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Microbial Mechanisms for Remediation of Hexavalent Chromium and their Large-Scale Applications; Current Research and Future Directions

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Abstract

The increase of anthropogenic activities has led to the pollution of the environment by heavy metals, including chromium (Cr). There are two common oxidative states of Cr that can be found in industrial effluents the trivalent chromium Cr(III) and the hexavalent chromium Cr(VI). While the hexavalent chromium Cr(VI) is highly toxic and can trigger serious human health issues, its reduced form, the trivalent chromium Cr(III), is less toxic and insoluble. Leather tanning is an important industry in many developing countries and serves as a major source of Cr(VI) contamination. Globally, tannery factories generate approximately 40 million m^3 of Cr-containing wastewater annually. While the physico-chemical treatments of tannery wastewater are not safe, produce toxic chemicals and require large amounts of chemical inputs, bioremediation using chromium-resistant bacteria (CRB) is safer, efficient and does not produce toxic intermediates. Chromium-resistant bacteria (CRB) utilise three mechanisms for Cr(VI) removal: biotransformation, biosorption and bioaccumulation. This review will evaluate the three Cr(VI) detoxification mechanisms used by bacteria, their limitations and assess their applications for large-scale remediation of Cr(VI). This can be helpful for understanding the nature of Cr(VI) remediation mechanisms used by bacteria, therefore, bridging the gap between laboratory findings and industrial application of microorganisms for Cr(VI) removal.

Keywords: Bioremediation, Chromium-Reducing Bacteria, Contamination, Tannery Effluents, Toxicity

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INTRODUCTION

The unprecedented growth of human populations, coupled with the increase of industrial activities, has led to the pollution of the environment by different organic and inorganic substances. Chromium (Cr) is a metal abundant in natural environments and in toxic concentrations in effluents generated from industrial activities, including in the fabricated metal industry, leather tanning, dying, coal combustion, oil combustion, the metal mining industry and so forth[1]. Notably, for the last two decades, Cr has been classified as one of the top 20 highly toxic metallic elements on the superfund priority list of hazardous substances[2]. In a report released in 2015, by the Blacksmith Institute (Pure Earth), Cr was considered as one of the top six toxic contaminants and was estimated to impact 16 million people and cause 3 million disability-adjusted life years (DALYs)[3]. Understanding the nature of Cr is, therefore, critical to developing strategies to ensure removal of this metal from the environment.

In the environment, there are two stable forms of Cr: the trivalent chromium Cr(III) and the hexavalent chromium Cr(VI). Cr(VI) has a high level of toxicity and can trigger serious human health issues, including cancers, liver damage and pulmonary congestion illness[4-7]. Meanwhile, Cr(III) is relatively less toxic and can be removed from wastewater[8]. The devastating effect of Cr(VI) results from the fact that it can use sulphate transport channels to enter into living cells[9]. Within the cells, the reduction of Cr(VI) to Cr(III) leads to the formation of reactive oxygen species (ROS), which can damage living organisms through interacting with nucleic acids and proteins[10,11].

In the conventional leather tanning industry, crude animal skin is treated to create various leather products and is a major source of Cr(VI) pollution. Skin treatment involves four main stages, including pre-tanning, chrome tanning, post-tanning and finishing. During these processes, Cr(III) is oxidised into Cr(VI), which is then discharged into the environment alongside other metals. As reviewed by Dabai & Mohammed, on a global scale tanning factories generate approximately 40 million m^3 of Cr-containing wastewater annually[12]. Leather tanning is an important industry in many developing countries, including India. In India, the massive leather tanning industry annually leaks between 2000 and 3000 tonnes of Cr into the surroundings, causing an estimated final concentration of 2000 to 5000 mg/L Cr in the environment[13]. A single operating tannery factory can create groundwater pollution in a 7 to 8 km radius[14]. Therefore, the removal of Cr is paramount to protect human health as well as to prevent the long-term irreversible damage that can occur to the environment.

The most common way of treating tannery wastewater is conventional methods (or physicochemical treatment), which include electrochemical treatment, reverse osmosis and ion exchange. Nevertheless, the use of such methods is usually associated with a high energy input and generates toxic by-products that cause secondary pollution[15,16]. Therefore, there is a dire need for an alternative, cost-effective and environmentally friendly approach to remove Cr(VI) contamination.

