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Detoxification and Removal of Hexavalent Chromium in Aquatic Systems: Applications of Bioremediation

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Abstract.
Chromium is a transition metal with a wide range of applications in leather tanning, textile, electroplating, stainless steel production, inorganic chemical production and wood preservation industries due to yellow colouration, corrosion resistance, higher melting-point and crystalline structure with raging of oxidation states from 0 to +6. Trivalent and hexavalent chromium are the most abundant forms of chromium discharged into the aquatic environment by industries. It has been reported that hexavalent chromium is highly toxic than trivalent chromium due to the higher solubility, mobility and tendency to accumulate in higher trophic levels, which, therefore, become bioavailable and causes carcinogenic, mutagenic and teratogenic effects on most microorganisms and animals, growth inhibition, morphological and physiological changes and yield reductions in plants. Therefore, it is essential to detoxify the above hazardous pollutants up to permissible limits, which local and international authorities have legislated concerning its threat towards biotic components. Hexavalent chromium detoxification is possible to achieve using three methods i.e. physical, chemical and biological methods. These remediation processes can eliminate highly toxic hexavalent chromium or transform it into a less toxic form of trivalent chromium, completely or partially by adsorption and reduction. Biological remediation is considered a cost-effective and eco-friendly method compared to physical and chemical remediation. Further, many biological agents have been identified as agents that can tolerate the hexavalent chromium toxicity up to certain higher levels depending on the internal and external environmental factors, indicating different metal tolerance mechanisms that are assumed to be applied in metal remediation aspects. According to the testimonies of novel bioremediation studies, some hexavalent chromium tolerant organisms such as plants, bacteria, unicellular and multicellular fungi and algae are promising eco-friendly alternatives in detoxification and hexavalent chromium removal perspective. This article reviews the bioremediation approaches available for hexavalent chromium detoxification and removal and highlights the strengths and weaknesses of current bioremediation methods.

Keywords: Hexavalent chromium, toxicity, biological remediation, chemical remediation, physical remediation, biofilms
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Introduction
Chromium is a highly valued industrial raw material with a wide range of industrial applications such as pigment production for paints, inks and plastics, anti-corrosion coating production, stainless steel production, wood preservation and leather tannins which are discharged both trivalent chromium (Cr(III)) and hexavalent chromium (Cr(VI)) in higher quantities [1,2]. According to toxicological studies, Cr(VI) is 100 times more toxic than Cr(III) due to solubility, mobility and permeability in biota [3–7]. From the view of toxicology, prolonged exposure to Cr(VI) can lead to carcinogenic, mutagenic and teratogenic effects on animals which has been clinically proved, and morphological and physiological effects on plants, algae and other microorganisms [8–12]. The working community in chromium based industries, including chrome mining, has the highest potential for chromium poisoning [8,13–16]. Therefore, international authorities for the occupational community, such as Occupational Safety and Health Administration (OSHA), has set the maximum limit for Cr(VI) exposures as similar to the conventional public health concerning local and international authorities; World Health Organization (WHO), United States Environmental Protection Agency (US EPA). The minimization and detoxification of Cr(VI) in industrial effluents can be achieved by Physical, Chemical, and biological methods. Among the above methods, biological remediation is considered the most cost-effective and environmentally friendly method. [17–20].

Chemical nature and uses of Chromium.
Chromium is the 21st most abundant element in earth's crust, which belongs to d-block in the periodic table with a molar mass of 51.9961 g mol^{-1} with a wide range of industrial applications based on chemical and physical properties such as inert nature, hardness, strength, high-temperature resistance and corrosion resistance etc. [21].

Cr(III) and Cr(VI) are the most stable and domain oxidation forms of chromium that exists in nature among the other oxidation forms of metallic chromium (Cr(0)), divalent chromium (Cr(II)), tetravalent chromium (Cr(IV)) and pentavalent chromium (Cr(V)).

The majority of chromium is used for metallurgical (67%) and refractories (18%), while the rest of 15 % are used for chromium induced chemical production, which is used for wood preservation, leather tanning, metal finishing, pigment production and textile industry as a raw material [2] (**Table 1**).

Table 1 – Industrial applications of Cr(0), Cr(III) and Cr(VI).

Oxidation state	Industrial application	Reference
Cr (0)	Stainless steel production Alloy production Metal manufacturing	[2]
Cr (III)	Metal and alloy production Textile and leather tanning Copy machine toners Brick lining Chrome plating Catalysts production Paint production	[2,22]
Cr (VI)	Chrome plating Leather tanning Textile industry Copy machine toners Dye/paint pigment production Wood preservation High temperature battery production Metal finishing Catalyst production Stainless steel production Plastic production	[2,22,23]

Toxicity of Chromium.

Among the most stable oxidation states of chromium in nature, Cr(III) is considered an essential micronutrient of higher organisms with less toxic effects due to lower solubility and impermeability [6,24]. Further, it has been reported that Cr(III) can assist in regulating the glucose level of the human body [25]. In contrast, Cr(VI) has been categorized as a carcinogenic agent by the USEPA and International Agency for Research on Cancer (IARC) due to high water solubility and mobility [26].

Toxicity of Cr(VI) to humans.

