Microbes in heavy metal remediation

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Heavy metal contamination due to natural and anthropogenic sources is a global environmental concern. Release of heavy metal without proper treatment poses a significant threat to public health because of its persistence, biomagnification and accumulation in food chain. Non-biodegradability and sludge production are the two major constraints of metal treatment. Microbial metal bioremediation is an efficient strategy due to its low cost, high efficiency and ecofriendly nature. Recent advances have been made in understanding metal-microbe interaction and their application for metal accumulation/detoxification. This article summarizes the potentials of microbes in metal remediation.

Keywords: Bioremediation techniques, Heavy metal contamination, Metal bioremediation, Microbial metal bioremediation.

Contamination of heavy metals in the environment is a major global concern because of their toxicity and threat to human life and environment1,2. Much research has been conducted on heavy metal contamination in soils from various anthropogenic sources such as industrial wastes3, automobile emissions4, mining activities5, and agricultural practices6. The group of heavy metals are about 65 and are defined in a number of criteria such as their cationic-hydroxide formation, specific gravity greater than 5 g/ml, complex formation, hard-soft acids and bases, and, more recently, association with eutrophication and environmental toxicity.

Roane and Pepper7 classified metals into three classes on the basis of their biological functions and effects: (1) the essential metals with known biological functions, (Na, K, Mg, Ca, V, Mn, Fe, Co, Ni, Cu, Zn, Mo, and W), (2) the toxic metals (Ag, Cd, Sn, Au, Hg, Ti, Pb, Al, and metalloids Ge, As, Sb and Se), and (3) the nonessential, nontoxic metals with no known biological effects (Rb, Cs, Sr, and T). Based on primary accumulation mechanisms in sediments, heavy metals are classified into five categories. (1) adsorptive and exchangeable, (2) bound to carbonate phases, (3) bound to reducible phases (Fe and Mn oxides), (4) bound to organic matter and sulfides, and (5) detrital or lattice metals8.

Need for metal bioremediation

Greater awareness of the ecological effects of toxic metals and their biomagnifications through food chain as well as highly publicized episodes such as mercury pollution in Minimata, Japan has prompted a demand for decontamination of heavy metals in the aquatic systems. However, essential metals are required for enzyme catalysis, nutrient transport, protein structure, charge neutralization and control of osmotic pressure9. Olson et al.10 reported incorporation of nickel in four microbial enzymes involved in ureolysis, hydrogen metabolism, methane biogenesis and acetogenesis. A number of heavy metals are required as micronutrients to plants. They act as cofactors as part of prosthetic groups of enzymes which are involved in a wide variety of metabolic pathways. However, when they are present in high levels, most heavy metals are toxic to plants11.

Metal concentration has been linked to birth defects, cancer, skin lesions, retardation leading to disabilities, liver and kidney damage and a host of other maladies11. The Center for Disease Control (CDC)12 and the Agency for Toxic Substances and Disease Registry (ASTDR)12 estimate that 15-20% of U.S. children have lead levels greater than 15 mg/dl in blood, which is considered potentially toxic. Changes in trace element profile of the soil causes physiological and genetic changes in various life, such as plants, aquatic and benthic fauna, insects, earthworms, fish, birds and mammals as evidenced by recent research work13. Tyagi et al.3 stated that the concentration of heavy metals in industrial areas in India is much higher than the permissible limit of World Health Organization. They have also reported that these metals in the ground water have caused various diseases in human beings and also disturb the metabolic functions. A brief list of heavy metals with
their pathological effects, which pose health hazard to environment (Table 1).14.

