Alleviation of hexavalent chromium by using microorganisms: insight into the strategies and complications
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ABSTRACT
Excessive industrialization and anthropogenic activities have resulted in widespread prevalence of heavy metals including hexavalent chromium in the environment. In addition to toxic properties, Cr(VI) possesses high stability and mobility, which in total makes it included in the list of priority heavy metals; thus it needs to be managed urgently. Among different methods available for remediation of Cr(VI), bioremediation is considered as one of the sustainable methods which could effectively be utilized for controlling Cr(VI) pollution. In this aspect, the treatment of Cr(VI)-containing wastewater originating from industries is noteworthy. The present review thus is an attempt to present a systematic overview dealing with studies on remediation of hexavalent chromium by using microorganisms and their application in treatment of Cr(VI)-containing industrial wastewaters. Various factors affecting the Cr(VI) removal and methods to enhance the bio-treatment are highlighted, which might act as a basis for researchers developing Cr(VI) bioremediation techniques.

INTRODUCTION
The term ‘heavy metals’ usually refers to the group of metals and metalloids with atomic density greater than 4 g cm\(^{-3}\) (Duruibe et al. 2007; Mishra & Bharagava 2016). These are also defined as high density metallic elements and show toxicity even at low concentrations (Duruibe et al. 2007). Heavy metals are naturally found in dispersed form in rock formations; however, with rapid industrialization, their concentration is tremendously increasing in the environment. One of the major problems associated with heavy metals is that unlike organic pollutants they cannot be degraded or completely removed from the environment (Bharagava & Mishra 2018; Fernandez et al. 2018). Their widespread distribution and accumulation along with high persistence and toxicity thus pose a great threat to living beings. Scientists worldwide are working to find solutions for treatment of various heavy metal contaminated matrices.

Among the various heavy metals, chromium is a common pollutant with high concentrations in the environment due to the discharge of chromium contaminated effluents from a variety of industries such as electroplating, tanning, steel production, ore refining, and automobile manufacturing (Owlad et al. 2009; Xiao et al. 2017; Fernandez et al. 2018).

Chromium is a transition element and placed in the periodic table in group VI-B with an atomic number of 24. It is a steel-gray, lustrous, hard metal that has a high melting point (Owlad et al. 2009). Chromium was first discovered in the Siberian red lead ore (crocoite) in 1798 by the French chemist Vauquelin (Losi et al. 1994a; Mishra & Bharagava 2016).

Chromium occurs in oxidation states ranging from –II to +VI and among various oxidation states the trivalent (+III) and hexavalent (+VI) states of chromium are the most stable ones (Wani et al. 2007; Polti et al. 2011; Chug et al. 2016). Compared to trivalent form, hexavalent chromium is considered as a highly hazardous metal due to its oxidizing, mutagenic and carcinogenic properties. Cr(VI) compounds are approximately 1,000-fold more cytotoxic and mutagenic than Cr(III) (Dhal et al. 2010; Mamais et al. 2016; Bharagava & Mishra 2018). Hence, chromium is a new entry in the toxic metal series after lead, cadmium, and mercury (Saha & Orvig 2010). Almost every statutory body in the world has listed Cr(VI) as a priority toxic
chemical for control (Cheung & Gu 2007). Moreover, chromium in hexavalent form is highly soluble and bioavailable as compared to other forms, thus gaining more attention (Wani et al. 2007; Xiao et al. 2017).

**Chromium in trivalent form**

Cr(III) is an essential trace element and has a role in maintenance of normal carbohydrate metabolism in mammals and yeasts. It is also associated with the tertiary structure of proteins and the composition of cellular RNA and DNA (Colin et al. 2012). Compared to its hexavalent form, Cr(III) is much less toxic, less soluble at neutral pH, and unable to cross the cell membrane (Narayani & Shetty 2013; Mamais et al. 2016). However, a high concentration of Cr(III) is toxic and known to affect cellular structures (Colin et al. 2012).

**Chromium in hexavalent form**

In the hexavalent state, chromium exists as CrO3 and CrO4−

In the hexavalent state, chromium exists as CrO3 and CrO4^2−/HCrO4 (pH > 6.5) and Cr2O7^2−/CrO4 (pH < 6.5) (Dhal et al. 2010).

Because of hardness and stability, hexavalent chromium and its compounds are widely used in different kinds of industrial processes such as leather tanning, electroplating or metal finishing, textile manufacturing, chrome ore processing, wood preservation, alloy making, and corrosion protection. The huge amount of wastewater resulting from these industries is the major source of chromium contamination. For instance, tannery effluents contain hexavalent chromium in the range of 0.33–3.46 mg L^−1 (Mahmood et al. 2015). Similarly, electroplating wastewaters have hexavalent chromium in the range of 17–81 mg L^−1 (Ahmad et al. 2010; Machado et al. 2010). Most of these effluents are discharged into the open environment without proper treatment. This results in contamination of water resources and nearby soil environments, and in the last few decades the level of chromium in the aquatic and terrestrial ecosystems has increased tremendously (Samuel et al. 2012).

In the present review, issues pertaining to excess Cr(VI) in the environment and the role of microorganisms in its remediation from synthetic as well as industrial effluents are discussed. Effects of various parameters on hexavalent chromium removal and mechanism for removal are also highlighted herein.

**ENVIRONMENTAL PROBLEMS AND TOXICITY ASSOCIATED WITH HEXAVALENT CHROMIUM**

Hexavalent chromium elicits several environmental and health problems because of its high toxicity, stability and mobility in aqueous system. In the environment, Cr(VI) alters the structure of aquatic and soil microbial communities by reducing microbial growth and their activities and thereby disturbing the normal functioning of the ecosystem. Cr(VI) is reported to induce acute and chronic toxicity, neurotoxicity, dermatotoxicity, genotoxicity, carcinogenicity, and immunotoxicity in humans and animals (Politi et al. 2011; Mishra & Bharagava 2016; Bharagava & Mishra 2018).