Prolonged Cr(VI) exposes through breathing, ingesting, and skin contacts can cause nasal irritations, nasal perforations, skin irritations, skin ulcerations, skin allergies, lung cancers, stomach upsets, convulsions, kidney and liver damages [2].

The working community in chromium-based industries (leather tanning, electroplating, mining and pigment production, etc.) has a high tendency to be affected by Cr(VI) toxicity. Cr(VI) can produce highly reactive hydroxyl radicals in blood vessels during the reduction into Cr(III), which can cause blood cell damages with organ degradations and cellular activity interruption by metal-DNA bindings [27]. Further it believes that Cr(VI) is responsible for causing teratogenic effects in human as it has proven with animal model trials [28].

Toxicity of Cr(VI) to plants.

Chromium being a non-essential element it does not have a specific mechanism of uptake into plants. It is believed that, the plants use a passive process to uptake Cr(III) and an active process for uptake Cr(VI) with carriers competing with iron, sulphur and phosphorus [29]. Part of the Cr(VI) is taken up into plants after reducing into Cr (III) on the root surface, and the rest of the Cr(VI) is taken up by plants by dissolving in water and without reducing [29,30]. In the toxicological point of view, Cr(VI) affect plants both morphologically and physiologically. It has been found that, high concentrations of Cr(VI) affect the seed germination negatively due to the depressive effects on enzyme activity and sugar transport to embryo axes [31,32], It also reduces the root growth due to inhibition of water absorption [33], and shoot growth due to chromium transportation in aerial parts [31]. Toxicological Studies done else ware using *Oryza sativa, Acacia holosericea, Leucaena leucocephala* and *Albizia lebbek* and *Phaseolus vulgaris* reported that leaf area and biomass can be adversely affected by Cr(VI) [31,34].

Plant physiological studies revealed that Cr(VI) can lead to yield reduction by decreasing chlorophyll a, chlorophyll b and carotenoid pigments and affecting water and mineral transportation due to high oxidative potential [31,35].

Toxicity of Cr(VI) to microorganisms.

Microorganisms are commonly exposed to many pollutants, including toxic metals, as they are widely dispersed in the environment, causing many toxic effects. Cr(VI) can become toxic to most bacterial strains causing cell enlargements, cell elongations and cell division inhibitions [23]. Cr(VI) can rapidly enter into the bacterial cytoplasm and reduces to lower oxidation states which are free radicals such as Cr(V), which leads to genotoxic effects by causing oxidative damages to DNA. Moreover, it has been found 400 – 800 μg of Cr(VI) can directly interact with bacterial DNA causing frameshift mutations and base-pair replacements [36].

The Cr(VI) tolerance limits of bacteria have not clearly been defined as it can depend on several factors, including the type of the strain and physio-chemical conditions of the habitat, nature of the waste etc. Providing evidence to the above assumption a study of reported that 10 – 12 mg/L of Cr(VI) was adequate to inhibit most soil bacteria [22], while some strains in activated sludge can tolerate up to 80 mg/L of Cr(VI) [37]. Further, they have reported that Cr (VI) was able to stimulate bacterial growth up to 25 mg/L of Cr(VI).

Compered to bacteria, fungi are less sensitive to Cr(VI) due to the decreased uptake and production of antioxidants [38,39,39–41]. However, some studies describe that, Cr(VI) can cause genotoxic and mutagenic effects on several strains of fungi, including *Saccharomyces cerevisiae, Sclerotium rolfsii and Pycnoporus sanguineus* leading to complex physiological changes and functional changes such as inhibition of oxygen uptake, induction of petite mutations and inducing mitochondrial functional damages [24,42–44]. Further studies on fungi have shown that effects of Chromium toxicity vary on the nature of carbon substrate [45].

Cr(VI) can affect PS II reaction centers of algae, which leads to inhibition of photosynthesis and cause significant morphological changes in some genera, including *Chlorella, Scenedesmas, Ulva, Isochrysis, Micrasterias,* and *Chlamydomonas* [36,46–51].

Disposal and remediation process of Chromium wastes.

The chromium-based industries i.e., electroplating, tanning, water cooling, textile, wood preservation, alloy manufacturing, dye and pigment production discharge large quantities of contaminated chromium containing waste to soil, air and water annually. Considerable proportions of used chromium as a raw material and / or a reagent for industries including tannery (40%), chrome plating (35%), academic, research and industry laboratories (100%) discharge Cr(III) and Cr(VI) as effluents [52].

These chromium contaminated effluents should be remediated before discharging into the environment, due to toxicity of chromium to the environment and public health. Therefore, rules and regulations have been legislated and implemented by national and international authorized bodies such as WHO, US EPA, and national environmental acts of host countries for industrial wastewater and drinking water.

According to the US EPA and WHO standards maximum permissible level of Cr(VI) in drinking water and industrial wastewater have been legislated to 0.05 mg/L and 0.10 mg/L, respectively. Considering the health hazard to the occupational community in chromium-based industries, Occupational Safety and Health Administration (OSHA) has set the maximum limit for Cr(VI) compounds for 8-hour work shifts and 40-hour workweeks as 0.052 mg/L [23,53].