Metal availability

Sposito15 categorised metals as bioavailable (precipitated, non-sorbed and mobile) and non-bioavailable (precipitated, sorbed, and non-mobile) metals. In nature, metals and metalloids exist mostly as cations, oxyanions, or both in aqueous solution and mostly as salts or oxides in crystalline (mineral) form or as amorphous precipitates in soluble form.16. The mobility of metals as hydrated ionic salts is dependent on two factors. 1. The metallic element that is participating as positively charged ions (cations) and 2. The one, which makes up negatively charged component of the salt. The cationic/anionic solubility relationship is in Table 2. Geochemical forms of heavy metals in soil affect their solubility, which directly influence their bioavailability.17. Lena and Rao18 reported mobility and bioavailability of metals in soils is in the order of Zn > Cu > Cd > Ni, based on their solubility and geochemical forms. The fate of toxic metals in soils depends mainly on the initial chemical form of the metal even though the environmental and edaphic conditions such as pH, redox status, and soil organic matter content have significant influence.19. Studies of Sauerbeck and Rietz20 have indicated the existence of different binding forms and strongly pH dependent solubility effects of the trace elements. Soil cation exchange capacity (CEC) affect metal bioavailability and it depends on an organic matter and clay content of the soil. The toxicity of metals within soils with high CEC is low even at high total metal concentration and in contrast the toxicity is vice-versa in low CEC. Under oxidized or aerobic condition (+800 to 0 mV) metals are usually found as soluble cationic forms (Cu2+, Cd2+, Pb2+ & Ca2+) and in reduced or anaerobic conditions (0 to -400 mV) they are found as CuS, PbS, and CdCO3 precipitates. At low soil pH, the metal bioavailability increases due to its free ionic species and in contrast, high soil pH decreases due to insoluble metal mineral phosphate and carbonate formation. However, in contrast to other metal bioavailabilities, at high pH range of 5-9, nickel may be adsorbed on iron and manganese oxides, or form complexes with inorganic ligands (OH, SO42−, Cl or NH3)21.

Metal microbe interaction

Microbial interactions with heavy metals were reviewed by, Ehrlich15 and Beveridge and Doyle22. Microbial interactions with small quantities of metals or metalloids do not exert a major impact on metal or metalloid distribution in the environment, whereas interactions with larger quantities are required in energy metabolism, for instance, to have noticeable impact. However, due to strong ionic nature metals

<table>
<thead>
<tr>
<th>Metal</th>
<th>Use</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hg</td>
<td>Coal, vinyl chlorides, electrical batteries</td>
<td>Neurotoxic</td>
</tr>
<tr>
<td>Pb</td>
<td>Plastic, paint, pipe, batteries, gasoline, auto exhaust</td>
<td>Livercirrhosis, mental disturbance, cancer, ulcer and hypoketonosis</td>
</tr>
<tr>
<td>Ar</td>
<td>Pesticides, coal, detergent</td>
<td>Kidney damage, injury in CNS and mental retardation</td>
</tr>
<tr>
<td>Cd</td>
<td>Fertilizer, plastic, pigment</td>
<td>Nephritic, cancer and ulceration</td>
</tr>
<tr>
<td>Cr</td>
<td>Tanning, paints, pigment, fungicide</td>
<td>Vomiting, renal damage and cramps</td>
</tr>
<tr>
<td>Zn</td>
<td>Fertilizer</td>
<td>Diarrhoea, low blood pressure and paralysis</td>
</tr>
<tr>
<td>Co</td>
<td>Vitamin B-12</td>
<td>Damage of liver, kidney, spleen and nervousness</td>
</tr>
<tr>
<td>Se</td>
<td>Coal, sulfur</td>
<td>Teratogenic, carcinogenic, genotoxic and mutagen</td>
</tr>
<tr>
<td>Ni</td>
<td>Electroplating</td>
<td>Carcinogen, acute and chronic poison</td>
</tr>
</tbody>
</table>