Hexavalent chromium generally enters the cells using membrane sulfate transport channels of sulfate-utilizing organisms (Cheung & Gu 2007). Inside the cell, Cr(VI) reacts with intracellular reductants (ascorbate and glutathione) and generates a variety of short-lived Cr intermediates (Cr(V), Cr(IV), free radicals and end products of Cr(III)) (Cheung & Gu 2007). The short-lived Cr intermediates undergo transformation to stable form of Cr and in the process generate reactive oxygen species (Cheung & Gu 2007). The free radicals and reactive oxygen species oxidize several components of cells like nucleic acids, proteins, and other biopolymers and thus produce toxic, mutagenic, and carcinogenic effects on biological systems (He et al. 2009).

Specifically, to humans, Cr(VI) causes nasal irritation and ulceration, skin irritation, eardrum perforation, lung carcinoma (Cheung & Gu 2007), asthma, bronchitis, and inflammation of larynx and liver (Saha & Orvig 2010; Fernández et al. 2017). Cr(VI) through its accumulation in the placenta also impairs fetal development in mammals (Cheung & Gu 2007; He et al. 2009). Thus, considering the above detrimental effects of hexavalent chromium on biological systems, Cr(VI) has become a well-recognized bio-hazard (He et al. 2009).

**CR(VI) REMOVAL METHODS**

Various physico-chemical methods are being practiced for removal of hexavalent chromium from the contaminated environment. The major ones among them are chemical
reduction followed by precipitation, ion exchange, electrochemical precipitation, solvent extraction, membrane separation, evaporation, reverse osmosis, and adsorption on coal, activated carbon, alum, or fly ash (Saha & Orvig 2010; Liu et al. 2012; Narayani & Shetty 2013; Bharagava & Mishra 2018). However, major drawbacks associated with these processes such as high operation cost, low efficiency, and non-environment-friendly nature make most of these processes unsustainable in practice (Bharagava & Mishra 2018). The other major disadvantage is that these methods are economically viable at high and moderate concentrations of metals but remain inappropriate for low metal concentrations (1–100 mg L⁻¹) (Narayani & Shetty 2013; Mamais et al. 2016).

Owing to aforementioned problems of physico-chemical methods, biological treatment is becoming a preferable choice for remediation of Cr(VI) nowadays. Among various biological methods of chromium remediation, microbial remediation has gained major attention due to its cheaper, efficient, and sustainable applications (Malaviya & Singh 2016; Fernandez et al. 2018).

MICROBIAL REMEDIATION OF CR(VI)

Although Cr(VI) is known to exhibit toxicity to microbes, resulting in their growth inhibition, few microbial strains are able to grow in or adapt themselves to the presence of various concentrations of Cr(VI). Some of these chromium-tolerant microbes also develop the ability to reduce the toxic concentration of Cr(VI) in the surroundings and hence play an important role in Cr(VI) bioremediation. In the last decade, numerous reports on isolation and characterization of various chromium-reducing microbial strains of bacteria (Pseudomonas spp., Bacillus spp. Enterobacter spp., Acinetobacter spp., Burkholderia spp.), fungi (Paecilomyces spp., Aspergillus spp., Phanerochaete spp., Penicillium spp., Rhizopus spp.), yeast (Candida spp., Saccharomyces spp.), and algae (Guha et al. 2001; Guha et al. 2003; Chen et al. 2016) have been reported in literature. The search for efficient Cr(VI)-tolerant and reducing microbial strains is still going on for further improvement of the remediation processes. The extent and mechanisms of Cr(VI) reduction are variable and depend on several factors including the microbes’ isolation source and their growth characteristics (Dhal et al. 2010; Samuel et al. 2012).

The microbial system uses a number of resistance mechanisms like metal efflux pumps, chelation, and absorption/adsorption to evade heavy metal toxicity. Among these, the following two mechanisms are by and large used by microbes for alleviating toxic Cr(VI) concentrations in the medium (Valls & de Lorenzo 2002; Sharma & Adholeya 2011; Rao et al. 2017; Vendruscolo et al. 2017; Fernandez et al. 2018):

• transformation of Cr(VI) to Cr(III) followed by precipitation of reduced product
• sorption by microbial cells (biosorption/accumulation and adsorption).

Microbial transformation of Cr(VI) to Cr(III)

Most of the chromium-resistant microbes reported in literature are known to use Cr(VI) transformation as a resistance mechanism. The Cr(III) produced as a result of transformation finally precipitates as insoluble chromium(III) hydroxide (Cr(OH)₃)₃ (Dhal et al. 2010; Field et al. 2018). Microbial reduction of Cr(VI) to Cr(III) occurs either directly with the help of the microbial enzyme chromium reductase or indirectly using microbial metabolic products (as electron donors). Since hexavalent chromium has high reduction potential (+1.3 V under standard conditions) it can easily be reduced to Cr(III) in the presence of various reductants (Cheung & Gu 2007; Silva et al. 2007); for example, H₂S produced during anaerobic metabolism works as a reductant in chromate reduction.

The chromate reductase enzyme, depending upon the microbial species, may occur either in soluble form (cytoplasm) or in membrane-associated form. The chromate reduction can occur both under aerobic and anaerobic conditions. In aerobic conditions, most of the Cr(VI) transformation occurs inside or outside the plasma membrane using soluble chromate reductase, whereas, under anaerobic conditions, Cr(VI) can serve as a terminal electron acceptor in the respiratory chain for a large array of electron donors, including carbohydrates, proteins, fats, hydrogen, NAD(P)H and endogenous electron reserves (Cheung & Gu 2007).