Based on these regulations, Cr(VI) contaminated wastes should be remediated before being discharged into the environment. Remediation of chromium containing waste can be carried out using three (03) methods i.e. chemical, physical and biological which are summarized in the **Table 2**.

Chemical methods of Cr(VI) remediation.

Chemical reduction and photocatalysis are the most common chemical remediation methods that have been applied in chemical remediation processes. The chemical reduction uses reducing agents such as sulfur dioxide (SO_2), calcium polysulfide (CaS_5), ferrous sulfate ($FeSO_4$), sodium metabisulfite ($NaHSO_3$), sodium sulfite (Na_2SO_3), barium sulfite ($BaSO_3$), hydrazine hydrate (N_2H_4), hydrogen peroxide (H_2O_2) and, calcium carbonate (Na_2CO_3) [19,54–56]. Redox reactions of above mentioned reducing agents are kinetically slow at low Cr(VI) concentrations [57]. Therefore, it may require different methods to remediate residual Cr(VI), which are even higher than the discharge limits. Further, it has been found that this reduction process is also influenced by physical and chemical characteristics of the discharging sites (pH, conductivity, soil type and texture, presence of transition metals) [58,59].

Semiconductor based photocatalysis is a developing technology for toxic metal remediation such as Cr(VI), Hg(II), As(V), Cu(II), and Pb(II) [62]. This technology is more advantageous as there are no requirements for secondary disposal methods. Titania based photocatalysts such as TiO_2 and $La_2Ti_2O_7$ are extensively used for photocatalytic reduction of Cr(VI) in specific values [19,61]. But these Titania based photocatalysts cannot be applied practically to mass-scale commercial reactor systems due to high cost and operational disturbances due to sunlight irradiation and highly acidic conditions [62].

Physical methods of Cr(VI) removal.

Physical remediation is achieved by techniques such as adsorption, electrolysis, ion exchange, membrane filtration and capping [19,63,64]. Adsorption is widely used for chromium removal in wastewater, consisting of significant advantages such as low cost, profitability, availability, high efficiency, and minimum effort operation than other physio-chemical methods. A range of synthetic and natural adsorbents, including activated carbon, zeolite, chitosan, treated wastes, biological materials of coconut shell, wood husk, orange peel, hazelnut shell, sawdust, are used for Cr(VI) removal with a wide range of removal percentages under different pH values which are mostly laid on the extreme acidic range [65–69]. As some of the above adsorbents are freely available in nature, so that, adsorption is considered as one of the cost-effective methods of physical remediation. Membrane filtration technology is implemented with reverse osmosis, which is considered as one of the best available technology for removing all forms of chromium [70–72]. Literature shows that different membrane technology modifications to enhance Cr(VI) removal effectiveness, including micellar enhanced ultrafiltration, polymer inclusion membranes, ion exchange membranes and nanofiltration [73,74]. However, membrane technology is considered as a costly method with generating a large volume of concentrated liquid toxic wastes [72].

The acidic and basic ion exchange resins have also reported as effective Cr(VI) removal methods from chromium contaminated wastewater. A study of [75] have developed a complete Cr (VI) removal process from real wastewater by using a strongly basic synthetic Dowex 2-X4 resin without affecting pH. Further studies of [57] indicate 99.5% of Cr(VI) removal from "synthetic wastewater" using solvent impregnated resins which are acidic.

Biological methods of Cr(VI) removal.

Remediation of chromium contaminated sources using biological agents including bacteria, fungi, algae, and plants play an important role in remediation approaches. Bacteria and fungi have shown efficient remediation agents than other agents. It has shown that organisms that can survive in a contaminated site may have the ability to remediate the contaminated site by themselves up to a certain level by transforming toxic pollutants into nontoxic forms [19,76–81]. This detoxification is achieved through biosorption, bioaccumulation and biotransformation.

Bioremediation is affected by several physio-chemical factors including, energy source (electron donors), electron acceptors, nutrients, pH, temperature and inhibitory substrates or metabolites [82]. Bioremediation of chromium is implemented in both in situ and ex situ depending on the nature and requirements of the contaminated site [83,84].

Biological methods are considered as more advantageous from the economic and environmental point of view as they are cost-effective due to low installation and operational cost, eco-friendly with generating much less secondary pollutants, convenient and straightforward operation compared to physiochemical methods [63,85–87].

Bioremediation of Cr(VI) by bacteria.

Bacterial bioremediation of Cr(VI) is explained in terms of chromium tolerance mechanisms such as biosorption and biotransformation/ bioreduction in both Gram-positive and Gram-negative strains [19]. During the bioreduction, highly toxic Cr(VI) is reduced into lesser toxic Cr (III) inside the bacterial cytoplasm, cell wall, or in both. The bacterial strains that can reduce Cr (VI) are usually named Chromium Reducing Bacteria (CRB). It is believed that Gram-positive CRB have a significant high tolerance to high Cr(VI) concentrations than gram-negative CRB [88].

According to previous studies, bacterial genera such as Pseudomonas, Bacillus, Enterobacter, Deinococcus, Shewanella, Agrobacterium, Escherichia, Thermus, Microbacterium, Desulfovibrio, Deinococcus, Brucella, and Staphylococcus have the potential to reduce Cr(VI) "directly" with enzymes and "indirectly" with metabolic end products [88–92]. It has also been reported that chromium tolerance and reduction are independent properties of bacteria, which means not all Cr(VI) resistant bacteria can reduce Cr(VI) into Cr(III) [88,93].

