(OH)_2$	Stimulation of cysteine, sulphur, methionine, As resistance and oxidoreductase production in the presence of Cr(VI) and As(III)	[55]
Community dominated by *Spirochaetaceae*, *Delftia*, *Azonexus*, *Acetoanaerobium*, *Methanobacterium*	Anaerobic sludge from a full-scale brewery wastewater treatment plant	• Anaerobic, neutrophilic, mesophilic • Media supplemented with H_2 and CH_4	Increased reduction rate of 10 mg/L Cr(VI) in the presence of H_2 and CH_4 as joint electron donors	• Detection of key genes (*chrA*, *hupL*, *mcrA*) related to Cr(VI) reduction and H_2 and CH_4 oxidation • More active electron transference from the increase in extracellular cytochrome *c* and intracellular NADH	[55]
Pseudomonas aeruginosa G-12	Sewage sludge	• Aerobic, neutrophilic, mesophilic • Simultaneous removal of nitrate and chromate in wastewater	Simultaneous removal of 500 mg/L nitrate (95%) and 10 mg/L Cr(VI) – 93%	• Nitrate removal by aerobic denitrification • Cr(VI) reduction in the cellular surface	[56]
Pseudomonas umsongensis CY-1	Chromium contaminated soil	• Aerobic, neutrophilic, mesophilic • Simultaneous reduction of Cr(VI) and Hg (II)	• More than 90% Cr(VI) and Hg (II) reduction in aerobic batch systems • Lactate and NADH were used as electron donors	Cytoplasmic reduction with Cr(III) and Hg^0 as final products	[57]
Microbial community	Activated sludge from wastewater treatment plant	• Aerobic, neutrophilic, mesophilic	• Complete reduction of Cr(VI) in contaminated drinking water	• *Comamonadaceae* - Cr(VI) reducers • *Methylophilaceae*, *Methylococcaceae* - methanotrophs	[58]

Table 1 (continued)

Species/consortia	Isolated from	Growth condition	Cr(VI) reduction capabilities	Related information	Reference
		• Methane/ oxygen-based membrane biofilm reactor • Aerobic CH_4 oxidation, denitrification and Cr(VI) reduction		• *Comamonadaceae*, *Cytophagaceae*, *Hyphomicrobiaceae*, *Alcaligenaceae* - denitrifiers	
Enterobacter sp. SL	Soil near a sewage treatment station of a smelting plant	• Anaerobic, wide range of pHs (optimum pH 6), moderate thermophilic • Anaerobic reactor with waste molasses as carbon source	Complete reduction of 100 mg/L Cr(VI) in 25 h	• Final Cr(VI) concentration much lower than discharge limit stipulated by Electroplating Pollutant Emission Standard	[59•]
Microbial community of bacteria and archaea	Activated sludge from a wastewater treatment plan	• Anaerobic, neutrophilic, mesophilic • CH_4 as a sole C source in batch reactor	Cr(VI) reduction coupled to CH_4 oxidation	• Detection of anaerobic methanotrophic archaea family ANME-2d • Cr(VI) reduction by ANME-2d archaea or unknown Cr(VI)-reducing microbes coupled with ANME-2d	[60]
Bacterial and archaeal microbial community	Not mentioned	• Anaerobic membrane biofilm reactor • CH_4 was used as electron donor	Cr(VI) reduction coupled to CH_4 oxidation	• *Archaeon Candidatus 'Methanoperedens'* as the only anaerobic methanotroph detected • Could be responsible for the chromate bio-reduction stimulated in the presence of methane	[38]
Arbuscular mycorrhiza *Rhizophagus irregularis* and grass *Brachiaria mutica*	Not mentioned	Mycorrhizal phytoremediation assay	Very low residual Cr(VI) in the contaminated soil after Cr(VI) reduction by teh mycorrhiza	• Symbiotic relationship between *R. irregularis* and *B. mutica* resulted in increased Cr(VI) tolerance and bioaccumulation in *B. mutica* • Due to increased production of antioxidants and photosynthetic capacity	[61]
Stenotrophomonas rhizophila DSM14405	Culture collection	• Aerobic, neutrophilic, mesophilic • Plant-growth promoting bacteria	• Complete reduction of 50 mg/L Cr(VI) in 28 h • High MIC value of 1000 mg/L for Cr(VI)	• Intracellular reduction and accumulation of Cr(III) • Insignificant effect from salt stress on the growth and Cr(VI) reduction efficiency	[62]
Cellulosimicrobium sp. NF2	Soil from rhizosphere area of indigenous plants in contaminated area	Aerobic, neutrophilic, mesophilic	• Complete reduction of 100 mg/L Cr(VI) in 48 h • High MIC value of 800 mg/L for Cr(VI)	Plant growth promotion (alfalfa) under metal stress and increase of metal uptake by the plants	[63]

dry weight of biomass, viable suspended biomass, attached biomass and computational simulation and parameter determination for proposed Cr(VI) reduction models was conducted using the Computer Program for the Identification and Simulation of Aquatic Systems AQUASIM 2.01 (AQUASIM™, EAWAG, Dübendorf, Switzerland).