Romanenko & Korenkov (1977) for the first time reported Cr(VI) to Cr(III) transformation in Pseudomonas sp. under anaerobic condition. Similarly Shen & Wang (1993) were the first to discover transformation of Cr(VI) under aerobic condition in Escherichia coli. Since the above discoveries, the search for Cr(VI)-reducing microorganisms (both aerobic and anaerobic) is being enthusiastically pursued, with numerous strains of genera, such as Pseudomonas spp., Acinetobacter spp., and Bacillus spp., Arthrobacter spp., being isolated and studied for chromate reduction potential (Cheung & Gu 2007).
A list of some chromate-reducing bacterial strains including their isolation source and Cr(VI) removal efficiency is presented in Table 1.

Biosorption and bioaccumulation

The cell surfaces of microorganisms are negatively charged due to the presence of various anionic structures, providing an advantage to bind positively charged metals from their surroundings (Sharma & Adholeya 2011). This process of binding metal to microbial surface from the surroundings is known as biosorption, which is a metabolism-independent process. The biosorption can be performed both by living and dead biomass, whereas uptake of metals ions by metabolically active growing cells is known as bioaccumulation. Microbial heavy metal accumulation often by metabolically active growing cells is known as bioaccumulation. Microbial heavy metal accumulation often comprises two phases: first, passive adsorption of metal at the cell surface and, second, a rapid phase involving active metabolism-dependent transport of metal into bacterial cells (Dermou et al. 2005; Polti et al. 2011). Metal sequestration or uptake in the case of bioaccumulation is followed by a number of processes such as localization of metal (Cr) within cell components, metallothionein binding, metal accumulation, extracellular precipitation, and complex formation (Polti et al. 2011). A large number of microbial strains, for instance Paenibacillus niger (Srivastava & Thakur 2006a; 2006b), Bacillus cereus IST105 (Naik et al. 2012), Zobellella denitrificans (He et al. 2016), and Bacillus mycoides 200AsB1 (Wang et al. 2016), have been reported to remediate Cr(VI) using biosorption and bioaccumulation mechanisms. A list of some of them including their isolation source and Cr(VI) removal efficiency is furnished in Table 2.

The above-mentioned resistance mechanisms adapted by some microbial cells is presented in the form of a schematic diagram in Figure 1.

FACTORS AFFECTING MICROBIAL Cr(VI) REMOVAL

pH of the media

The pH of the media plays a major role for the growth and metal bioaccumulation properties of the microbial strains by influencing metal binding sites (Narayani & Shetty 2015; Wang et al. 2016). Both biosorption and Cr(VI) reduction is dependent on initial pH of the media. Since Cr(VI) reduction is a proton consumption process, there is an increase in pH with Cr(VI) reduction. Hence, Cr(VI) reduction is generally higher at low pH values (Narayani & Shetty 2015). Enzymatic reduction of chromate is also affected by changes in pH, as pH alteration affects enzyme activities (Narayani & Shetty 2015; Xiao et al. 2017). However, depending upon microbial growth and their chromate reduction potential, different optimum pH values for chromate reduction have been reported by various researchers.

Similarly, the optimum initial pH for biosorption also depends on type of microbial species because of presence of different absorptive sites on different microorganisms (Aksu & Akpinar 2001). However, biosorption of chromium or other metals is generally favored at acidic pH values as, at lower pH, the overall surface charge becomes positive promoting sorption of anionic Cr(VI) species (CrO4^2-, Cr2O7^2-, HCrO4^-) (Alam & Ahmad 2011).

Temperature

Temperature seems to affect biosorption to a lesser extent within the range from 20 to 35 °C (Vijayaraghavan & Yun 2008). Higher temperatures usually enhance sorption due to the increased surface activity and kinetic energy of heavy metals (Vijayaraghavan & Yun 2008; Wang et al. 2016). At low temperature, fluidity of the membrane decreases which results in non-functioning of the transport system. However, physical damage to the biosorbent can also be expected at higher temperatures (Vijayaraghavan & Yun 2008). Similarly, microbial chromate reduction is also affected by temperature, as temperatures below and above the optimum (value depends upon the microbe used and varies from microbe to microbe) affect cell growth and viability along with chromate reductase activity.

Initial chromate concentration

Generally chromate biosorption and reduction by living biomass decreases with increase in initial chromate concentration (Srivastava & Thakur 2006a; Wani et al. 2007). This is due to increased chromate toxicity with rise in concentration which results in low viability and less growth of biomass. However, this limiting chromate concentration depends on different microbial species according to their tolerance and resistance properties.

