MICROBIAL BIOREMEDIATION OF HEAVY METALS AND RADIONUCLIDES

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Abstract:
Microorganisms are ubiquitous in all kinds of environments. These can be found in extreme conditions including heavy metal/radionuclide enriched milieus. Such microbes have developed various mechanisms to adapt to such niches and survive under metal stress. The various strategies adopted by these organisms include sorption of metal to the cell surface, biofilm formation, accumulation of metal with intracellular molecules, mineralization of metals to form metal precipitates and reduction of metals to a lesser oxidative state. These mechanisms result in decreasing the overall bioavailability of the metals thereby diminishing their toxic effects. Efforts have been made at the Bio-science Group (BSG), BARC to gain a fundamental understanding of metal/uranium-microbe interactions that can be utilized for remediating metal contamination from the environment. Such activities to harness the bioremediation potential of indigenous and recombinant microbes for alleviating metal/uranium contamination have been detailed in this chapter.

1. Introduction:

The elements with an atomic density of more than 5 g/cm^3 are termed heavy metals. These can be essential and non-essential for cellular functions. Metals in microbial cells either act as cofactors for enzymes in various biochemical reactions or are important for the folding and stabilization of structural proteins. Essential heavy metals include zinc, copper, iron, cobalt, manganese, and nickel amongst others. At elevated concentrations, even essential metals are toxic to the cells. Non-essential metals like cadmium, mercury, and lead can cause mis-

metalation of proteins[1]. Thus, the cells need to balance the levels of essential metals and efflux out the toxic metals to function optimally. Metal homeostasis involves the regulation of the uptake, sequestration, and efflux of metals.

Various human activities including mining, industrial applications, agricultural practices, production of pharmaceuticals, nuclear fuel processing and technological applications have contributed to large amounts of heavy metals including uranium (U) being released into the environment, thereby contaminating air, soil and water. These then enter into the biological system exerting toxic effects. The major problem associated with metal pollution is that metals cannot be degraded completely and persist in the environment forever. Physico-chemical methods of remediation include membranes based on ion exchange and reverse osmosis, electrochemical treatment, chemical precipitation, evaporation, and chelation[2]. These strategies require high reagent volume making them expensive and often resulting in toxic by-products. In contrast, biological remediation or bioremediation offers an economical and eco-friendly alternative solution for removing metal contamination.

Microbes have developed various strategies to combat heavy metal stress and thus maintain metal homeostasis. These metal alleviation strategies have been exploited for bioremediation. This chapter discusses the various mechanisms employed by microbes for surviving heavy metal stress with special reference to uranium. Known for its physiological irrelevance in biological systems, uranium induces chemical toxicity rather than radiotoxicity. Uranium causes oxidative damage, suspension of DNA metabolic processes like replication, transcription and translation leading to loss in cell viability[3]. Heavy metal or uranium bioremediation by microbes results in immobilization of the metal/U and primarily results in volume reduction of the contaminating solution. Some of the studies in this context have been undertaken at the Bio-science Group (BSG) at Bhabha Atomic Research Centre (BARC) and their potential for practical purposes have also been explored, which will be described in the following sections.

2. Biosorption:

Biosorption is the first line of defense employed by microbes against heavy metal toxicity. It is defined as the passive sorption of metals on the surface of biomass[4]. The biomass can be living or dead. It is a rapid phenomenon of metal sorption which is a metabolically independent process and takes place in two phases: biosorbent in the solid phase and the solvent containing the heavy metal pollutant in the liquid phase[5].

The physico-chemical interaction between the various functional groups present on the cell surface is responsible for biosorption. These could be ion exchange, adsorption, electrostatic and hydrophobic interactions. Microbial surfaces composed of polysaccharides, proteins and lipids are rich in moieties necessary for interaction with the metals. The various functional groups present on the microbial cell surfaces for metal interaction include hydroxyl, carboxyl, amino, ester, sulfate, carbonyl and phosphates[6].

2.1. Factors affecting biosorption:

pH: It is the most important parameter that governs metal sorption. pH determines the type of metal complex formed, thereby the oxidation state of the metal and its solubility. pH also affects the charge on the functional groups on the cell surface. Biosorption is significantly

reduced at lower pH due to the competition between metal cations and protons for binding sites[7]. At physiological near-neutral pH, the various moieties on the microbial cell surface are present primarily as anionic species that are targets for binding to cationic heavy metals.