Bioremediation is a microbially driven approach which uses microorganisms to remove toxic pollutants from the environment. Certain microorganisms, such as bacteria, fungi, archaea and algae, are capable of tolerating and reducing Cr(VI), hence remediating Cr contamination[17,18]. This review will primarily focus on the use of bacteria to detoxify Cr(VI) contamination. The mechanism used to remove Cr(VI) can vary on the basis of biogeochemical conditions, means of nutrient utilisation by bacteria and the presence/absence of oxygen in the environment. Understanding these mechanisms used by bacteria to reduce the toxicity of Cr(VI) will enhance future applications of microbial communities to remove Cr contamination from an environment. This review will evaluate the three Cr(VI) detoxification mechanisms used by bacteria, their limitations and assess their applications for large-scale remediation of Cr(VI).

Microbial Remediation of Hexavalent Chromium

Bioremediation of Cr(VI) using bacteria is a promising approach owing to the fact that it is safe, efficient and does not produce toxic intermediates. In numerous research studies, bacteria have shown the potential to detoxify Cr(VI) by three different mechanisms, including biotransformation, biosorption and bioaccumulation[19-21]. Biotransformation involves

direct and indirect reduction of toxic Cr(VI) to Cr(III)[9]. The transformation process principally relies on the availability of oxygen and an appropriate electron donor[9]. Biosorption is a passive physico-chemical process between Cr(VI) and bacteria in which both dead and living cells can participate. Nevertheless, the dead cells are proven to be more effective[20]. Bioaccumulation is active uptake of Cr(VI) by live bacteria that depends on the concentration of the metal and the time of contact with the microbe[22]. CRB using any of the three mechanisms can be a potential biological agent for large-scale microbial remediation of Cr(VI). A large number of bacteria were isolated and tested for their ability for Cr(VI) bioremediation (Table 1). In the following subsections, the three major bacterial mechanisms for Cr(VI) detoxification are scrutinised, and their use in large-scale remediation in bioreactors is critically evaluated.

Biotransformation

In the biotransformation approach, the highly toxic hexavalent Cr(VI) is chemically transformed to the less toxic and more stable form of trivalent Cr(III) via the reduction reaction of Cr-reducing bacteria (CRB) using a gene called *chrR*. The reduced Cr(VI) is then expelled from the cells by a mechanism called "efflux pumps" by the actions of the *chrA* gene[23]. Bacterial transformation of Cr(VI) can occur either directly through an enzymatic reduction reaction or indirectly by the metabolic end products of other microbes, such as Fe(II) and H_2S of sulfate-reducing bacteria (SRB) and iron-reducing bacteria (IRB)[24,25]. The efficiency of Cr(VI) reduction differs on the basis of the carbon source available to the bacteria[26].

Under aerobic conditions, some CRB have shown a unique ability to detoxify Cr(VI), using industrial waste as a carbon source. A study demonstrated that a locally isolated CR-resistant bacterium known as *Acinetobacter haemolyticus* was able to resist 100 mg/L of Cr(VI) and reduce more than 90% of Cr(VI) as it grew on sugarcane bagasse waste, compared to Luria Bertan medium, which showed only 25% reduction[27]. Bacterial species have different preferences for carbon sources. Although glucose can promote the growth of many species, it can also limit the Cr(VI) reduction ability of some CRB. In a study that tested the detoxification ability of *Pannonibacter phragmitetus* LSSE-09 using different carbon sources noted the high growth rate of the species on glucose; however, limited reduction was observed compared to with using acetate as the carbon source[28]. On the contrary, glucose can enhance Cr(VI) reduction for other species. Evidently, the addition of 1% of glucose to a culture of *Bacillus* sp. (strain XW4) increased the reduction of Cr(VI) significantly[29]. Although the *Bacillus* sp. showed a complete reduction under a concentration of 40 mg/L CR(VI), it was not able to reduce one of 100 mg/L of Cr(VI)[29]. Apparently, 100 mg/L of Cr(VI) was high enough to halt the metabolic activity of the bacterium leading to the failure of the reduction pathway.

Under anaerobic conditions, Cr(VI) is used as terminal electron acceptor[30]. In an experiment that used *Burkholderia cepacia* MCMB-821 to detoxify Cr(VI) found that the addition of 2% of lactose facilitated the reduction of 75 mg/L of Cr(VI) by 98%[31]. Meanwhile, other electron donors such as ethanol, methanol and sodium acetate decreased the transformation ability of the species[31]. In addition, anaerobic species such as sulphate- and iron-reducing bacteria (SRB and IRB) are significant contributors to the reduction of Cr(VI). The combination of IRB and SRB is estimated to provide reduction that is roughly 100 times faster than when the CRB are the only species used for bioremediation[24,32]. Nevertheless, the activities of many important hydrogenases enzymes required for the reduction were inhibited under high concentrations of Cr(VI)[24]. Altogether, the efficiency of the biotransformation approach is based on the availability of an appropriate electron donor and is significantly affected under high concentration of Cr(VI) in the environment.