Inoculum size

Rate of Cr(VI) reduction and biosorption increases with increase in cell density and a linear relationship exists...
<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Isolation source</th>
<th>Optimum culture conditions</th>
<th>Initial [Cr(VI)]</th>
<th>Removal efficiency</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Arthrobacter</em> sp.</td>
<td>Tannery waste contaminated soil</td>
<td>pH: 6.9; Temp: 21 °C Agitation: 100 rpm; Minimal salt medium with 0.5% glucose</td>
<td>20 mg L(^{-1})</td>
<td>100% in 46 h</td>
<td>Megharaj <em>et al.</em> (2005)</td>
</tr>
<tr>
<td><em>Bacillus</em> sp.</td>
<td>Tannery waste contaminated soil</td>
<td>pH: 6.9; Temp: 21 °C Agitation: 100 rpm; Minimal salt medium with 0.5% glucose</td>
<td>10 mg L(^{-1})</td>
<td>100% in 72 h</td>
<td>Megharaj <em>et al.</em> (2005)</td>
</tr>
<tr>
<td><em>Bacillus</em> sphaericus</td>
<td>Contaminated soil</td>
<td>pH: 6.0; Temp: 25 °C Agitation: 120 rpm; Minimal medium with 1.0 g L(^{-1}) glucose</td>
<td>20 mg L(^{-1})</td>
<td>62% in 48 h</td>
<td>Pal &amp; Paul (2004)</td>
</tr>
<tr>
<td><em>Bacillus</em> sp.</td>
<td>Chromate contaminated soil</td>
<td>pH: -; Temp: 35 °C Agitation: 200 rpm; Nutrient medium</td>
<td>10 mg L(^{-1}); 40 mg L(^{-1}) K(_2)Cr(_2)O(_7)</td>
<td>&gt;95% in 24 h (10 mg L(^{-1})); &gt;95% in 72 h (40 mg L(^{-1}))</td>
<td>Elangovan <em>et al.</em> (2006)</td>
</tr>
<tr>
<td><em>Providencia</em> sp.</td>
<td>Contaminated soil</td>
<td>pH: 7.0; Temp: 37 °C Agitation: 200 rpm; LB broth</td>
<td>100, 200, 300 mg L(^{-1}) K(_2)Cr(_2)O(_7)</td>
<td>100% in 30 h (100 mg L(^{-1})); 100% in 96 h (200 mg L(^{-1})); 100% in 108 h (300 mg L(^{-1}))</td>
<td>Thacker <em>et al.</em> (2006)</td>
</tr>
<tr>
<td><em>Acidithiobacillus ferrooxidans</em></td>
<td>–</td>
<td>pH: 1.8; Temp: 30 °C Agitation: 150 rpm; Sulfur-containing medium</td>
<td>5.0 mg L(^{-1})</td>
<td>100% in 3 d</td>
<td>Cabrera <em>et al.</em> (2007)</td>
</tr>
<tr>
<td><em>Acidithiobacillus thiooxidans</em></td>
<td>–</td>
<td>pH: 2.5; Temp: 30 °C Agitation: 150 rpm; Sulfur-containing medium</td>
<td>2.5 mg L(^{-1})</td>
<td>100% in 1 d</td>
<td>Cabrera <em>et al.</em> (2007)</td>
</tr>
<tr>
<td><em>Burkholderia cepacia</em> MCMB-821</td>
<td>Alkaline crater lake</td>
<td>pH: 9; Temp: 35 °C; In presence of 2% (w/v) NaCl; 2% (w/v) lactose</td>
<td>75 mg L(^{-1})</td>
<td>98% in 36 h</td>
<td>Wani <em>et al.</em> (2007)</td>
</tr>
<tr>
<td><em>Acinetobacter haemolyticus</em></td>
<td>Textile effluent</td>
<td>pH: -; Temp: 30 °C</td>
<td>50 mg L(^{-1})</td>
<td>100% in 36 h</td>
<td>Zakaria <em>et al.</em> (2007)</td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp. G1DM21</td>
<td>Chromium industrial landfill</td>
<td>pH: 7.0; Temp: 37 °C; Agitation: 150 rpm; LB broth</td>
<td>500 μM</td>
<td>99.7% in 48 h</td>
<td>Desai <em>et al.</em> (2008)</td>
</tr>
<tr>
<td><em>Ochrobactrum</em> sp. CSCr-3</td>
<td>Chromium landfill</td>
<td>pH: 10; Temp: 35 °C</td>
<td>200 mg L(^{-1})</td>
<td>80% in 30 h</td>
<td>He <em>et al.</em> (2009)</td>
</tr>
<tr>
<td><em>Bacillus</em> sp. CSB-4</td>
<td>Chromite mine soil</td>
<td>pH: 7.0; Temp: 35 °C; Agitation: 100 rpm</td>
<td>100 mg L(^{-1})</td>
<td>&gt;90% in 144 h</td>
<td>Dhal <em>et al.</em> (2010)</td>
</tr>
<tr>
<td><em>Bacillus atrophaeus</em> MM20</td>
<td>Tannery waste contaminated soil</td>
<td>pH: -; Temp: 21 °C; Agitation 100 rpm</td>
<td>10 mg L(^{-1})</td>
<td>94% after 50 h</td>
<td>Patra <em>et al.</em> (2010)</td>
</tr>
<tr>
<td><em>Serratia</em> sp. Cr-10</td>
<td>Chromium contaminated soil</td>
<td>pH: 7.0; Temp: 37 °C; 1% (w/v), fructose</td>
<td>10, 20 mg L(^{-1})</td>
<td>100% after 12 h</td>
<td>Zhang &amp; Li (2011)</td>
</tr>
<tr>
<td><em>Arthrobacter</em> sp. SUK 1201</td>
<td>Chromite mine overburden</td>
<td>pH: 7.0; Temp: 35 °C; Agitation: 120 rpm; Minimal medium with 1.0 g L(^{-1}) glucose</td>
<td>2.0 mM</td>
<td>67% in 7 days</td>
<td>Dey &amp; Paul (2012)</td>
</tr>
<tr>
<td><em>Cellulosimicrobium</em> sp. MWM81</td>
<td>Contaminated soil</td>
<td>pH: 7.0; Temp: 37 °C</td>
<td>10 mM</td>
<td>45% in 48 h</td>
<td>Rehman <em>et al.</em> (2013)</td>
</tr>
</tbody>
</table>