### Table 1 — Application of heavy metals and their toxic effects

### Table 2 — Summary of cation/anion solubility relationship of metals

<table>
<thead>
<tr>
<th>Anions</th>
<th>Cations</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>All anions</td>
<td>Na+, K+, NH4+</td>
<td>Soluble</td>
</tr>
<tr>
<td>NO3−, NO2−, C2H4O2−</td>
<td>All metals</td>
<td>Soluble</td>
</tr>
<tr>
<td>MnO4−, ClO4−</td>
<td>All metals except Pb2+, Ag+, Hg2+</td>
<td>Soluble</td>
</tr>
<tr>
<td>Cl−, Br−, I−</td>
<td>All metals except Ba2+, Sr2+, Pb2+</td>
<td>Soluble</td>
</tr>
<tr>
<td>SO42−</td>
<td>All metals except Ca2+, Ba2+, Sr2+</td>
<td>Insoluble</td>
</tr>
<tr>
<td>CO32−, PO43−, SO42−</td>
<td>All metallic salts except Na+, K+, NH4+</td>
<td>Insoluble</td>
</tr>
<tr>
<td>BO3−, F, SiO32−</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
bind to many cellular ligands and displace native essential metals from their normal binding sites, which are toxic. Eubacteria and archaeeae are able to oxidize Mn(II), Fe(II), Co(III), AsO$_4$$^2$- or reduce Mn(IV), Fe(III) Co(II) AsO$_4$$^3$- , SeO$_3$$^2$- on a large scale and conserve energy in these reactions. Some microbes reduce ions such as Hg$^{2+}$ or Ag$^+$ to Hg$^0$ and Ag$^0$ respectively, but do not conserve energy from these reactions. Prokaryotes methylate metal and metalloid compounds producing corresponding volatile metal derivatives. The bacterial oxidation of AsO$_3$$^-_4$ to AsO$_5$$^-_4$ by a strain of Alcaligenes faecalis and reduction of CrO$_4$$^2$- to Cr(OH)$_3$ by Pseudomonas fluorescens LB 300 or Enterobacter cloaceae are examples of redox reactions involving enzymatic microbial detoxification of harmful metals or metalloids. Microbes secrete inorganic metabolic products such as sulfide, carbonate or phosphate ions in their respiratory metabolism and with them precipitate toxic metal ions as a form of non-enzymatic detoxification. In nature microbes immobilize metals through cellular sequestration and accumulation, or through extracellular precipitation.

**Conventional method of heavy metal removal from soil and water**

Various methods are available for the removal and management of heavy metals, which involve technical inputs. Current methods used for treating soils contaminated with toxic metal are 1) Land filling: the excavation, transport and deposition of contaminated soil in a permitted hazardous waste land, 2) Fixation: the chemical processing of soil to immobilize the metals, usually followed by treatment of the soil surface to eliminate penetration by water and 3) Leaching: using acid solutions as proprietary leaching agent to desorb and leach metals from soil followed by the return of clean soil residue to site.

Approaches to reduce heavy metal contamination in water include: 1) precipitation or flocculation, followed by sedimentation and disposal of the resulting sludge, 2) ion exchange, 3) reverse osmosis, 4) microfiltration, 5) electrodialysis and 6) evaporation. Apart from these, other methods like foam flotation, liquid membrane technique, solvent extraction and crystallization can also be employed.

**Bioremediation techniques**

The goal of microbial remediation of heavy metal contaminated soils and sediments are to immobilize the metal in situ to reduce metal bioavailability and mobility or to remove the metal from the soil. Pazirandeh et al. described the metabolic pathways resulting in bioprecipitation of heavy metals or their biotransformation. Low cost and higher efficiency at low metal concentrations make biotechnological process very attractive in comparison to physico-chemical methods for heavy metal removal. Metal remediation strategies using microorganisms can minimize the bioavailability and biotoxicity of heavy metals. Biostimulation, stimulation of viable native microbial population, bioaugmentation, artificial introduction of viable population, biocaccumulation use of live cells, and biosorption, use of dead microbial biomass, are the cost-effective innovative bioremediation technologies. Biological approach for metal detoxification affords the potential for selective removal of toxic metals and operation flexibility and easy adaptability for in situ and ex situ application in a range of bioreactor configurations. In the past few decades, new metal treatment and recovery techniques based on biosorption have been explored using both dead and living microbial biomass with remarkable efficiency. Prokaryotic and eukaryotic microbes are capable of accumulating metals by binding them as cations to the cell surface in a passive process.

**Microbes for metal remediation**

The mechanisms by which metal ions bind to the cell surface include electrostatic interactions, Van der Waals forces, covalent bonding, redox interactions, and extracellular precipitation, or combination of these processes. The negatively charged groups (carboxyl, hydroxyl, and phosphoryl) of the bacterial cell wall adsorb metal cations, which are then retained by mineral nucleation. Biosorption studies of U, Zn, Pb, Cd, Ni, Cu, Hg, Th, Zn, Cs, Au, Ag, Sn and Mn, showed that the extent of sorption varies markedly with the metal as well as with the microorganisms (Table 3).