Biosorption

The removal of Cr(VI) through biosorption mechanism is based on a passive physico-chemical process between the heavy metal species and the bacteria in which microorganisms can trap Cr(VI) and make it immobile and unavailable for biological uptake[33]. In the biosorption mechanism, no energy is needed for metal uptake. Furthermore, the uptake of Cr(VI) continues until it reaches equilibrium between absorbed ions and the ions in the solution[30,33].

The ability of dry microbial biomass to take up Cr(VI) from the environment is more

efficient in dead than in living cells. Evidently, a comparative analysis of uptake of Cr(VI) ions in dead and living cells of *Bacillus sphaericus* in a controlled environment found that metabolically inactive cells were 13 to 20% better than living cells at pH 2.5 at absorbing Cr(VI)[20]. In this study, *B. sphaericus* OT4b31 showed a 44.5% uptake of 30 mg/L of Cr(VI), compared to a 25% uptake for living cells, and *B. sphaericus* IV(4)10 showed 32% and 45% for living and dead biomass, respectively[20]. In addition to this, using dead microbial biomass to take up Cr(VI) is the most effective approach to overcome the pH barrier. In a study that used dried biomass of *Bacillus thuringiensis* under 250 mg/L of Cr(VI) and at pH 2 revealed that *B. thuringiensis* was able to absorb 24.1% of Cr(VI)[34].

Nevertheless, the use of the biosorption approach might not be an effective solution when Cr(VI) is not the only metal in the effluents. Alongside with Cr(VI), industrial effluents contain large amounts of cadmium, or Cd(II)[35]. The presence of Cd(II) in the environment can reduce the biosorption of Cr(VI), as Cd(II) is preferred by some types of microbial biomass. In a binary system experiment in which *Staphylococcus xylosus* and *Pseudomonas* sp. were grown on a medium enriched with Cr(VI) and Cd(II) demonstrated a profound selectivity for Cd(II) ions against Cr(VI) ions[36]. Therefore, biosorption is an effective approach when Cr(VI) is the dominant metal in the effluents. This can be problematic for *in situ* applications, as the biosorption might shift towards another metal.

Bioaccumulation

Bioaccumulation of Cr(VI) from the environment is a metabolism-dependent mechanism that requires energy to be spent for the transportation of Cr(VI) reversibly across the membrane. Therefore, only living microbial cells can be used to take up Cr(VI) from an environment. As the chemical structure of Cr(VI) ions resembles that of other ions such as tetrahedral sulphate ($SO4^{2-}$) (Fig. 1), Cr(VI) ions move across the bacterial membrane by utilising the $SO4^{2-}$ transport pathways[9].

Several studies documented the use of microbial communities to take up Cr(VI) from an environment through the bioaccumulation approach. Certain bacterial species have a distinct surface protein referred to S-layer that can entrap metallic ions on the cell membrane[20]. In a lab-scale study, two bacterial species – *B. sphaericus* OT4b31 and *B. spaericus* IV(4)10 – were able to accumulate 25 to 32 mg/L Cr(VI)[20]. Bacteria can show a different ability in accumulating Cr(VI) ions. In an experiment that used indigenous microbial strains to remove Cr(VI) from the environment showed that *Klebsiella pneumoniae* MB361 was able to accumulate an 83.51% Cr(VI) concentration between 500 and 1000 ug/ml under a neutral pH condition[37]. In another study, two species of bacteria isolated from tannery effluents which contain 0.96 mg/L of Cr(VI), – *Bacillus megaterium* (strain A) and *Bacillus coagulans* (181) – showed remarkable capacity, accumulating 15.7 and 23.8 mg Cr/g of the microbial dry weight, respectively, in just 24 hr[21]. Some metabolically active microbial cells can tolerate a high concentration of Cr(VI) when it is incubated with 5% of industrial effluents. B. cereus strain IST105 isolated from electroplating effluent demonstrated >75% removal of Cr(VI) within a five-day period when living cells were used[38].