(continued)
<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Isolation source</th>
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<th>Initial [Cr(VI)]</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger (CICC41115)</td>
<td>Commercial</td>
<td>pH: 7.0; Temp: 37 °C; Agitation: 150 rpm</td>
<td>50 mg L⁻¹</td>
<td>100% in 84 h</td>
<td>Gu <em>et al.</em> (2015)</td>
</tr>
<tr>
<td>Acinetobacter guillouiae SFC 500-1A</td>
<td>Tannery sludge</td>
<td>pH: 10; Temp: 28 ± 2 °C; Agitation: 150 rpm (with phenol as carbon source)</td>
<td>10 mg L⁻¹</td>
<td>~62% in 72 h</td>
<td>Ontañon <em>et al.</em> (2015)</td>
</tr>
<tr>
<td>Pseudomonas sp. JF122</td>
<td>Contaminated soil</td>
<td>pH: 6.5; Temp: 30 °C; Agitation: 150 rpm (with phenol as sole carbon source)</td>
<td>2.0 mg L⁻¹</td>
<td>100% in 72 h</td>
<td>Zhou &amp; Chen (2016)</td>
</tr>
<tr>
<td>Bacillus subtilis MNU16</td>
<td>Soil from coal mining site</td>
<td>pH: 7.0; Temp: 30 °C</td>
<td>50 mg L⁻¹</td>
<td>75% within 72 h</td>
<td>Upadhyay <em>et al.</em> (2017)</td>
</tr>
<tr>
<td>Cellulosimicrobium sp. KX710177</td>
<td>Tannery wastewater</td>
<td>pH: - ; Temp: 35 °C; Agitation: 120 rpm</td>
<td>300 mg L⁻¹</td>
<td>62% after 96 h</td>
<td>Bharagava &amp; Mishra (2018)</td>
</tr>
<tr>
<td>Arthrobacter sp. LLW01</td>
<td>Contaminated soil</td>
<td>pH: - ; Temp: 22 °C; Agitation: 150 rpm; Minimal medium with 15 mM glucose</td>
<td>50 μM</td>
<td>50% in 144 h</td>
<td>Field <em>et al.</em> (2018)</td>
</tr>
<tr>
<td>Penicillium oxalicum SL2</td>
<td>Indoor air</td>
<td>pH: Temp: 30 °C; Agitation: 200 rpm</td>
<td>1,000 mg L⁻¹</td>
<td>100% within 144 h</td>
<td>Long <em>et al.</em> (2018)</td>
</tr>
<tr>
<td>Bacillus sp. SFC 500-1E</td>
<td>Bacterial consortium native to tannery sediments</td>
<td>pH: 7.0; Temp: 28 °C Agitation: 150 rpm</td>
<td>50 mg L⁻¹</td>
<td>43% after 72 h</td>
<td>Ontañon <em>et al.</em> (2018)</td>
</tr>
</tbody>
</table>
Table 2 | Some microbial strains reported to bioaccumulate Cr(VI) in synthetic medium, their isolation source, and culture conditions

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Isolation source</th>
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<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus circulans</td>
<td>Tannery effluent</td>
<td>pH: 9.0; Temp: 20 °C; Nutrient broth containing 4% NaCl</td>
<td>50 mg L⁻¹</td>
<td>Permissible limit in 24 h</td>
<td>Srinath et al. (2002)</td>
</tr>
<tr>
<td>Mixed culture</td>
<td>Industrial saline wastewater</td>
<td>pH: 2.5; Temp: 28 °C</td>
<td>100 mg L⁻¹</td>
<td>29.9 mg g⁻¹ at 120 min</td>
<td>Alam &amp; Ahmed (2011)</td>
</tr>
<tr>
<td>Exiguobacterium sp. ZM2</td>
<td>Tannery effluent contaminated soil</td>
<td>pH: 7.0; Temp: 30 °C; Agitation: 220 rpm; Mineral medium with glycerol</td>
<td>50 mg L⁻¹</td>
<td>52% in 72 h</td>
<td>Poli et al. (2011)</td>
</tr>
<tr>
<td>Streptomyces sp. MC1</td>
<td>Contaminated sediment</td>
<td>pH: 7.0; Temp: 30 °C; Agitation: 220 rpm; Mineral medium with glycerol</td>
<td>50 mg L⁻¹</td>
<td>52% in 72 h</td>
<td>Poli et al. (2011)</td>
</tr>
<tr>
<td>Acinetobacter sp. AB1</td>
<td>Tannery</td>
<td>pH: 10; Temp: 30 °C; Nutrient broth</td>
<td>50 mg L⁻¹</td>
<td>100% at 72 h</td>
<td>Essahale et al. (2012)</td>
</tr>
<tr>
<td>Acinetobacter sp. PD12S2</td>
<td>Tannery effluent</td>
<td>pH: 7.0; Temp: 37 °C; Mineral medium with 4.0 g L⁻¹ glucose</td>
<td>8.86 mg L⁻¹</td>
<td>0.19 mg L⁻¹ h⁻¹ (uptake)</td>
<td>Panda &amp; Sarkar (2012)</td>
</tr>
<tr>
<td>Enterobacter aerogenes T2</td>
<td>Tannery effluent</td>
<td>pH: 7.0; Temp: 37 °C; Mineral medium with 4.0 g L⁻¹ glucose</td>
<td>8.86 mg L⁻¹</td>
<td>0.35 mg L⁻¹ h⁻¹ (uptake)</td>
<td>Panda &amp; Sarkar (2012)</td>
</tr>
<tr>
<td>Acinetobacter junii VITsUKMW2</td>
<td>Chromite mining water</td>
<td>pH: 7.5; Temp: 30 °C; Nutrient broth</td>
<td>20 mg L⁻¹</td>
<td>40% in 8 h</td>
<td>Samuel et al. (2012)</td>
</tr>
<tr>
<td>Bacillus subtilis VITsUKMW1</td>
<td>Chromite mining water</td>
<td>pH: 7.5; Temp: 30 °C; Nutrient broth</td>
<td>20 mg L⁻¹</td>
<td>40% in 8 h</td>
<td>Samuel et al. (2012)</td>
</tr>
<tr>
<td>Escherichia coli VITsUKMW3</td>
<td>Chromite mining water</td>
<td>pH: 7.5; Temp: 30 °C; Nutrient broth</td>
<td>20 mg L⁻¹</td>
<td>40% in 5 h</td>
<td>Samuel et al. (2012)</td>
</tr>
<tr>
<td>Acinetobacter sp. B9</td>
<td>Wastewater of CETP</td>
<td>pH 7.0; Temp: 30 °C; Agitation: 200 rpm</td>
<td>7.0 mg L⁻¹</td>
<td>67% in 24 h</td>
<td>Bhattacharya &amp; Gupta (2013)</td>
</tr>
<tr>
<td>Exiguobacterium sp. KSKE41</td>
<td>Contaminated soil</td>
<td>pH: 7.0; Temp: 28 °C</td>
<td>10 mM</td>
<td>35% in 48 h</td>
<td>Rehman et al. (2013)</td>
</tr>
<tr>
<td>Enterobacter sp. DU17</td>
<td>Tannery waste dump site</td>
<td>pH: 7.0; Temp: 37 °C; Agitation: 180 rpm; LB broth containing 0.2% glucose or fructose</td>
<td>50 mg L⁻¹</td>
<td>~79%</td>
<td>Rahman &amp; Singh (2014)</td>
</tr>
<tr>
<td>Arthrobacter sp. Sphe3</td>
<td>–</td>
<td>pH: 8.0; Temp: 30 °C; 10 g L⁻¹ glucose</td>
<td>45 mg L⁻¹</td>
<td>100%</td>
<td>Ziegova et al. (2014)</td>
</tr>
<tr>
<td>Streptomyces wernaensis LD 22</td>
<td>Animal fecal matter</td>
<td>pH: 7.0; Temp: 41 °C; Agitation: 100 rpm</td>
<td>250 mg L⁻¹</td>
<td>51.7% after 7 d</td>
<td>Latha et al. (2015)</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>Mining soil</td>
<td>pH: 6–9; Temp: 37 °C; Agitation: 180 rpm</td>
<td>0.2 mM</td>
<td>&gt;90% in 48 h</td>
<td>Zheng et al. (2015)</td>
</tr>
<tr>
<td>Bacillus mycoides 200AsB1</td>
<td>Rhizosphere of Pteris vittata</td>
<td>pH 7.0; Temp: 30 °C; Agitation: 180 rpm</td>
<td>25 mg L⁻¹</td>
<td>100% in 25 h</td>
<td>Wang et al. (2016)</td>
</tr>
<tr>
<td>Arthrinium malaysianum</td>
<td>Contaminant culture (heat treated)</td>
<td>pH 3.0; Temp: 30 °C; Agitation: 150 rpm</td>
<td>1,000 mg L⁻¹</td>
<td>67% in 20 h</td>
<td>Majumder et al. (2017)</td>
</tr>
<tr>
<td>Aspergillus sydowii</td>
<td>Mangrove sediment</td>
<td>pH 5.0; Temp: 28 °C; Agitation: 80 rpm</td>
<td>50 mg L⁻¹</td>
<td>24.9% after 7 d</td>
<td>Lotlikar et al. (2018)</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>Commercial NaOH &amp; heat treated (121 °C for 60 min)</td>
<td>pH 5.0; Temp: 25 °C; Agitation: 100 rpm; Biosorbent conc: 5.0 g L⁻¹</td>
<td>90 mg L⁻¹</td>
<td>99.66% in 3 h</td>
<td>Rossi et al. (2018)</td>
</tr>
</tbody>
</table>