Temperature: Biomolecules like proteins gets denatured at high temperatures above 45ºC thus decreasing overall metal binding.

Metal affinity: The presence of multiple metals in the wastewater can compete for the same binding groups. In such cases, the binding affinity decides the order of metal adsorption[5].

Biosorbent and metal concentration: An optimum biomass and metal concentration is required for maximum metal binding due to the interference between the binding sites. As biosorption is a surface phenomenon, this in turn affects the surface area to-volume ratio available for binding[5].

2.2. Advantages and limitations of biosorption:

Biosorption is a widely used mechanism for practical applications. The main advantage is that there is a significant reduction in the volume of waste as large volumes of contaminated water containing multiple metals can be treated simultaneously that are adsorbed onto the cell surface. It is one of the cheapest methods of bioremediation as microbial biomass can be generated easily at a low cost. As dead biomass can also act as effective biosorbents, it can be used over a range of temperatures and other external conditions. The microbial biomass can be regenerated for further usage by treating it with appropriate metal chelating agents like EDTA, mineral acids like HCl etc. The major limitation seen with biosorption is that the microbial surfaces can get saturated easily, hence it can be used only for treating contamination containing low levels of heavy metals. As many metals can bind to the same sites, essential metals like zinc which are non-toxic at low concentrations also inhibit the binding of toxic metals like cadmium.

2.3. Lab-scale studies of U/heavy metal remediation by biosorption:

2.3.1. Naturally occurring microbes for biosorption:

Biosorption of uranium has been demonstrated by micro-organisms isolated from subsurface soils of uranium ore deposits in Domiasiat in North-East India. *Serratia marcescens* strains from these areas showed superior uranium tolerance with minimum inhibitory concentrations (MIC) in the range of 3.5-4 mM. These isolates showed >90 % U (VI) binding at concentrations ranging from 100-500 µM uranyl nitrate by 24 h at pH 3.5 retaining their viability[8]. Another bacterial isolate from the same site, *Chryseobacterium* sp. strain PMSZPI with MIC of 4 mM for uranium[9] showed 50–60% sequestration of uranium within 1 h which increased to ~90% by 24 h and loading up to 21.42 mg U g^{-1}dry wt of biomass when challenged with 100 µM uranyl carbonate at pH 7.0[10]. Microbial cells immobilized in various matrices have also shown potential for bioremediation. *Yarrowia lipolytica,* a marine yeast, encapsulated in calcium alginate beads showed maximum uranium binding of 73% within 1 h at pH 7.5 with a loading capacity of 1.15 mg U g^{-1} dry wt. biomass on exposure to 50 µM uranyl carbonate[11]. Under batch mode, sorption equilibrium followed the Langmuir isotherm model. Continuous flow through system studies have also been carried out with *Y. lipolytica* cells immobilized in polyacrylamide gels packed in fixed bed volume columns. In the first cycle of biosorption, ~56.29% (~134 mg/L) U was removed. Recovery of bound uranium and

regeneration of column up to five cycles by eluting bound uranium in 0.1 N HCl was also successfully demonstrated[11]. Uranium sequestration studies by unicellular marine cyanobacterium, *Synechococcus elongatus* strain BDU/75042 have also shown great potential to be used for remediation[12]. This strain could remove 72% of uranium within 1 h from 100 µM uranyl carbonate with a maximum adsorption capacity of 124 mg U g^{-1}dry wt of biomass. Fourier transform infrared (FT-IR) spectroscopy confirmed that extracellular polysaccharides (EPS) containing amide and deprotonated carboxyl groups were involved in interaction with uranium. The binding kinetics suggested monolayer adsorption on the cell surface which fitted into the Langmuir adsorption isotherm[12]. These studies were taken further to develop eco-friendly options for uranium recovery from simulated sea water[13]. Even though the uranium concentration in seawater is low i. e. 13 nM or 3 µg/L, by virtue of the large volume, seawater holds 1000 times more uranium (4.5 billion tonnes) than terrestrial deposits. Long-term experiments carried out with continual exposure of *Synechococcus* to simulated sea water (~30 L) showed a loading of 2.9 mg U/g in 4 weeks. 85-90 % desorption of uranium was achieved using 0.1 N HCl[13]. Apart from unicellular cyanobacterium, a marine filamentous, heterocystous cyanobacterium, *Anabaena torul*osa which was isolated from saline paddy fields of Trombay, Mumbai has also been studied for uranium sequestration. The uranium binding studies were carried out under phosphate-limited conditions mimicking the aquatic environments that are devoid of phosphates. *A. torulosa* cells showed a biphasic mode of uranium binding-initially fast binding of 48% uranium by 30 min (56 mg Ug^{-1} dry wt.) and then gradual phase, binding of 65% uranium with loading of 77.35 mg U g^{-1}dry wt. in 24 h from 100 µM uranyl carbonate solutions at pH 7.8[14]. In yet another study, a strain of *Arthrobacter* isolated from uranium-rich environment exhibited a high MIC of 400 mM for Cs and Sr. It sequestered 9,612 mg Cs g^{-1} dry wt in 12 h and 9,989 mg Sr g^{-1} dry wt of cells in 18 h on being exposed to 75 mM of Cs or Sr and showed tolerance to 1 kGy of ^{60}Co gamma radiation as well[15].