Nonetheless, the physiological activity of many metabolically active microbial cells can be negatively impacted at high concentrations of the

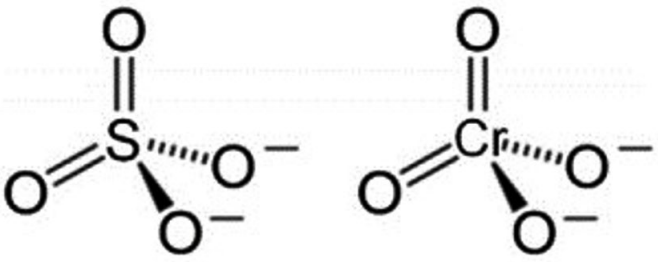

Fig. 1. Structural similarity of chromate and sulfate ions; adapted from Thatoi et al.[9]

pollutant as well as at low pH levels. In a study that used living cells of *Acinetobacter junii* VITSUKMW2 under pH levels between 5 and 11 to remove Cr(VI) reported that the growth and the reduction ability of the bacterium were significantly reduced when the pH was adjusted to 5 and reached a peak at a pH of 9[39]. Furthermore, as the bioaccumulation mechanism is energy-dependent, under high concentrations of the pollutant, the metabolic activity can be significantly affected[29]. Therefore,

Table 1. List of bacteria and their mechanisms for Cr(VI) detoxification

Name of the bacteria	Bioremediation mechanism	Reference
Bacillus sphaericus OT4b31	Biosorption/Bioaccumulation	20
Bacillus sphaericus IV(4)10	Biosorption/Bioaccumulation	20
Bacillsu cereus IST105	Biosorption	38
Pseudomonas aeruginosa	Biosorption	40
Bacillus subtilis	Biosorption	40
Acinetobacter haemolyticus	Biotransformation	27
Enterococcus casseliflavus	Biosorption	41
Corynebacterium paurometabolum	Biosorption/Biotransformation	42
Bacillus megaterium	Biosorption/Bioaccumulation	21
Bacillus coagulans	Biosorption/Bioaccumulation	21
Pseudomonas gessardii LZ-E	Biotransformation	19
Shewanella putrefaciens	Biotransformation	43
Thiobacillus ferrooxidans DSM 11477	Biotransformation	44
Methanothermobacter thermautotrophicus	Biotransformation	45
Staphylococcus xylosus	Biosorption	36
Bacillus sp. MGG-83	Biosorption	46
Bacillus amyloliquifaciens	Biotransformation	47
Microbacterium spp.	Biotransformation	48
Staphylococcus aureus K1	Biotransformation	49
Stenotrophomonas maltophilia	Biotransformation	50
Escherichia coli FACU	Biotransformation	51
Acidiphilium cryptum JF-5	Biotransformation	52
Cellulosimicrobium funkei strain AR6	Biosorption/Bioaccumulation	53
Mesorhizobium amorphae	Biosorption	54
Arthrobacter rhombi	Biotransformation	55
Acinetobacter baumannii L2	Biosorption/Biotransformation	56
Pseudomonas stutzeri L1	Biosorption/Biotransformation	56
Leucobacter sp. G161	Biotransformation	57
Bacillus amyloliquefaciens	Biotransformation	58
Stenotrophomonas sp.	Biotransformation	59
Bacillus endophyticus	Biotransformation	60
Virgibacillus sp.	Biotransformation	61
Pediococcus acidilactici	Biotransformation	62
Providencia sp. UTDM314	Biotransformation	63
Serratia sp.	Biotransformation	64
Acinetobacter junii	Biosorption	65
Pseudomonas aeruginosa A2Chr	Biotransformation	66
Burkholderia cepacian MCMB-821	Biotransformation	31
Desulfovibrio vulgaris Hildenborough	Biotransformation	24
Tenotrophomonas sp. MB339	Bioaccumulation	37
Klebsiella pneumoniae MB361	Bioaccumulation	37
Staphylococcus sp. MB371	Bioaccumulation	37
Pannonibacter phragmitetus LSSE-09	Biotransformation	28
Bacillus thuringiensis	Biosorption	34

the low level of pH as well as the high concentration of pollutants in the environment can lead to the failure of the bioaccumulation approach.

Advantages and Limitations of Cr(VI) Remediation Using Microbes

Bioremediation using bacteria offers a cost-effective, efficient and sustainable approach to clean the environment from wastewater containing Cr(VI) through biotransformation, biosorption and bioaccumulation mechanisms.

The biotransformation approach is the most-utilised method for bioremediation of Cr(VI). Given that the reaction in the biotransformation mechanism is based on the carbon source available for microbes, it needs to be chosen carefully according to the preference of the inoculum[26]. There are many advantages for using the biotransformation approach, such as the reusability of the reduced Cr(VI) as well as maintaining the viability of the inoculum for future applications. Another benefit is that it allows the use of microbial consortia to catalyse the reaction[9,24]. A microbial consortium of CRB alongside IRB and SRB is a powerful tool to ensure not only faster and complete but also sustainable technology for future application[9]. However, there are disadvantages that need to be addressed, including the loss of cell viability under high concentrations of Cr(VI) (Table 2)[24]. The reduction pathway is also energy-dependent, and poor selection of an electron donor can limit the biotransformation ability of the bacteria[28,29,31].