between these two (Narayani & Shetty 2013). An increase in the biomass concentration generally increases the amount of solute biosorbed, due to the increased surface area of the biosorbent, which in turn increases the number of binding sites (Vijayaraghavan & Yun 2008).
Agitation

In stationary phase, an external film development around the absorbent influences the rate of biosorption. With appropriate agitation, the diffusion rate of a solute from the bulk liquid to the liquid boundary layer surrounding particles becomes higher due to the enhanced turbulence and the decrease in the thickness of the liquid boundary layer (Vijayaraghavan & Yun 2008). However, too vigorous agitation would result in desorption of metals from the sorption sites (Ibrahim et al. 2006). Similarly, agitation enhances dissolved oxygen or aeration which in turn strongly favors microbial growth and hence increases chromate reduction. However, there may be mechanical damage to microbial cells as a result of high agitation speed (Tripathi & Garg 2013). Hence, optimization of shaking speed is important for effective microbial Cr(VI) removal from the contaminated medium.

Electron donors

Supply of inorganic or organic reductants (electron donor) mainly in the form of low molecular weight carbohydrates, amino acids, fatty acids, NADPH or hydrogen, and nano zero-valent iron is known to be one of the attractive strategies for accelerating microbial chromate reduction (Cheung & Gu 2007; Narayani & Shetty 2013). These electron donors may also occur in endogenous form (NADPH) in some microorganisms. Losi et al. (1994b) reported enhanced chromate reduction by supplying organic manure as reductant to the contaminated system. Similarly, Tokunaga et al. (2005) reported enhanced microbial chromate reduction after addition of tryptic soy broth or lactate as nutrients into Cr(VI) contaminated soil.

Effect of co-contaminants

Co-contaminants in the form of other heavy metals or toxic organic compounds drastically affect the removal of chromium(VI) from the environment. Generally, co-presence of these contaminants creates additional toxic stress on microorganisms which subsequently lowers growth rate and viable cell count. Co-presence of Cd(II), Zn(II), Co(II), and Ni(II) in the media were observed to reduce the reduction of Cr(VI) to Cr(III) using Arthrobacter sp. SUK 1201 (Dey & Paul 2012). However, these authors observed enhanced chromate reduction in co-presence of Cu. Structural and physiological changes due to one stress, especially organic co-pollutants, sometimes helps microbes in combating toxicity of heavy metals. For instance, increased membrane saturation due to the presence of organic compound toluene helps Bacillus sp. ORAs2 in combating arsenic toxicity (Pepi et al. 2008). Occasionally, the organic compound itself or its intermediate degrading products might work as electron donor for reduction of Cr(VI) to Cr(III). Song et al. (2009) and Zhou & Chen (2016) suggested intermediate phenol degradation products as electron donors for reduction of toxic Cr(VI) to less toxic Cr(III) during their studies with bioremediation of phenol and Cr(VI).
APPROACHES TO ENHANCE MICROBIAL CR(VI) REMEDIATION

Use of zerovalent iron particles

Use of zerovalent iron as a reducing agent for reduction of Cr(VI) to Cr(III) and its subsequent precipitation/sorption is reported by a number of researchers for remediation of Cr(VI) from various environmental matrices. Zerovalent iron acts as an electron donor and results in oxidation-reduction reactions without any energy requirement (Guha & Bhargava 2005). Zerovalent iron (Fe⁰) and its corrosion product Fe(II) act as reductants for the removal of Cr(VI), resulting in oxidation of Fe⁰ to Fe(III) while Cr(VI) is reduced to Cr(III) (Zhong et al. 2017). This technique is coupled with the bioremediation method by a number of researchers for improving the remediation efficiency and removal of lower Cr(VI) concentrations (Guha & Bhargava 2005; Galdames et al. 2017).