Bacterial Cr(VI) reduction is achieved under aerobic, anaerobic and both conditions [94]. Aerobic reduction is associated with soluble proteins and NADH as electron donors to enhance the reduction process, while anaerobic Cr(VI) reduction is associated with cell membrane bound reductase (flavin reductase, cytochromase, hydrogenases) and soluble reductase or both [95,96]. Bacterial bioreduction rate of Cr(VI) is influenced by initial cell density/ concentration, initial chromium concentration, initial pH, temperature, electron donors, oxyanions, salt concentration, presence of other heavy metals, metabolic inhibitors and oxidation-reduction potential of culture [96,97]. Further, the bacterial strains in the same species have different Cr(VI) tolerance and removal potentials depending on the level of the contaminants in the environment. This phenomena was evidenced in a comparative study carried out between uncontaminated and Cr(VI) polluted environments [98].

Table 2. Chemical, physical and biological method of Cr(VI) removal

Method	Technique	Mechanism	Type of contaminated source (Tested)	Cr, Cr (VI) removal percentage	Time	pH	Temp. (°C)	Reference
Chemical	Reduction	Cr(VI) reduction and adsorption by ED-RGO.	Synthetic wastewater	100%	24 hrs.	2.0	N/A	[120]
	Reduction	Cr(VI) reduction by Calcium polysulfide (CaS_x).	Contaminated ground water	90%	4 days	8 - 12.5	N/A	[121]
	Reduction and biosorption	Cr(VI) reduction and biosorption by chemically treated brown seaweed (*Ecklonia* sp.).	Synthetic wastewater	100%	12 hrs.	2.0	25	[122]
	Reduction	Cr(VI) removal by chemically and electrochemically.	Synthetic wastewater	99.99%	10 min.	8.5 – 10.0	N/A	[123]
	Reduction	Cr(VI) reduction by Sodium corboxymethyl stabilized nanoscale zero valent iron.	Synthetic waste soil sample	80%	72 hrs.	4.73 – 7.36	N/A	[124]
	Reduction and coagulation	Cr(VI) removal by Ferrous sulfate ($FeSO_4$).	Spiked ground water	95%	46 hrs.	> 7.5	N/A	[125]
Physical	Adsorption	Chromium Removal by fly ash.	Industrial waste	97.86%	12 hrs.	N/A	25	[126]
	Adsorption	Cr(VI) removal by Ragi husk powder.	Synthetic wastewater	81.34%	2 hrs.	1.75	N/A	[127]
	Adsorption	Cr(VI) removal by green algae and activated carbon.	Waste water	99.52%	2 hrs.	1.0	25	[110]
	Adsorption	Cr(VI) removal by treated waste newspaper (TWNP).	Synthetic wastewater	64%	1 hrs.	3.0	25	[65]
	Adsorption	Cr(VI) removal by green coconut shell.	Synthetic wastewater	95%	30 min.	6.5	28	[66]
	Adsorption	Cr(VI) removal by agriculture wastes. Maize corncob. Cane bagasse. Jatropha oil cake.	Synthetic wastewater	62% 92% 97%	1 hrs.	2.0	30	[128]
	Adsorption	Cr(VI) removal by *Mangifera indica* leaves.	Synthetic wastewater	91%	2 hrs.	2.0	30	[129]
	Retention/ filtration	Cr(VI) removal by Aromatic polymide thin film membrane.	Synthetic wastewater	77%	N/A	8.0	25	[73]
	Adsorption	Cr(VI) removal by anion exchange resins.	Synthetic wastewater	99.4%	30 min.	3.0 – 5.0	25 60	[130]
	Adsorption	Cr(VI) removal by hydrophobic resin.	Synthetic wastewater	99.5% 92%	24 hrs.	3.0	25	[131]
	Adsorption	Cr(VI) removal by boiled rice husk.	Synthetic wastewater	71%	3 hrs.	2.0	27	[132]
	Adsorption	Cr(VI) removal by formaldehyde treated rice husk.	Synthetic wastewater	76.5%	3 hrs.	2.0	27	[132]