When the cost of providing nutrient media is a feasibility issue, the biosorption approach is a preferred alternative remediation approach. In particular, using dead microbial biomass to adsorb Cr(VI) from contaminated matrices is an effective way that does not require energy and thus reduces the cost of Cr(VI) removal, as dead cells are immune to low pH levels and allow the recovery of the metal from microbial biomass[33]. One disadvantage is that applied biomass cannot be used for future applications because it is generally converted to powder for Cr(VI) recovery[20]. Given that the behaviour of microbial biomass under low pH values can vary, so does its ability to eliminate Cr(VI) in an acidic environment. To ensure Cr(VI) removal in the biosorption mechanism, metabolically inactive biomass is used under low pH values between 2.0 and 5.0, depending on the physico-chemical characteristic of the biomass used[20,34]. However, the presence of multiple metal ions in wastewater can pose a major challenge, as some ions show more affinity to microbial biomass than others[36].

The bioaccumulation mechanism relies only on using active microbial biomass. Compared to the biotransformation and the biosorption approaches, microbial accumulation of metals received less attention, owing to the fact that it is

Table 2. Comparison of the three Cr(VI) removal mechanisms

Feature	Biotransformation	Biosorption	Bioaccumulation
Definition	The direct or indirect reduction of Cr(VI) to Cr(III)	The passive uptake of Cr(VI) ions by the biomass until reaching equilibrium	The active accumulation of the Cr(VI) ions by living cells
Reduction efficiency	High efficiency	High efficiency	Low efficiency
Nature of cells	Only living cells	Both living and dead cells	Only living cells
Biomass reusability	Multiple times	Single time	Single time
Cr(VI) recovery	Recovered as Cr(III)	Recovered as dry microbial biomass, which is then converted to powder	Recovered as dry microbial biomass, which is then converted to powder
Cr(VI) uptake	-	Rapid accumulation	Slower than biosorption
Energy requirement	Spend energy	No energy is needed	Spend energy
pH requirement	Vary based on the biomass used	Better Cr(VI) removal under low pH	Mostly neutral pH
Major limitations	High concentration of Cr(VI) and poor selection of carbon source can limit the reduction pathway	Biosorption is a pH dependent approach and can shift towards other metals when Cr(VI) is not the dominant pollutant	High concentration of Cr(VI) and low level of pH can inhibit the accumulation of Cr(VI)

a slower process than the biosorption mechanism and can be interrupted under low pH levels. In the biosorption mechanism, a low pH value is needed to compensate the lack of protons important for ion exchange. This cannot be done efficiently in living cells for bioaccumulation[20]. Although the use of metabolically active microbes can present a significant advantage over the use of dead cells by performing different metabolic activities, including formation of an extracellular complex, precipitation and transport, using living cells can complicate the recovery of Cr(VI), which poses a subsequent challenge, particularly if they were precipitated or compartmentalized inside the cells[67]. A major limitation of the bioaccumulation approach is that cell growth can be inhibited as Cr(VI) accumulates to a toxic level in a cell[25,68].

In summary, approaches for Cr(VI) bioremediation are complicated mechanisms and can be affected by many factors, including Cr(VI) concentration in the effluents, surface charge, the physico-chemical properties of industrial waste (such as pH), the interaction between biomass and metal ions and the interaction within metal ions. In the use of dead microbial biomass, the pH barrier does not constitute a problem. However, to maximise the Cr(VI) reduction in using living biomass, the pH level must be adjusted to the optimum level for boosting the physiological activity of the employed microbe. Bioreactor systems that utilise different Cr detoxification mechanisms need detailed characterization of waste and inocula for maximum efficiency of Cr removal.

Large-scale Cr(VI) Bioremediation Using Bioreactors

For large-scale removal of Cr(VI), bioreactors such as fed-batch or continuous reactors are used in which CRB are exploited as biological agents. Bioreactor systems are classified into four different systems based on their applications: a. Stirred-tank reactors; b. Fixed-bed reactors; c. Fluidized-bed reactors; d. Airlift reactors.