2.3.2. Cyanobacterial biofilm for metal remediation:

Cyanobacteria have multiple strategies, for their survival under extreme abiotic stresses. A principal visible adaptation mechanism employed by cyanobacteria is biofilm formation by themselves or in association with different bacterial species thus creating a protecting shield against various abiotic stresses[16]. Exopolysaccharides have been known to be involved in microbial mat system formation and harbor organisms like cyanobacteria that are also capable of generating exopolysaccharides[17]. Cyanobacterial exopolysaccharides possess two important unique characteristics (a) their anionic nature that can be attributed to presence of uronic acid[18] and that (b) they happen to be complex heteropolysaccharides with multiple types of monosaccharide units[18]. Commercially, cyanobacterial extracellular polymeric substances possess various applications such as drug delivery, metal sequestration, etc.[18]. Cyanobacterial biofilms and their exopolysaccharides have been explored for their potential to sequester heavy metals. These mats and the exopolysaccharides content in them as seen above have been the naturally evolved mechanism of heavy metal sequestration in soil and aqueous environments. Microbial mat systems have been used effectively for the bioremediation of heavy metals from solutions[19, 20]. Along similar lines exopolysaccharides released from cyanobacteria have been shown to be effective in sequestering Pb(II), Cu(II) and Cd(II) from water bodies[21].

Research on biofilm/exopolysaccharides mediated heavy metal tolerance and sequestration has shown that *Nostoc muscorum* cultures forming biofilms exhibited propensity to remove cadmium from aqueous solutions under laboratory conditions[22]. The cyanobacterium *N. muscorum* (Nm) formed biofilms that strongly adhere to glass surface. The Nm biofilm challenged with 1 ppm Cd(II) could sequester ~62% and ~70% Cd after1 and 3 h respectively at pH 7[22]. The Nm biofilm was found to sequester Cd(II) from solutions with as low as 0.05 ppm Cd(II) and as high as up to 100 ppm[22]. The ability of Nm biofilm to sequester Cd(II) at various pH showed that the Nm biofilm sequestered between 55-80% Cd(II) from pH 5 to 9[22]. *N. muscorum* biofilm did not exhibit any sign of lysis even after 24 h exposure to 100 ppm Cd and the adsorption capacity of the biofilm system ranged from 0.3-1mg/g for 20 ml to 1000 ml of Cd(II) solution[22]. High-resolution atomic force microscopy revealed changes on the cell surface such as a 2-fold increase in surface roughness and an 18% increase in cell size due to Cd(II) treatment. Biophysical techniques such as SEM-EDS, XPS and FTIR analysis showed that >C=O and >C=N functional groups are involved in the chemisorption of Cd(II) on to the biofilm[22]. The *N. muscorum* based biofilm system could also sequester multiple metal species from aqueous solutions[23].

Studies carried out on *N. muscorum* biofilm system showed binding of heavy metal with functional groups present on the surface that are possibly contributed by exopolysaccharides[22]. Studies were initiated to improve the exopolysaccharide content and understanding their relevance to heavy metal in *Nostoc* PCC 7120. In this regard, the gene encoding the protein ExoD (*alr2882*) was overexpressed in *Nostoc* PCC7120. This strain was designated (*AnexoD*$^+$) and it was observed that exopolysaccharide content in this strain was 3.28-fold higher in comparison to the corresponding control culture suggesting a direct correlation between exopolysaccharide synthesis, export and *exoD* expression in *Nostoc* 7120[24]. The *AnexoD*$^+$ strain formed tightly adhering biofilm. *AnexoD*$^+$ also produced nearly 4-fold more intracellular mono/polysaccharide[24]. Overexpression of ExoD also showed no change in the monosaccharide content of exopolysaccharide when analysed with NMR and HPLC. *AnexoD*$^+$ cells exhibited higher heavy metal tolerance in comparison to non-transformed cells suggesting the role of exopolysaccharides in providing tolerance against heavy metals[24].