Surfactants such as rhamnolipid produced by Pseudomonas aeruginosa show specificity for certain metals such as Cd and Pb. Higher molecular weight (~10$^6$) bioemulsifiers such emulsan, can also aid in metal removal. Studies of Sand et al. revealed that Thiobacillus ferroxidans and Leptospirillum ferroxidans are capable of oxidizing iron and sulfur. Joeger et al. reported that metal accumulating bacterium Pseudomonas stutzeri AG 259 is capable of producing silver based single crystals, which can reduce the toxicity of metals.
Mechanisms of metal tolerance

Organisms respond to heavy metal stress using different defense systems (Fig. 1) such as exclusion, compartmentalization, formation of complexes and synthesis of binding proteins like metallothioneins (MTs) and phytochelatins (PCs). Ochard has divided general toxicity mechanism for metal ions into three categories, 1. blocking the essential biological functional groups of biomolecules especially proteins and enzymes, 2. displacing the essential metal ion in biomolecules and 3. modifying the active conformation of biomolecules resulting the loss of specific activity. Microorganisms can affect heavy metal concentrations in the environment because they exhibit a strong ability for metal removal from solution; this can be achieved through either enzymatic or non-enzymatic mechanisms. Avoidance, restriction of metal entry into the cell, either by reduced uptake/active efflux or by the formation of complexes outside the cell and sequestration, reduction of free ions in the cytosol either by synthesis of ligands to achieve intracellular chelation or by compartmentalization are the two major strategies of organisms to protect themselves against heavy metal toxicity. The general mechanisms of metal tolerance in microorganisms are shown in Table 4.

Metallothioneine

Metallothioneines, discovered about 45 years ago, play a central role in heavy metal metabolism and in the management of various forms of stress. Metallothioneines (MTs) are low molecular weight (6-7 kDa), cystine-rich proteins, divided into three different classes on the basis of their cystine content and structure. The Cys-Cys, Cys-X-Cys and Cys-X-X-Cys, motifs (in which X denotes any amino acid) are characteristic and invariant for metallothioneine. Studies of Lichtlen and Schaffner show metal

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Elements</th>
<th>Uptake (% dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zoogloe spp.</td>
<td>Co</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Ni</td>
<td>13</td>
</tr>
<tr>
<td>Citrobacter spp.</td>
<td>Cd</td>
<td>170</td>
</tr>
<tr>
<td></td>
<td>U</td>
<td>900</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td>Cu</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>14</td>
</tr>
<tr>
<td>Chlorella vulgaris</td>
<td>Au</td>
<td>10</td>
</tr>
<tr>
<td>Rhizopus arrhizus</td>
<td>P</td>
<td>10</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>Hg</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Th</td>
<td>19</td>
</tr>
</tbody>
</table>

Table 3 — Example of microorganisms that take up heavy metals

Fig. 1 — Heavy metal toxicity mechanisms to microbes
Table 4.—Mechanism of metal tolerance in microorganisms

<table>
<thead>
<tr>
<th>Metal</th>
<th>Tolerance mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>AsO₄³⁻</td>
<td>Anion efflux (ATPase)</td>
</tr>
<tr>
<td>AsO₄³⁻ &amp; Sb³⁺</td>
<td>Efflux (ATP ase)</td>
</tr>
<tr>
<td>Cd²⁺ &amp; Zn²⁺</td>
<td>Efflux</td>
</tr>
<tr>
<td>Hg²⁺</td>
<td>Reduction</td>
</tr>
<tr>
<td>Co²⁺ &amp; Ni²⁺</td>
<td>Efflux</td>
</tr>
<tr>
<td>CrO₂⁶⁻</td>
<td>Decreased uptake</td>
</tr>
<tr>
<td>Cd²⁺</td>
<td>Cation efflux</td>
</tr>
<tr>
<td>CrO₂⁶⁻</td>
<td>Decreased uptake</td>
</tr>
<tr>
<td>Cu²⁺</td>
<td>DNA damage</td>
</tr>
</tbody>
</table>

regulatory transcriptional factor-I (MTF-I) was essential for basal and heavy metal induced transcription of the stress-responsive metallothionein-I and metallothionein-II. Metallothionein-like proteins have been isolated from the Cyanobacterium, Syneococcus spp. as well as Escherichia coli and Pseudomonas putida. Plasmid-encoded energy dependent metal efflux systems involving ATPases and chemiosmotic ion/proton pumps are associated to Ar, Cr and Cd resistance in Staphylococcus aureus, Bacillus subtilis, Listeria spp., E.coli, A.eutrophus, P.putida, some Cyanobacteria, fungi and algae. E.coli use a two-component membrane bound ATPase complex, ArsA and ArsB responsible for the active efflux of arsenite form the cell. In contrast Gram-positive bacteria lack the ArsA protein.