Stirred Tank Bioreactors (STRs)

The STR system has a stirrer that works in two modes: batch or continuous. Furthermore, this type of bioreactor makes it possible to study the removal of Cr(VI) at different concentrations. In an experiment to study the

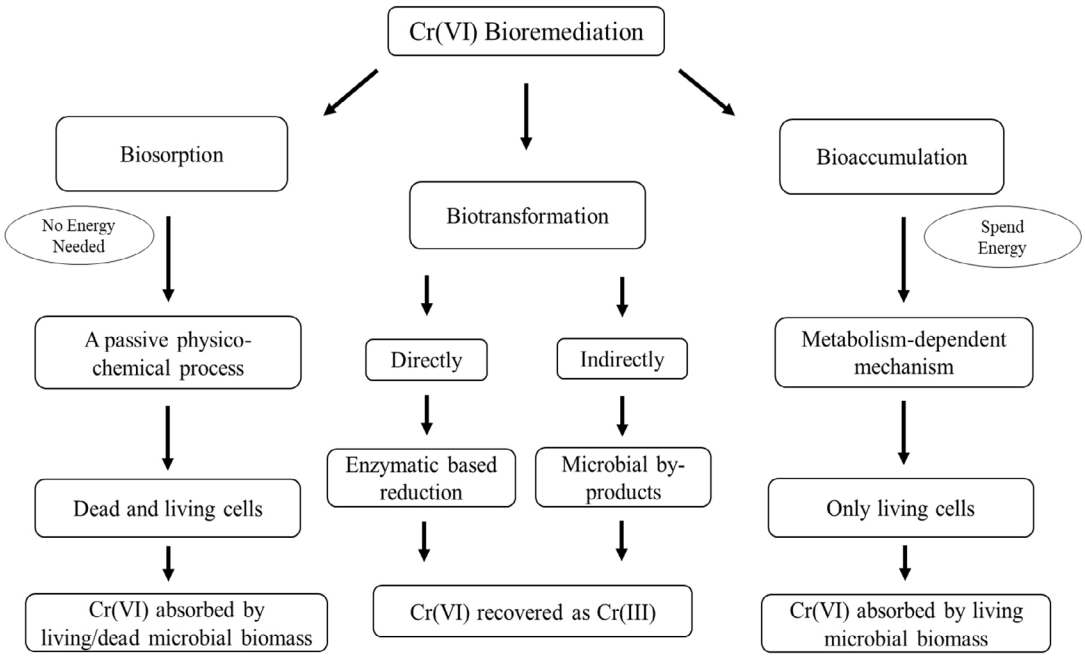

Fig. 2. Schematic illustration of the three Cr(VI) bioremediation mechanisms.

Cr(VI) biotransformation ability of *Arthrobacter rhombi-RE* strain MTCC7048 grown on molasses as a carbon source using three different growth systems, including an anoxic attached growth, aerobic attached and aerobic suspended system, found that the aerobic attached approach was the most effective, reaching 95% reduction of Cr(VI) in an initial concentration of 20 mg/L and chemical oxygen demand (COD) reduction of between 90 and 95% in an initial concentration of 3000 mg/L[55]. Nevertheless, there are drawbacks which may limit the application of the STRs for large-scale remediation. These drawbacks include the cost of high energy consumption, STRs can only be used to treat small quantities of effluents, loss of the viability of the metabolically active microbes and the fact that mixing of different contaminants might shift the remediation pathway[36,69,70].

Fixed-bed Reactors (FXRs)

The FXR system is characterised by its simplicity in construction and operation. In this system, the biosorbent is contained in a bed fixed on a column which passes the industrial effluents to be treated[71]. In a study that involved using FXR with immobilized agar-agar that contains a consortium of CRB – namely, *Pseudomonas aeruginosa* and *Bacillus subtilis* – the bacteria were able to remove a large amount of heavy metals, including Cr(VI), from textile effluents in 15 days and to significantly reduce COD from 1200 mg/L to 200 mg/L[40]. In another study that involved the use of SRB growing on ethanol in FXR revealed that the bacteria removed 95% of 50 mg/L of Cr(VI) using the biosorption approach[72]. Yet, there are some challenges that may limit the use of FXRs. These challenges include the need for multiple columns to maintain the optimal conditions for Cr(VI) remediation, and the need to generate a fixed bed when the biosorbent reaches its maximum capacity[25].