Immobilization of Cr(VI)-removing microorganisms

In order to reduce the toxic effect of Cr(VI) on microbial cells, Cr(VI)-removing microbial strains can be immobilized into various matrices. Immobilization protects the cells from direct damage due to toxicants and thus is reported to enhance the removal rate in some cases (Bayat et al. 2015). Various matrices, viz. agar, agarose, alginate, polyvinyl alcohol–alginate, polyacrylamide, polyethylene glycol etc., have been used by a number of researchers worldwide for immobilization of microbial cells capable of alleviating Cr(VI) levels (Narayani & Shetty 2013; Hora & Shetty 2016). In comparison to free cells, the immobilized cells have higher capacity to tolerate the metal toxicity and sudden changes in local physico-chemical parameters. Furthermore, reusability is another important property associated with immobilized preparation, as the cells immobilized on a suitable matrix could be used repeatedly.

Use of consortia/binary culture

A mixture of different indigenous microbial strains capable of tolerating or reducing chromium(VI) is ascribed as one of the important methods to reduce the toxic level of Cr(VI) (Joutey et al. 2011; Sharma & Malaviya 2016). Here microbes work synergistically to reduce Cr(VI) concentrations and thereby improve the rate of removal. In consortia, some microbial strains reduce Cr(VI) to Cr(III), while others lower the levels of both the species (Cr(VI) or Cr(III)) in a contaminated environment through sorption or uptake. The technique is very relevant and effective for remediation of Cr(VI) from real industrial wastewater.

Sequential treatment

In sequential treatment, first a microorganism or set of microorganisms possessing the ability to convert Cr(VI) to Cr(III) is added to the contaminated system, followed by inoculation of another microorganism or set of microorganisms capable of removing the Cr(III) from the system through biosorption. This technique is also used to reduce the level of particular pollutant (heavy metals/species of metals or organic compounds) to enhance the removal or working efficiency of microbial strains concerned with Cr removal from co-contaminated systems.

Microbial induced calcite precipitation based approach

In microbial induced calcite precipitation, one or more urease-producing microorganisms are added to Cr or other heavy metal contaminated systems. Urease produced by microbe(s) acts on substrate (urea) to ultimately form carbonic acid (H₂CO₃), which further dissociates to form bicarbonate (HCO₃⁻) and carbonate (CO₃²⁻) ions. The carbonate then reacts with free Ca ions (either naturally present or extraneously added) to form calcium carbonate (calcite). During the process of formation of calcite, metals such as Cr also get co-precipitated to form metal–calcite composites, thereby lowering the concentration of free heavy metals (Bhattacharya et al. 2018). The detail about the utility of this technique in remediation of heavy metals is well described in the review of Anbu et al. (2016). Other approaches, like use of biosurfactants, algae–bacteria symbiosis, and rhizoremediation, are also widely reported in literature for remediation of chromium contaminated systems (Kavamura & Esposito 2010; Rengifo-Gallego et al. 2012; Chug et al. 2016; Usman et al. 2016; Mishra et al. 2017).

BIOREMEDICATION OF CR(VI) FROM INDUSTRIAL WASTEWATER

Several microorganisms which show potential for remediation of target pollutants in synthetic medium often do not work well with real, harsh, complex contaminated systems i.e. industrial effluents/wastewater. These effluents are complex in the sense that they are characterized by the presence
of a mix of contaminants and may also have interfering native microflora. Nevertheless, an advantage of biological treatment of wastewater over physico-chemical methods is the use of efficient chromate- and other heavy-metal-reducing microorganisms in heavy metal-rich industrial wastewater. Various authors have demonstrated microbial-

### Table 3 | Microbial treatment for removal of Cr(VI) from industrial wastewater, mode of treatment, and treatment conditions