Results obtained suggested that cell physiology contributed to the extent and mode of biotransformation in the bacterial culture. A delayed response in Cr(VI) reducing activity was observed in Gram negative cells compared to that for the Gram positive cells. In this study, we propose that this difference could be due to mass transport effects imposed by the outer membrane of the Gram negative cell and the fact that the reductase containing peptidoglycan layer in much thinner in Gram negative cells than in Gram positive cells. Gram negative cells acquired an optimum Cr(VI) reduction capability at around 38 h, Cr(VI) reduction occurred rapidly until the concentration in the liquid medium was near neutral with respect to Cr(VI). This observation was validated by the observed trend in *E. coli* ATCC 33456 which followed a similar trend as the Gram negative *Microbacterium* spp. The culture harvested as consortium at 16 to 24 h performed best within the 8 h period. The better performance of the consortium culture compared to the pure culture was attributed to it having retained the best attributes of both the Gram positive and Gram negative cultures resulting in faster initial Cr(VI) reduction within 60 h followed by further increase in reduction rate due to the presence of the *B. thuringiensis* and *B. cereus* cells followed by the activity of *Microbacterium spp.* after 60 h. Results showed advanced enzymatic Cr(VI) reductase structures in *B. thuringiensis* never observed in other microbial species before. Blocking of electron carrier enzymes suggested the involvement of dissolved thioredoxin in the cytosol and bulk media as possible catalysts for Cr(VI) reduction in resting cells.

Other researchers also reported on the evaluation of Cr(VI) reducing ability by environmental isolate either in batch [39, 89] or continuous bacterial biofilm system [77, 90–94]. One example is the ability of a locally isolated gram negative aerobic *Acinetobacter haemolyticus* (*A. haemolyticus*) EF369508 that can carry out both Cr(VI) resistant and reducing properties [89]. Using the shake-flask technique, complete Cr(VI) reduction was achieved until 30 mg/L Cr(VI) where initial specific reduction rate increased with Cr(VI) concentrations. It was observed that Cr(VI) reduction was not affected by the presence of 1 or 10 mM sodium azide (metabolic inhibitor), 10 mM of PO_4^{3-}, SO_4^{2-}, SO_3^{2-}, NO_3^- or 30 mg/L of Pb(II), Zn(II), Cd(II) ions. However, heat treatment caused significant dropped in Cr(VI) reduction to less than 20% only. *A. haemolyticus* cells loses its shape and size after exposure to 10 and 50 mg Cr(VI)/L as revealed from the TEM examination. One interesting point noted from the study was the effect of time of Cr(VI) addition to culture broth and the rate of Cr(VI) reduction [39]. It was observed that the ability of *A. haemolyticus* to reduce Cr(VI) depends strongly on the number of surviving cells present in the solution. Higher Cr(VI) reduction can be achieved when a high number of bacterial cells are present. When 10–100 mg/L Cr(VI) was added at time of inoculation, extended time was required to reach stationary phase of cells growth, with total growth inhibition at 70 mg/L Cr(VI) and a Cr(VI) reduction drop to 35% at 100 mg Cr(VI)/L. However, bacterial growth was uninterrupted when 10-100 mg Cr(VI) L^{-1} were added at the early stationary phase (after 12 h of bacterial growth) indicated by a high bacterial count of 10^8 CFU mL^{-1}. More than 95% of Cr(VI) was reduced at an initial Cr(VI) of 50 mg L^{-1}. *A. haemolyticus* was then evaluated for its Cr(VI) resistant-reducing performance in a continuous flow-through bacterial biofilm system [39]. In the glass column used, wood-husk was used as support material for bacterial attachment. Around 97% of the 15 mg L^{-1} Cr(VI) present in the Cr(VI)-containing electroplating wastewater was reduced at a flow rate of 8.0 mL min^{-1}. Liquid pineapple waste was used as nutrient for the bacteria. Electron microscopic examinations of the wood-husk after 42 days of column operation showed gradual colonization of the wood-husk by bacterial biofilm. The use of 0.1% (v/v) formaldehyde as a disinfecting agent inhibited growth of bacteria present in the final wastewater discharge.