	Adsorption	Cr(VI) removal by modified montmorillonite clay nanocomposite.	Synthetic wastewater	99.9%	24 hrs.	2.0 - 6.6	25	[133]
	Adsorption	Cr(VI) removal by Fe_2O_3/ graphene adsorbents.	Synthetic wastewater	70.33%	N/A	3 - 4	25	[134]
	Adsorption	Cr(VI) removal by synthesized hydroxyapatite microfibrillated cellulose (CHA/MFC)	Synthetic wastewater	94%	5 min.	7 - 5	25	[135]
	Adsorption	Cr(VI) removal by Magnetite nanoparticles	Synthetic wastewater	66%	2 hrs.	3.0	25	[136]
Physical	Adsorption	Cr(VI) removal by mixed waste tea and coffee ground	Synthetic wastewater	95%	3 hrs.	2.0	50 - 65	[137]
	Adsorption	Cr(VI) removal by natural adsorbents. Wool Olive cake Sawdust Pine needles Almond Coal Cactus	Synthetic wastewater	69.3% 47.1% 53.5% 42.9% 23.5% 23.6% 19.8%	2 hrs.	2.0	30	[138]
	Adsorption	Polypyrrole – montmorillonite clay composite	Synthetic wastewater	100%	24 hrs.	2.0	25	[139]
	Adsorption	Nanocomposite of ZnO with cotton stalks biochar	Synthetic wastewater	96.19%	1 hr.	2-4	25	[140]
	Filtration	Green emulsion liquid membrane	Synthetic wastewater	97-99%	0.5 hrs.	0.45	30	[71]
	Filtration	Green synthesized CuO nanoparticles	Synthetic wastewater	88.08%	2 hrs.	6.9	25	[70]
	Reduction	Cr (VI) bioreduction by effluent bacteria *Staphylococcus cohnii*	Synthetic wastewater	90%	96 hrs.	7.2	37	[141]
	Reduction	Cr (VI) bioreduction by *Pseudomonas umsongensis*	Synthetic wastewater	93.9%	72 hrs.	7.0	30	[142]
	Reduction Adsorption	Cr (VI) bioreduction and biosorption by *Bacillus* sp.	Synthetic wastewater	97.04%	96 hrs.	7.0	37	[143]
Biological	Reduction	Cr (VI) bioreduction by *Aeromonas hydrophila*	Synthetic wastewater	88%	72 hrs.	7.2	30	[144]
	Reduction	Cr(VI) reduction by *Bacillus thuringiensis*	Synthetic wastewater	86.42%	96 hrs.	7.0	35	[91]
	Reduction	Cr(VI) reduction by *Staphylococcus capitis*	Synthetic wastewater	97.34%	96 hrs.	7.0	35	[91]
	Reduction	Cr(VI) reduction by *Bacillus cereus*	Synthetic wastewater	98.5%	72 hrs.	7.1	26	[98]

	Reduction Sorption	Cr(VI) reduction and sorption by *Enterobacter* sp.	Synthetic wastewater	99.1%	25 hrs.	6.0	45	[145]
	Reduction	Cr(VI) reduction by *Morganella morganii*	Synthetic wastewater	92%	48 hrs.	7.0	37	[146]
			Raw tannery effluent	90%	48 hrs.	7.0	37	
	Reduction Sorption	Cr(VI) reduction and sorption by *Stenotrophomonas rhizophila*	Synthetic wastewater	100%	28 hrs.	7.5	30	[147]
	Reduction	Cr(VI) reduction by *Cellulosimicrobium* sp.	Synthetic wastewater	100%	48 hrs.	7.0	30	[148]
	Reduction	Cr(VI) reduction by *Geobacter sulfurreducens*	Synthetic wastewater	99%	2hrs.	N/A	30	[149]
	Reduction	Cr(VI) reduction by *Pseudomonas aeruginosa*	Synthetic wastewater	93%	96 hrs.	7-8	30	[150]
	Sorption	Cr(VI) biosorption by *Shewanella putrefaciens*	Synthetic wastewater	85.68%	17 hrs.	8.0	38.44	[151]
	Reduction	Cr(VI) bioreduction by mixed bacterial consortium.	Synthetic wastewater	100%	120 hrs.	8.0	30	[152]
	Reduction Adsorption	Cr(VI) bioreduction and biodorption by *Corynebacterium paurometabolum*,	Synthetic wastewater	55%	2 hrs.	3.0	30	[153]
	Reduction	Cr(VI) bioreduction by *Cellulosimicrobium funkei*	Synthetic wastewater	80.43%	120 hrs.	7.0	35	[154]
Biological	Reduction	Cr(VI) bioreduction by *Pseudomonas stutzeri*	Synthetic wastewater	97%	24 hrs.	7.0	37	[155]
	Reduction	Cr(VI) bioreduction by *Acinetobacter baumannii*	Synthetic wastewater	99.58%	24 hrs.	8.0	37	[155]
	Reduction	Cr(VI) bioreduction by *Ochrobactrum* sp.	Synthetic wastewater	96.5%	N/A	7.0	30	[156]
	Adsorption	Cr(VI) biosorption by *Trichoderma* sp.	Synthetic wastewater	97.39%	2 hrs.	5.5	25	[103]
	Reduction Adsorption	Cr(VI) biosorption and bioreduction by *Paecilomyces lilacinus*	Synthetic wastewater	100%	120 hrs.	5.5	25	[157]
	Adsorption	Cr(VI) biosorption by *Phanerochaete chrysosporium*	Synthetic wastewater	99.7%	72 hrs.	7.0	40	[158]
	Adsorption	Cr(VI) biosorption by *Pleurotus ostreatus*	Synthetic wastewater	80%	12 hrs.	2.0 – 11.0	65	[159]
	Adsorption	Cr(VI) adsorption by Cationic surfactant-modified, *Kazachstania yasuniensis* *Kodamaea transpacifica* *Saturnispora quitensis* *Saccharomyces cerevisiae*	Synthetic wastewater	80.70% 85.80% 85.40% 75.80%	4 hrs.	4.5	25	[105]