Genetics of metal resistance

Recent environment pollution with anthropogenic sources of metals has increased the need for research concerning microbial metal resistance as well as remediation. Large plasmids (165-250 kb), encoded specific metal conferring resistance to a variety of metals including Ag⁺, AsO₄³⁻, AsO₃³⁻, Cd²⁺, CO₃²⁻, CrO₂⁶⁻, Cu²⁺, Hg²⁺, Ni²⁺, Pb²⁺, Sb³⁺, TeO₂⁻, Ti⁴⁺, and Zn²⁺. Metal resistance systems have been encoded by chromosomal genes in some organisms like, Bacillus.spp (Hg-resistance) and E.coli. (Ar-efflux). Roane and Pepper categorized mechanism of metal resistance as 1) general and do not require metal stress 2) dependent on a specific metal for activation and 3) general and are activated by metal stress.

The plasmid-borne cadA gene encodes a cadmium specific ATPase in several bacterial genera, including Staphylococcus, Pseudomonas, Bacillus and E.coli. Schmid and Scheget reported the czc and nec operons are responsible for cadmium resistance in Alcaligenes eutrophus CH34 (now renamed as Ralstonia eutropha) and in A. xylosoxidans, respectively. The genetic determinants for heavy metal resistance in A.eutrophus CH34 are present in two plasmids, pMOL 28 and pMOL 30. Nickel resistance is encoded by pMOL 28 (163 kb) which occur by an efflux pathway via cation proton-antiporter chemiosmotic system. This removes the toxic metal, which is accumulated by the uptake of essential divalent cations (e.g. Mg²⁺, Mn²⁺). Taghavi et al. showed that nickel resistance is inducible and is due to an energy-dependent efflux system driven by chemiosmotic proton-antiporter system.

Genetic engineering for metal remediation

Genetic engineering allows introduction of desired traits into cells to metal clean up techniques, and this approach has already been used to construct cells for the bioremediation of mercury. Krishnasamy and Wilson constructed an E.coli strain that accumulated Ni²⁺ by introducing the mxa gene (coding for a nickel transport system) from Helicobacter pylori into E. coli JM109 that expressed a glutathione S-transferase metallothionein fusion protein. The recombinant E.coli strain accumulated four times more nickel than the wild type. Bang et al. engineered E.coli to produce sulphide by heterologous expression of the thiosulphate reductase gene from Salmonella enteritica, which enhanced protein synthesis leading to the precipitation of cadmium sulphide. Metal resistant R. eutropha isolate, engineered to produce metallothionein accumulated more Cd²⁺ than its wild type counterpart, and offered tobacco plants some protection form Cd²⁺ when inoculated into contaminated soil. Studies of Pazirandeh showed periplasmic expression of a Neurospora crassa metallothionein in E.coli generated cells that were superior to bacteria with cytoplasmic metallothionein location in terms of metal ion adsorption. Kotriha et al. engineered metal binding peptides that contain either histidines (GHHPHG) (HP) or cystines (GCGPCGCG) (CP) into LamB and expressed on the surface of E.coli. Surface display of CP and HP increased the bioaccumulation four and two fold, respectively.

Samuelson et al. reported recombinant Staphylococcus xylosus and S. carnosus, had gained Ni²⁺ and Cd²⁺ binding capacity and suggested that they could be used in bioremediation of heavy metals. They evaluated surface display systems for expression of two different polyhistidyl peptides, His₆-Glu-His₆ and His₄, which had good metal binding activity. The nickel resistant bacteria from anthropogenically polluted biotypes and naturally nickel-rich soils have been hybridized with various probes carrying our
from plasmid pMOL28 (A. eutrophus CH34), ncc from pTOM8 (A. xylosoxidans 31A) nre from pTOM8 (A. xylosoxidans 31A) and nre from Klebsiella oxytoca 15788. With respect to their hybridization signals, the nickel resistant determinants of the strains could be assigned to enr/ncc type, enrch/ncc type Klebsiella oxytoca type and others showing no hybridization\(^{40}\). Bacterial communities in soil amended for many years with sewage sludge that contained heavy metals were assessed using molecular tools such as, rRNA analysis, FISH (Fluorescence in-situ hybridization), cloning and sequencing\(^{55}\).