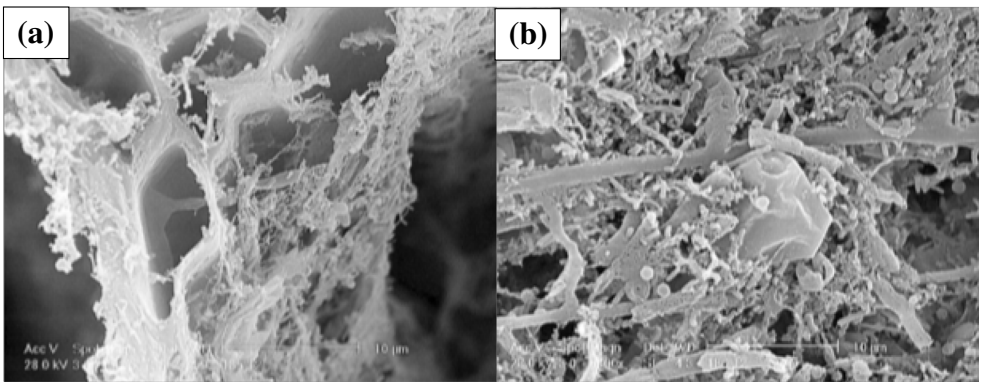

Fig. 1 SEM micrographs for column bioreactor containing sugarcane bagasse (**a**) without and (**b**) with the addition of *A. haemolyticus* cells after 61 days of column operation (6400× magnification)

Following this, wood-husk immobilised *A. haemolyticus* was evaluated for Cr(VI) resistant-reducing properties at the 0.2 m^3 bioreactor [77] and 2.0m^3 bioreactor scale [90]. For the 0.2 m^3 bioreactor, complete Cr(VI) reduction to Cr(III) was obtained immediately after the start of bioreactor operation when 17–81 mg Cr(VI) L^{-1} was fed into the bioreactor at flowrates of 0.11–0.33 m^3 h^{-1}. Using the laboratory-optimized flocculation and coagulation dosages [92], less than 0.02 mg Cr(VI) L^{-1} and 1 mg total Cr L^{-1} were recorded in the outflow flow. By using nonsterilized operating condition, bioreactor containing wood husk-immobilised *A. haemolyticus* showed remarkable robustness as demonstrated by the uninterrupted Cr(VI) reduction, i.e., complete Cr(VI) reduction, even though fluctuations in influent parameters were recorded as follows: pH 6.2–8.4, Cr(VI) 17–81 mg L^{-1}, liquid pineapple waste 1–20% (v/v), and temperature 30–38 °C. When the Cr(VI) resistant-reducing system was evaluated at a larger bioreactor of 2 m^3 capacity, higher influent Cr(VI) concentrations were able to be tolerated (15–240 mg L^{-1}), and complete reduction of Cr(VI) was achieved even after 3 months of bioreactor operation. Cr(VI) was not detected in the final effluent fraction indicating complete removal of Cr from solution from the flocculation/coagulation step, as well as the unlikely reoxidation of Cr(III) into Cr(VI). The organic-rich sludge obtained from the flocculation and coagulation stage was determined for its potential use as soil additive for the growth of ornamental plants. Results obtained showed that *Impatiens balsamina* L. and *Gomphrena globosa* L. showed better growth in the presence of soil-sludge mixture compared to *Coleus scutellarioides* (L.) *Benth*. Acid digestion of dried plant parts revealed that significant amounts of Cr were accumulated that indicate its potential application in Cr phytoremediation effort. The bacterial-based Cr(VI)-reducing system was also reported not to be detrimental to human health based on the low levels of Cr detected in the hair and nail samples of the plant operators. A lab-scale study was also carried out using sugarcane bagasse in view of elucidating the possible replacement of wood husk as support material as well as reducing the usage of liquid pineapple waste in influent bioreactor mixture [93]. In the study, *A. haemolyticus* was successfully immobilised onto sugarcane bagasse and was able to use carbon source from sugarcane bagasse to reduce 92–99% of 10–100 mg/L Cr(VI) (Fig. 1). However, 16S rRNA identification for bacterial species present in the formed biofilm on the sugarcane bagasse (after more than 30 days of operation), did not reveal substantial presence of *A. haemolyticus* with *Chitinophaga terrae*, *Laribacter hongkongensis*, *Ottowia thiooxydans*, *Rhizobium cellulosilyticum* and *Candidate* division OP10 being the dominant species. Some important characteristics for the chromate reductase activities for *A. haemolyticus* have been reported [94] where results obtained are as follows: not NADH-dependent, associated with CFE with notable contribution from the membrane fraction, enhanced in the presence of glucose, optimal at pH 7.0, 30 °C, in the presence of 1 mM Co^{2+} (highest) with Michaelis–Menten constant, K_m, and maximum reaction rate, V_{max}, of 184.47 μM and 33.3 nmol/min/mg protein, respectively. The copresence of Ag^+ and Hg^{2+} ions inhibited the enzyme activity.

Conclusion

Chromium remains as one of the more important minerals utilised in various industrial processes. Incomplete treatment of residual chromium is of great concern due to its high solubility and toxicity. Review of recent advancement of knowledge in the biological treatment of Cr(VI)-containing wastewater as presented in this work forms only a small part of the continuous effort to ensure that technologies developed are efficient, feasible, and robust enough. Nevertheless, more studies at the fundamental, laboratory, and pilot scale levels need to be carried out prior to a more widespread application of the biotechnological approach for Cr(VI) reduction.

Declarations

Conflict of Interest The authors declare that they have no conflicts of interest in the content and materials published in this work.

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