	Adsorption	Cr(VI) adsorption by a hydroxyl-functionalized magnetic *Aspergillus niger* nanocomposite	Synthetic wastewater	64.91%	4 hrs.	5.0	50	[160]
Biological	Adsorption	Cr (VI) adsorption by *Chlorella sorokiniana.*	Synthetic waste water	99.68%	72 hrs.	7.0	40	[161]
	Reduction Adsorption	Cr (VI) removal by green algal strain *Cladophora albida*	Synthetic waste water industrial waste water	100%	120 hrs.	0.5	25	[162]
	Adsorption	Cr(VI) removal by marine algae *Turbinaria ornata*	Synthetic wastewater	95.25%	3.5 hrs.	4.7	33.6	[163]

N/A – Not applicable

Table 3. Microorganisms and substrates used in biofilm formation for bioremediation of Cr(VI)

Organism	Adhesion substrate	Cr (VI) removal percentage (%)	pH	Temp. (°C)	Time	Reference
Arthrobacter viscosus	Granular activated carbon	99.9%	5 – 5.5	28	30 days	[190]
Pseudomonas sp. *Bacillus* sp. *Azotobacter* sp. *Acremonium* sp.	Glass wool	90%	5.6-6.1	30	10 days	[172]
Streptomyces strain CG252	Glass bead	100%	N/A	30	48 – 72 hrs.	[191]
Arthrobacter sp.	Gravel packed bed reactors	100%	N/A	30	26 hrs.	[192]
Morganella morganii STB5	Polystyrene Polysulfone	99.47% 90.78%	7.0	30	72 hrs.	[193]
Arthrobacter sp. SUK 1205	Glass beads	100%	7.0	37	96 hrs.	[194]
Halomonas sp.	Pumic particle stones	94.5%	6.5	28	48 hrs.	[195]
Bacillus subtilis *Escherichia coil* *Acinetobacter junii*	Alginate bead	97.84%	7.0	25	7 hrs.	[196]
Wickerhamomyces anomalus	Wood husk	92.5%	3.72	30	N/A	[171]
Acinetobacter haemolyticus	Wood husk	97%	7.0	25	72 hrs.	[69]
Streptococcus salivarius	Stainless steel AISI 316L	42%	N/A	37	72 hrs.	[197]
Pseudomonas fluorescens LB 300	Glass beads	100%	6.8-7.0	30	8 days	[198]
Cellulosimicrobium sp.	PVC Rubber tubing Sand Small stone	99.5% 90.0% 96% 88.4%	N/A	25	11 days	[199]
Escherichia coli	Kaolin	100%	4.6-5.1	37	10 days	[200]
Nostoc sp.	Polystyrene	86.49%	7.0	25	7 days	[201]
Shewanella xiamenensis	Zeolite	100%	3.0	22-25	35 days	[202]
Cunninghamella elegans	Stainless steel compression springs	98.6%	7.0-3.0	28	40 hrs.	[203]
Arthrobacter sp. SUK 1201	Glass beads	100%	7.0	37	3 days	[204]
Lysinibacillus sphaericus RTA-01	Glass slide	82.8%	5.2	37	72 hrs.	[205]
Ochrobactrum pseudintermedium ADV31	Polyurethane foam	82%	7.0	45	5 days	[206]

N/A – Not applicable

Bioremediation of Cr(VI) by fungi.

Similar to the bacterial remediation of toxic metals, some fungal strains have been investigated for bioremediation with the same metal removal techniques used with bacteria, i.e. biosorption, bioaccumulation and biotransformation/ bioreduction.

Fungi *Aspergillus* sp. was reported to remove chromium through bioreduction from contaminated effluents [99]. This study also indicates 65% of chromium removal from tannery effluent and 85% of Cr(VI) removal from the synthetic medium at pH 6 within 07 days. The similar bioreduction of Cr(VI) has been also reported by [19,100–102] with *Hypocrea tawa, Trichoderma inhamatum* and isolated Yeast strains. However, the Cr(VI) reduction capability of fungal strains can be changed with the initial Cr(VI) concentration and initial biomass of the strain.

Fusarium oxysporum and *Trichoderma* sp. have shown to adsorb Cr(VI) on to their cell surface by forming chemical bonds with cell surface proteins with analytically verified evidence using FT-IR spectrum [19,103]. Comparison of Cr(VI) removal in synthetic and raw wastes was found to be 77% and 85% of Cr(VI) removal respectively, with 200-1000 mg/L of initial Cr(VI) concentrations using immobilized Baker's yeast strain (*Saccharomyces cerevisiae)* in Biomass/Polymer Matrices Beads (BPMM) through biosorption [104]. A study comparing Cr(VI) biosorption by native Ecuadorian yeast species, reported that *Kazachstania yasuniensis, Kodamaea transpacifica,* and *Saturnispora quitensis* have the ability to remove Cr(VI). Furthermore, they have reported that efficient Cr(VI) removal can be achieved by inducing belzalkonium chloride (BZK) to cell surface as a chemical modification to the applying bio agent [105].

Bioremediation of Cr(VI) by algae.