**Biosorption**

Biosorption, utilization of inexpensive dead or live microbial biomass to sequester metals from industrial effluents has gained importance in recent years due to their good performance, low cost, specificity, minimum sludge generation and amenability for repeated use\(^{34,56}\). Several active groups of cell constituents like acedamido group of chitin, structural polysaccharide of fungi, amine (amino and peptidoglycodies), sulfhydryal and carboxyl groups in protein, phosphodiester (teichoic acid), phosphate, hydroxyl in polysaccharides, participate in biosorption\(^{57}\). The general microbial mediated heavy metal sorption mechanisms are in the Fig. 2. Hernandez et al.\(^{3}\) isolated three species of bacteria belonging to family Enterobacteriaceae, which were capable of accumulating nickel and vanadium. Biosorption for metal removal has been studied extensively using various species of live and inactivated biomass of bacteria, algae, fungi or yeast\(^{57}\). The technology involving surface complexation, ion exchange and microprecipitation is a potential alternative to current metal treating processes that extend the duration of exposure of the clean up crews\(^{58}\). Research in the area of heavy metal removal from wastewater and sediments has focused on the development of microbial materials with increased affinity, capacity and selectivity for target

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**Fig. 2 — Mechanism of microbial metal tolerance**
metals\textsuperscript{28,54}. Fungal biomass is finding increasing
application in current biotechnological procedures for
the cleanup of nickel-polluted effluents and for the
recovery of metal ions\textsuperscript{1}. Rajendran et al.\textsuperscript{59} reported
\textit{A. niger} mutant M3 capable of absorbing 50\% more
nickel at 1.7 mM concentration than its parent strain.
The waste fungal biomass from industrial fermenters
may prove to be cost effective metal biosorptive
agents on a large scale\textsuperscript{60}. Fouvert and Roux\textsuperscript{61}
studied the use of mycelia of \textit{Mucor nicheri}, \textit{Aspergillus niger}
and \textit{Penicillium chrysogenum} from fermenters in the
removal of nickel, zinc, cadmium and lead by bio-
adsorption. Gad\textsuperscript{31} reported chemical modification
of biomass might create derivatives with altered metal
binding abilities and affinities. Pseudomonads have
been shown to be efficient in heavy metal
bioaccumulation from polluted effluents in the
immobilized state\textsuperscript{62,63}.

\textbf{Immobilization for metal remediation}

Biosorption technique for metal removal and
accumulation can be even with a low degree of
understanding of the metal binding mechanisms.
However, a better understanding of the technique will
be useful for effective and optimized application of
the method\textsuperscript{64}. Free cells can provide valuable
information in laboratory experimentation but have
limitations in industrial applications\textsuperscript{65}. The use of
freely suspended microbial biomass suffers with
disadvantages like small particle size, low mechanical
strength and difficulty in sequestering biomass and
effluent. The immobilized biosorbent biomass
packed in sorption columns, which are effective for
continuous removal of heavy metals can operate on
cycles consisting of loading, regeneration and
rinsing\textsuperscript{66}. Ibanez and Umetsu\textsuperscript{67} have demonstrated the
ability of protonated alginic beads in the removal of
chromium, copper, zinc, nickel and cobalt ions from
dilute aqueous solutions. Biomass immobilized in a
range of inert materials like, silica, polyacrylamide,
polymethane and polysulfone has been used in a
variety of bioreactor configurations, including
rotating biological contractors, fixed reactors, trickle
filters, fluidized beds and air lift bioreactors\textsuperscript{68}. Karna
et al.\textsuperscript{69} immobilized \textit{Phomopsis valderianum} BDU
30501 in polyvinyl foam and used for the removal of
\textit{Ca}\textsuperscript{2+}, \textit{Co}\textsuperscript{2+}, \textit{Cu}\textsuperscript{2+} and \textit{Ni}\textsuperscript{2+}. Ashiana et al.\textsuperscript{63}
reported that Ca-alginate immobilizing agent is very
effective in nickel biosorption and used inorganic salts like
\textit{NaCl} and \textit{Cu (NO3)}\textsubscript{2} for desorption of nickel from
immobilized microbial biomass. HCl and EDTA are
the most efficient desorption agents for nickel
removal\textsuperscript{62,68}.