Fluidized-bed Bioreactors (FBRs)

In FBR system, microbes grow to form a biofilm on a solid surface, and the suspension state of a bed is maintained by continuously movement of the particles the reactor[25]. In an experiment that used an FBRs, *E. coli* supported kaolin to remove a number of metal ions, including Cr(VI). The study found that the presence of kaolin increased the biosorption of Cr(VI) to 100% at a lower concentration (8 mg/L) and to approximately 26% at 116 mg/L[73]. In another study that used an FBR system and ethanol as a carbon source, SRB showed high efficiency in Cr(VI) removal[74]. In this study, SRB was able to remove up to 93% of 45 gm/L of Cr(VI) from textile wastewater[74]. Despite all the benefits, there are some limitations for using FBRs for large-scale Cr(VI) remediation, which include the loss of microbial viability as well as potential contamination with other microbes[75].

AirLift Reactors (ALRs)

The function of ALR system is based on reducing shear stress by using an air bubble column alongside an airlift. The system has aeration, which ensures that the amount of needed oxygen has been met and enhances aeration mixture as well as mass transfer[25]. Furthermore, this type of bioreactor works best when the microorganisms used for bioremediation are fungi. In fact, it allows the formation of fungal mycelium, which in turn increases the area of contact. The system has been extensively studied using fungi, including filamentous fungi and yeast, to remove Cr(VI), but it has been poorly studied for bioremediation by bacteria[76,77]. Additionally, the system offers many benefits, among them lower power consumption, a lack of moving parts, rapid mixing, low risk of contamination and easy sterilization[78]. However, the use of the system is limited to the low-density liquids.

Advantages and Limitations for Using Bioreactors

Using bioreactors is the most efficient approach to scale up the bioremediation ability of the three microbial mechanisms for Cr-removal. The benefits and drawbacks for four bioreactors are discussed below.

Stirred-tank reactors (STRs) comprise one system that can enhance the ability of microbes to remove Cr(VI) from industrial wastewater via optimising the conditions to boost the microbial performance. There are many advantages to using STRs, including the simplicity of the system, which allows repeatability of experiments, as well as the ability to study the efficiency of the microbial removal approaches at different concentrations of a pollutant[78]. Yet several disadvantages limit the use of STRs for large-scale applications (Table 3). Poor energy efficiency is among these drawbacks, and thus use of STRs often comes with a high operational cost. Another disadvantage is that the system can reach maximum performance only

Table 3. Summary of the advantages and disadvantages of bioreactors

Bioreactors	Characteristics	Advantages	Disadvantages
Stirred Tank Bioreactors (STRs)	The reactor has stirrer to maintain the suspension of the biomass.	i. Simple system and is easy to operate. ii. Allows the repetition of the experiment.	High energy consumption leading to high operation cost.
Fixed-Bed Reactors (FXRs)	Mostly used for the removal of chromium using biosorption approach. Characterised by containing a column that passes the industrial effluents to be treated.	i. Simple to construct and operate. ii. Increases the working life of the microbial biomass.	The reaction conditions need to be closely monitored. It requires more than one inoculum to ensure a new fixed bed is generated when reaching the maximum capacity of the biomass.
Fluidized-Bed Bioreactors (FBRs)	This type of bioreactors is maintained in a suspension state through supplying contaminated effluents. Moreover, microbial biofilm is used and is grown on a slide surface.	i. The clogging effect is reduced significantly. ii. Take short retention hydraulic time.	Drawbacks of this type of bioreactors include the loss of the viability of the biomass used and microbial contamination.
AirLift Reactors (ALRs)	The function of this system is based on reducing the shear stress by using air bubble column alongside with airlift. Furthermore, the system is frequently used in the bioprocesses that rely on gas-liquid contact.	i. Consume low power, lack of moving parts, rapid mixing, high oxygen solubility and relatively better homogenisation ii. It reduces pellet formation thus allowing efficient surface contact.	Not efficient when used for effluents that are highly viscous or contain highly dense particles.

when a small amount of industrial wastewater is used, thus limiting large-scale application[62,79].

Fixed-bed reactor (FXR) systems are usually used to optimise the Cr(VI) uptake by microbial biomass. There are many positive aspects of using FXRs, including the simplicity of construction and operation, longer working life for microbial biomass and the allowance of Cr(VI) recovery through cycles of desorption (Table 3). Additionally, FXRs facilitates the use of large particles for immobilization of biosorbents 66. Some disadvantages, however, might restrict large-scale applications of FXR system, including the need to maintain the reaction condition and the need for multiple inocula to be supplied when the bed has reached its full capacity[80,81].

Fluidized-bed bioreactors (FBRs) support microbial biomass to form a biofilm on a solid surface. An important feature of FBR system is that the clogging effect is reduced considerably, which allows better flow of supplies to the system and thus achieves better results capacity[25]. Moreover, a shorter retention time makes it more efficient than the other systems (Table 3). Nevertheless, loss of the viability of microbial biomass is a major issue for FBR system. In addition, as the system required constant aeration, there is a high potential of microbial contamination through air flow[75].