It is evident that both freshwater and marine algal species such as Cladophora sp., Selenastrum sp., Spirogyra sp., Ceramium sp, Chlorella sp. and Ulva sp. can be used to remediate chromium contaminated wastewater by applying as cultures or incorporating with other physio-chemical methods following biosorption and bioreduction [19,106–109]. Unlike other organisms, algae have been used in both living and non-living forms for Cr(VI) remediation. Introducing an efficient Cr(VI) removal method [110] reports that dried Ulva lactuca incorporated into activated carbon can be used to remediate highly acidic and halophilic wastewater.

Chlorella sp. has been used in most Cr(VI) algal remediation bioreactors as it is widely dispersed in the aquatic environment with higher Cr(VI) tolerance [109,111–114]. Constructing a hybrid remediation system [115] has introduced efficient and reusable alumina hollow fibers immobilized with TiO2 and *Chlorella vulgaris* cells. Additionally, this hybrid system has been achieved greater than 90% of Cr(VI) removal after five sequential reuses.

However, similar to bacterial bioremediation, algal Cr(VI) bioremediation is influenced by physio-chemical parameters including pH, temperature, initial biomass, initial Cr(VI) concentration, light intensity, contact time of cells and wastes of the treatment process bioremediation [19,116].

Bioremediation of Cr(VI) by plants.

Limited studies have reported that the Cr(VI) detoxification and removal potential of plants compared to the other biological agents. Green plants detoxify many pollutants using various mechanisms followed by uptake, known as phytoremediation [117]. Plants can either store heavy metals in roots or partially translocate to shoot through the xylem after getting diffused into the root system. Further, it has been reported that upward translocation of heavy metals is retarded by the cation exchange process in plant tissues and leads to considerable heavy metal accumulation in roots compared to axial parts of plants. In the point of view of Cr(VI) and other chromium forms, this phenomenon has been reported else ware using *Phragmitus australis*, *Ailantus altissima* and *Salix viminalis* [118]. This may be due to the encapsulation in vacuoles of root cells based on natural counteraction of plants against chromium toxicity [119]. According to observations over 360 days, a study suggests that *Salix viminalis* used for large scale phytoremediation application for removal of Cr(VI) and other chromium forms from contaminated sources as *S.viminalis* were removed 70% of total chromium and 90% of Cr(VI) removal with indicating higher translocation capacity [118].

In vitro study of *Nopalea cochenillifera* found that it has the potential to accumulate a wide range of Cr(VI) (600 – 26,000 mg/ Kg) from the growth medium. As *N. cochenillifera* is a non-consuming plant for diets, the risk of bioaccumulation can be avoided in the ecosystem. Furthermore, the above study has also reported plant species with different chromium accumulation potentials including *Gynura pseudochina*, *Brassica napus*, *Prospis juliflora*, *Leersia hexandra*, *Urtica dioica*, *Salix matsudana, Brassica napus, Helianthus annuus, Lycopersicon lycopersicum* and *Saponaria officinalis* perhaps considered for the phytoremediation [117].

Biofilms

An aggregated community of prokaryotic and eukaryotic microorganisms adhering to substance/matrix surface and submerged/embedded in self-produced extracellular polymeric substances (EPS) is termed a "biofilm" [164,165]. These aggregates are omnipresent in the biosphere, including soil, water, plant and animal tissues, abiotic substances like pipelines, ship hulls and filters. These biofilms can be developed in solid–water, water–air and solid–air interfaces with composing EPS, multivalent cations, biogenic particles, colloidal and dissolved compounds [166]. Biofilms are comprised of both single and multiple microbial species. Among them, multiple species biofilms are the most dispersed biofilm type in the environment [167].

Biofilm formation is a subsequent process that consists of 03 main steps; surface attachment, biofilm maturation and dispersal [165]. In surface attachment, microbial cells undergo reversible attachment at cell poles by involving cell appendages (flagella, pilli and fimbriae) followed by irreversible attachment. After the reversible attachment stage, microbial cells can be adapted to biofilm lifestyle or left the matrix. During the irreversible attachment, stage cells adhere to the matrix by EPS and surface proteins (Sad B and Lap A).

Biofilm maturation starts after the irreversible attachment with developing microcolonies. At this stage, previous microbial cells are assembled and proliferated along with producing EPS. Further studies explain that biofilm structure, including thickness and cell density, is dynamically changed according to environmental conditions such as temperature, presence of oxygen, pH and amounts of nutrients [164,165].

The immobilized microbial cells are transferred back to planktonic growth as the final stage of the biofilm lifecycle and as an initial step of a naval forming biofilm. This dispersal can be happened "actively" by cell motility and EPS degradation or "passively" by external physical forces.

Environmental applications of biofilms

Even though free-living microorganisms are capable of bioremediating polluted environments, the remediation process can be disrupted due to high concentrated toxic compounds, availability of nutrients and environmental stress. Applying sessile or floating biofilms for remediation is highly advantageous as biofilm communities have higher tolerance towards environmental stress, including lack of nutrient availability, high concentrated chemical exposures, pH and temperature fluctuations, lack of moisture content than free-living microorganisms [168].

When selecting biofilms for remediation purposes, several factors must be considered: the capability to tolerate environmental stress, exchange of genetic materials, growth rates, metabolic diversity, and symbiotic relationships. Based on above factors bacterial, algal and fungal biofilms are used in bioreactors to remediate contaminated sources by a wide range of pollutants including, organic pollutants (polyaromatic hydrocarbons, chlorinated aromatic compounds, aromatic amine compounds, polyethylene and polythene), heavy metals (Cu, Zn, Cd, Ni, As, Fe, Hg, Mn), inorganic pollutants (nitrate ions and synthetic dyes) contaminates which can adversely affect the eco-systems [168–170].