\textbf{Plant-microbe interaction in metal remediation}

Heavy metal toxicity to plants can be reduced by
the use of plant growth promoting bacteria, free-living
soil bacteria that exert some beneficial effects on
plant development when they are applied to seeds or
incorporated to seeds\textsuperscript{70}. Plants can accelerate
bioremediation in surface soils by their ability to
stimulate soil microorganisms through the release of
nutrients from the soil and transport of oxygen to the
rhizosphere\textsuperscript{70}. Plant growth promoting bacteria that
contain ACC deaminase may act to insure that the
ethylene level does not impair root growth and
facilitate the formation of larger roots, which enhance
seedling survival\textsuperscript{71}. Burd et al.\textsuperscript{72} reported that a metal-
resistant soil bacterium \textit{Kluyvera ascorbata} SUD 165
promoted the growth of canola (\textit{Brassica campestris}) in
the presence of high concentrations of nickel and
the increase in growth rate is due to the ability of the
bacterium to lower the level of ethylene stress in the
seedlings. They further isolated siderophore producing
mutants, \textit{K. ascorbata} 165/26 which was able to provide sufficient ions for the growth of Indian
mustard (\textit{Brassica juncea}) and tomato (\textit{Lycopersicon
esculentum}) in the presence of high concentrations of
\textit{Ni}, \textit{Pb}, and \textit{Zn}. Studies of Burd et al.\textsuperscript{73} suggested the
best method to prevent plants from becoming
chlorotic in the presence of high levels of heavy
metals was to provide them with a siderophore-
producing bacterium, \textit{Kluyvera ascorbata} SUD 165.
Mycorrhizal fungi also reduce metal toxicity to their
host plant by binding heavy metals to their cell wall or
surrounding polysaccharides. Binding sites within
cells of ectomycorrhizal fungi are metallothionein-
like proteins, which have high cystine content and
perhaps polyphosphate granules\textsuperscript{74}.

\textbf{Field applications}

The most important biotechnological application of
metal-microbe interaction is in bioleaching,
bioremediation of polluted sites and mineralization of
polluting organic matter\textsuperscript{16}. Uranium is extracted from
uranite ore through bioleaching by indirect action
with \textit{T. ferrooxidans}. In this case, ferric iron in acidic
solution, which the organism generate when oxidizing
pyrites (\textit{FeS}2), oxidizes insoluble \textit{UO}2\textsuperscript{2+} to soluble
\textit{UO}2\textsuperscript{4+}. The extraction of metals such as \textit{Co}, \textit{Mo}, \textit{Ni},
\textit{Pb}, and \textit{Zn} from sulfide ores by bioleaching is
technically feasible. Gencor (South Africa) is currently
developing the BioNIC process to recover nickel from low-grade sulfide ores75. Signet Technology Inc. and Signet engineering Pvt. Ltd. (Western Australia) are currently developing a process to recover cobalt from pyrite ore from the kares cobalt project in Uganda76. Various microbially reducible metals, especially ferric iron in complexed form to keep it soluble at circum neutral pH, can be used as terminal electron acceptors in situ anaerobic bioremediation of sites polluted with toxic organics77. It is possible to bioremediate in situ sites polluted with chromate or dichromate [Cr (VI)] by stimulating the reduction of the Cr (VI) to Cr (III) by bacteria85. Fungi can convert oxidized selenium to volatile-methylated selenides for escape into the atmosphere, a removal by volatilization88. Beseker89 reported Penicillium joniculosum was able to extract more than 50% Ni and 75% Zn from test solutions containing 100 mg l⁻¹ of metal at pH 6.6 and 6.5 respectively. A potent algal biosorbent Alga SorbTM developed using a fresh water alga Chlorella vulgaris and AMT-BIOCLAIMTM (MRA) developed using Bacillus biomass are used to treat wastewater and metal recovery respectively89.

Future prospects

Microbes can significantly affect the distribution of metals in the environment, since they have developed means to use them for their benefit. This clearly holds promise for effective, economical and eco-friendly metal bioremediation technology for industrial exploitation and pollution free environment. A good and efficient metal biosorbent can replace the commercial ion exchange resins that have been used conventionally for metal removal. However, the basic knowledge of microbial metal bioremediation mechanism is inevitable for the development of commercially viable potent biosorbent. Although several microbial metal bioremediation approaches are established to combat heavy metal pollution from anthropogenic and natural sources, none is yet in wide spread use. Innovative, economically feasible and novel biomass regeneration and conversion of the recovered metal into usable form are the best options to attract more usage of biosorbents. It is time to initiate more comprehensive interdisciplinary approach between biotechnologists and metallurgists to bring lab scale bioremediation process to land scale technology that will be acceptable for industrialists.

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