<table>
<thead>
<tr>
<th>Industrial wastewater</th>
<th>Initial Cr(VI), mg L(^{-1}) (removal efficiency)</th>
<th>Microorganism</th>
<th>Treatment mode</th>
<th>Treatment conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannery</td>
<td>40 (60% at 35 h)</td>
<td><em>Pseudomonas aeruginosa</em> A2Chr</td>
<td>Batch</td>
<td>pH: 7.0; Temp: 37 °C; Agitation: 150 rpm; Supplemented with C, N, P.</td>
<td>Ganguli &amp; Tripathi (1999)</td>
</tr>
<tr>
<td>Electroplating</td>
<td>15 (100% at 30 h)</td>
<td><em>Pseudomonas aeruginosa</em> A2Chr</td>
<td>Batch</td>
<td>pH: 7.2; Temp: 37 °C; Agitation: 150 rpm; Supplemented with C, N, P.</td>
<td>Ganguli &amp; Tripathi (1999)</td>
</tr>
<tr>
<td>Electroplating</td>
<td>10 (76% at 4 h)</td>
<td><em>Pseudomonas aeruginosa</em> A2Chr</td>
<td>Dialysis bioreactor</td>
<td>pH: 7.2; Temp: 37 °C; Supplemented with C, N, P.</td>
<td>Ganguli &amp; Tripathi (2002)</td>
</tr>
<tr>
<td>Electroplating</td>
<td>10 (93% at 8 h)</td>
<td><em>Pseudomonas aeruginosa</em> A2Chr (biofilm)</td>
<td>Laboratory-scale rotating bioreactor</td>
<td>pH: 7.2; Temp: 37 °C; Supplemented with C, N, P.</td>
<td>Ganguli &amp; Tripathi (2002)</td>
</tr>
<tr>
<td>Tannery</td>
<td>557 Cr (65% at 7 d)</td>
<td><em>Aspergillus</em> sp. FK1</td>
<td>Laboratory-scale bioreactor</td>
<td>pH: 5.0–5.5; Agitation: 250 rpm; Aeration: 500 mL min(^{-1})</td>
<td>Srivastava &amp; Thakur (2006b)</td>
</tr>
<tr>
<td>Electroplating</td>
<td>18 ± 1.0 (&gt;98% at 30 min)</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td>Batch</td>
<td>pH: 2.3; Temp: 25 °C; Agitation: 150 rpm</td>
<td>Machado et al. (2010)</td>
</tr>
<tr>
<td>Electroplating (various)</td>
<td>8–30 (94–100%)</td>
<td><em>Candida lipoiytica</em> &amp; sewage sludge</td>
<td>Laboratory-scale bioreactor</td>
<td>pH: 1.92–5.22; Temp: 25 °C</td>
<td>Ye et al. (2010)</td>
</tr>
<tr>
<td>Electroplating</td>
<td>17–81 (&gt;99%)</td>
<td><em>ChromeBac™</em> system (Acinetobacter haemolyticus)</td>
<td>Pilot-scale bioreactor</td>
<td>–</td>
<td>Ahmad et al. (2010)</td>
</tr>
<tr>
<td>Tannery</td>
<td>1.24 (100% at 18 h)</td>
<td><em>Paecilomyces lilacinus</em></td>
<td>Batch</td>
<td>pH: 8.0; Supplemented with cane sugar</td>
<td>Sharma &amp; Adholeya (2011)</td>
</tr>
<tr>
<td>Tannery</td>
<td>50* (100% at 48 h)</td>
<td><em>Paecilomyces lilacinus</em></td>
<td>Batch</td>
<td>pH: 8.0; Supplemented with cane sugar</td>
<td>Sharma &amp; Adholeya (2011)</td>
</tr>
<tr>
<td>Electroplating</td>
<td>968 (76% in 3d)</td>
<td><em>Bacillus cereus</em> IST105</td>
<td>Batch</td>
<td>pH: 7.0; Temp: 30 °C</td>
<td>Naik et al. (2012)</td>
</tr>
<tr>
<td>Tannery</td>
<td>1.3 (84% at 72 h)</td>
<td><em>Enterobacter aerogenes</em> T2</td>
<td>Batch</td>
<td>–</td>
<td>Panda &amp; Sarkar (2012)</td>
</tr>
<tr>
<td>Electroplating</td>
<td>30 (93% in 144 h)</td>
<td><em>Acinetobacter</em> sp. B9</td>
<td>Batch</td>
<td>pH: 7.0; Temp: 30 °C; Agitation: 200 rpm</td>
<td>Bhattacharya &amp; Gupta (2013)</td>
</tr>
<tr>
<td>Tannery</td>
<td>2.41 (TC)** (73% in 48 h)</td>
<td><em>Bacillus cereus</em> Cr1</td>
<td>Batch</td>
<td>pH: 8.4; Temp: 35 °C; Agitation: 120 rpm</td>
<td>Kumari et al. (2016)</td>
</tr>
<tr>
<td>Tannery</td>
<td>9.86 (100% in 36 h)</td>
<td>Fungal consortia (immobilized on nylon mesh)</td>
<td>Stirred tank bioreactor</td>
<td>pH: 4.0; Temp: 28 °C; Supplemented with glucose &amp; ammonium nitrate</td>
<td>Sharma &amp; Malaviya (2016)</td>
</tr>
<tr>
<td>Leather industry</td>
<td>2.41 (30% in 24 h)</td>
<td><em>Arthrinium malaysianum</em></td>
<td>Batch</td>
<td>pH: 7.3; Temp: ambient under shaking condition</td>
<td>Majumder et al. (2017)</td>
</tr>
<tr>
<td>Electroplating</td>
<td>40.6 (100% in 48 h)</td>
<td><em>Penicillium oxalicum</em> strain SL2</td>
<td>Batch</td>
<td>pH: 7.0; supplemented with dextrose &amp; potato extract Temp: 30 °C; Agitation: 200 rpm</td>
<td>Long et al. (2018)</td>
</tr>
<tr>
<td>Electroplating</td>
<td>96.1 (100% in 96 h)</td>
<td><em>Penicillium oxalicum</em> strain SL2</td>
<td>Batch</td>
<td>pH: 7.0; supplemented with dextrose &amp; potato extract Temp: 30 °C; Agitation</td>
<td>Long et al. (2018)</td>
</tr>
</tbody>
</table>

C, Carbon; N, Nitrogen; P, Phosphorus.

*Extraneously added to make final Cr(VI) concentration.

**TC, Total chromium.
assisted remediation of Cr(VI) and other heavy metals from electroplating, tannery, and heavy metal-rich industrial wastewaters. Table 3 presents some of the studies related to bioremediation of Cr(VI) from various industrial wastewaters using microbial systems.

A recent review by Fernandez et al. (2018) very well covered the aspect of scaled-up or pilot-scale bioremediation of chromium from industrial wastewater. Nevertheless, in addition to microbial systems, various other systems, namely nano zerovalent iron (Němeček et al. 2014) and constructed wetland (Sultana et al. 2014; Amin et al. 2018; Ashraf et al. 2018), have been successfully explored by various researchers for remediation of chromium from industrial wastewater at large scale.

CONCLUSIONS

Microbial remediation of Cr(VI) in the environment is one of the viable and sustainable methods for attenuation of excess Cr(VI) accumulation in the environment. Based on the overall study, it could be said that heavy metal and specifically Cr(VI) bioremediation is a technique in its infancy and much more work at bigger spatial and temporal scales is needed, so as to develop reliable and cost-effective technologies for this problem.

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