Cr(VI) remediation by biofilms

Cr(VI) bioremediation is achieved using bacterial, algal and fungal single species and multi-species biofilms growing on either natural or artificial substrates through bioremediation and biosorption techniques [171–174].

Biofilms are more effective for Cr(VI) remediation than planktonic cells. The study investigating *Streptomyces* sp. strain CG252 and *Pseudomonas aeruginosa* A2Chr, respectively, reported evidence for the above phenomena [102,175]. Another study using three (03) different biosorbents including lyophilized *Escherichia coli* AUS 7 cells, granulated activated carbon (GAC) and biofilm of *Escherichia coli* AUS 7 on GAC exhibited that biofilms are able to achieve a higher adsorption of Cr(VI) than GAC and lyophilized cells in according to Langmuir and Freundlich isotherm models from aqueous solutions under acidic conditions [176]. However, contradictory results were also reported else ware, that the planktonic cells have more significant potential for Cr(VI) reduction than biofilms based on their study of *Bacillus subtilis* ATCC-6633. Furthermore above study revealed that, biofilm debris are susceptible to immobilized reduced Cr(III) ions completely [177].

Compared to bacteria, reports on Cr(VI) remediation by fungal biofilms are scarce in the literature. Immobilized cells of *Aspergillus niger, Coriolus versicolor, Saccharomyces cerevisiae,* and *Lentinus sajorcaju* have been used in sorption and reduction techniques [99,178–180]. Moreover, immobilized algal cells on different matrixes have been used for Cr(VI) remediation by sorption of metal ions to the cell wall components [181–185].

Algal-bacterial biofilms/consortia have also been used for Cr(VI) bioremediation. Further, it is reported that these consortia have symbiotic effects on each other by supplying each other's nutritional needs such as O_2 for aerobic bacteria by algae and CO_2 for algae by bacteria during the remediation process. Therefore, algal-bacterial consortia are termed as a self-sustaining system [186–188]. The algal-bacterial system has the potential to remove higher Cr(VI) contents such as 100 mg/L, 75 mg/L and 50 mg/L providing a carbon source to the mixed consortium of chromium reducing bacterial (CRB) cultures of *Escherichia coli*, *Bacillus thermoamylovorans* and *Citrobacter sedakii* from the algal strain of *Chlamdomonas reinhartii*. Furthermore, the above study suggests that the algal-biofilm consortia as a cost-effective method that prevents cost of carbon sources as it fulfils by algae even though algal-bacterial consortia takes a longer time duration for the Cr(VI) removal [189]. **Table** 3 illustrates some of the microorganisms and substrates used in bioremediation of Cr(VI).

Limitations and remedial actions of the current bioremediation methods in Cr(VI) removal

The notable limitations of biological methods available for Cr(VI) removal have been identified including, varying Cr(VI) tolerance and removal levels environmental conditions and nutritional requirements of biological components used, toxic substances present in wastewater which can interfere with the biological components, disposal of the accumulated Cr in the biological component, and practical difficulties in extrapolating bench/pilot-scale to full-scale field application [207–209]. Customized solutions need to be sought by assessing the remediation requirements at individual level, because the environmental conditions and the nutritional requirements of the biological component vary depending on the contaminated site. Moreover, to overcome the Cr disposal after bioremediation, it is possible to percolate the biotransformed Cr(III) through reduction at low pH conditions and tend to be reoxidised to Cr(VI) in the presence of manganese oxide and chlorine in treated effluent or discharging environment [210,211]. In order to prevent the discharge of higher amounts of chromium and to enhance the sorption capacity of biomasses, the metal desorption process should be followed. This desorption can be done by acid digestions [212–215] and alkaline treatments [216–220] as a hybrid Cr(VI) remediation process, which has many benefits such as reduction of generation of secondary pollutants and recovery of valuable metals. These recovered Cr(VI) and Cr(III) can be applied for tannery and chromium-based chemical production as raw materials [221].

Conclusion

The wide industrial and research application of Chromium followed by emitting considerable amounts of Cr(VI), coupled with the fact that it leads to serious problems to all components of the ecosystem. Therefore, it has been legislated to remediate Cr(VI) contaminated effluents by national and international authorities before it being discharged to the environment. This remediation is carried out by chemical, physical and biological methods. Biological remediation is considered as the most environment-friendly and cost-effective method rather than chemical and physical remediation. However, considering the limitations of the current bioremediation processes, hybrid remediation processes combining the bioremediation with other chemical and physical methods are being used for the effective remediation of Cr(VI) in aquatic systems.

Authors Contribution

The authors confirm contribution to the paper as follows: study conception and design: A.M.K.C.B. Aththanayake, I.V.N. Rathnayake, and M.P. Deeyamulla; draft manuscript preparation: A.M.K.C.M. Aththanayake; Review, and editing the final draft: A.M.K.C.B. Aththanayake, I.V.N. Rathnayake; All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no any competing interest.

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