AirLift reactors (ALRs) work by reducing shear stress via an air bubble column coupled with an airlift. The many advantages of using ALR system include low power consumption (cost- effective), mixing of contents at faster rate than in the other systems, low risk of microbial contamination and a lack of moving parts (Table 3)[77]. Although there are many benefits for ALR bioreactors, some disadvantages might limit the use of ALRs for large-scale bioremediation. These drawbacks include poor efficiency in dealing with viscous solutions as well as dense particles[78].

Future Directions

Several studies were conducted to improve the efficiency of the microbial mechanism for removing Cr. Among these studies, genetic engineering, using microbial consortium, using nano-material and designing higher efficiency bioreactors showed promising results in detoxifying Cr from the environment. In the following subsections, findings of these studies will be highlighted.

Designing Super-reduction Pathway

Employing genetic engineering to improve the microbial ability to tolerate high stress resulting from metallic ions is a promising approach. The capabilities of some microbial species to survive in highly toxic environments are attributed to their genetic adaptation to extreme environmental conditions[25]. Therefore, the genetic manipulation of CRB to design a super-reduction pathway can be achieved, specifically for plasmid-mediated Cr resistance. In a lab-scale study aimed at developing a recombinant bacterium able to reduce elevated levels of Cr, the gene *nemA* of *E.coli* and the gene *phaC* of *Ralstonia eutropha* were fused to create a novel Cr reduction system and were transferred to a recombinant microorganism, resulting in the expression of a Cr-reducing enzyme with 200-fold higher reduction efficiency[82]. Such approaches can be used to construct more efficient microbes that can tolerate multiple metals while reducing Cr(VI).

Using Microbial Consortium

The application of a microbial consortium is one of the most effective approaches to remove Cr(VI) from contaminated sites. The use of microbial consortia for large-scale Cr removal is evident in bioreactors[83]. One application that uses a microbial consortium to remove Cr(VI) is bioaugmentation, which involves adding metal resistance strain or a consortium to a site of contamination, allowing it to remove contaminants from the site (*in suit*)[84]. In a study that used *Aeromonas hydrophila* strain LZ-MG14 isolated from textile wastewater to develop bioaugmentation strategy in membrane bioreactor revealed that *A. hydrophila* was able to colonise the activated sludge and improve the ability of other microbes to reduce 0.5 mmol/L of Cr(VI) by 93.71% in just 12 hr[85]. Therefore, bioaugmentation using microbial consortia can be an effective tool for Cr(VI) remediation because it can activate diverse microbial pathways.

Using Nano-material

Additionally, the use of nanotechnology to improve microbial remediation is another alternative solution. This approach includes using immobilized microbial cells and metabolic enzymes alongside nanotechnology. It not only enhances the stability of the enzymes used for Cr(VI) reduction but can also promote the remediation of contaminated matrices at a nanometre scale by

combining CRB with nano-materials that can act as electron donors for the reaction[86].

Designing Higher Efficiency Bioreactors

Led by the desire to develop a low-cost, biologically based treatment for Cr-contaminated aquatic environments. Williams et al. developed the foundation for building a fixed film pilot bioreactor, which involved using microbial consortia such as *Enterobacter cloacae, Flavobacterium* sp. and *Ralstonia* sp. to remove Cr(VI)[87]. Notably, this was the first effective illustration of upscaled removal of > 99% of Cr(VI) from 24,000 L of contaminated groundwater[87]. Another promising means of Cr removal that can be applied is slurry-phase bioremediation. This slurry-phase reactor is characterized by treating soil in a bioreactor[88]. Adopting these strategies could help in developing an effective large-scale reactor for industrial wastewater treatment.

CONCLUSION

The widespread discharge of highly toxic Cr(VI) warrants development of efficient and rapid remediation technologies. Bacteria offer a promising solution to tackle Cr(VI) contamination in the environment. Three naturally evolved Cr-reducing mechanisms– namely, biotransformation, biosorption and bioaccumulation – are already being utilised, each with advantages and limitations. For an improved remediation of Cr(VI) using bacteria in bioreactors, multiple factors, including Cr(VI) concentrations, pH levels and carbon sources, need to be adjusted to achieve a considerable reduction level. Microbial consortia, genetically modified bacteria and well-designed bioreactors can be the most efficient ways to ensure the removal of hexavalent Cr from the environment.

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The authors declare that there is no conflict of interest.

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All the listed author(s) have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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