3. Bioaccumulation:

Many studies often use biosorption and bioaccumulation interchangeably. However, the main difference between bioaccumulation over biosorption is that the former is a metabolically active process. The first step is internalization of the heavy metals inside the cell which is mediated by energy-dependent metal transporters present on the cell membrane. Once internalized, these are sequestered by metal binding proteins like metallothioneins, peptides and other intracellular molecules like polyphosphates. Factors like temperature and pH affect bioaccumulation in a similar manner like that of biosorption. The additional nutritional constraints also affect this process as metabolically active living cells are required.Further, bioaccumulation of heavy metals poses toxic effects on the live cells that are to be used for practical applications. This can be circumvented by using genetically modified organisms or using engineered peptides for metal binding.

3.1. Metallothioneins (MTs) for metal detoxification

Metallothioneins as the name suggests, bind metals via thiolate clusters. These are cysteine-rich, low molecular weight intracellular proteins that are induced under metal elevated conditions. These were first identified as cadmium binding proteins[25]. MTs play a key role in the detoxification of toxic metals and the homeostasis of essential metals. The first prokaryotic metallothionein to be fully characterized was cyanobacterial metallothionein. Unlike eukaryotic MTs, in which only cysteine residues are involved in metal binding, bacterial metallothionein, SmtA was shown to coordinate to four zinc ions via nine cysteine residues and two histidine residues[26]. SmtA has been shown to interact with uranyl ion, UO_2^{2+} via carboxyl groups of glutamate and aspartate residues. The protective role of SmtA against uranium toxicity was also demonstrated when it was overexpressed in *E. coli*[27]. A SmtA homolog from *Anabaena* sp. PCC 7120, NmtA was identified and characterized[28]. Transcriptional induction of *nmtA* was seen in the presence of metals like cadmium, zinc and copper. NmtA protected against cadmium toxicity when overexpressed both in *E. coli* and its native strain *Anabaena* 7120[28]. However, increased cadmium accumulation was not seen following *nmtA* overexpression. This could be due to other active mechanisms of metal efflux pathways in the cell. Such constraints could be circumvented by adopting strategies like surface display of metal binding protein. SmtA when expressed as a fusion protein with Hpi on the surface of *Deinococcus radiodurans* showed cadmium loading of 1.2 mg g^{-1} dry wt biomass and 3-fold higher Cd^{2+} removal than control cells[29].

3.2. Intracellular polyphosphates (polyP):

Polyphosphates are present in almost every form of life. They are short and long-chain polymers comprising residues of orthophosphates ranging in order from ten to hundreds linked to each other linearly through high-energy phosphoanhydride bonds like ATP. PolyP are associated with multiple functions including inorganic phosphate and energy storage, ATP substitution, motility, stress adaption, biofilm development, quorum sensing and metal chelation[30]. The regulation of intracellular polyphosphate level is via polyphosphate kinase, PPK wherein there is reversible transfer of the phosphate group of ATP to polyP[31]. The exopolyphosphatase (PPX) catalyzes the removal of the terminal phosphate of polyphosphate irreversibly[32]. Poly P has been shown to bind toxic metals intracellularly thereby enhancing resistance to metals. Various bacterial strains including *Acidithiobacillus ferrooxidans* isolated from U mining waste found to accumulate U within polyphosphates intracellularly[33]. Our laboratory reported the uranium sequestration by unique surface associated polyphosphate bodies (SAPBs) in cyanobacterium, *Anabaena torul*osa[34]. Scanning electron microscope (SEM) with Energy Dispersive X-ray (EDX) confirmed the presence of uranium and phosphate on the cell surface. The acid labile nature of the uranium associated with surface polyphosphates was observed by SEM that appeared as 'holes' on the cell surface[34]. Recently, the role of polyphosphate in uranium tolerance was studied in *Anabaena torulosa*[35]. Polyphosphate rich ($PolyP^+$) and deficient ($PolyP^-$) cells were generated by altering the concentrations of phosphate in growth medium. $PolyP^+$ cells showed an increase in phosphate by ~6-7 times as compared to wild-type cells. Accumulation of polyphosphate in *Anabaena torul*osa provided significant tolerance towards the U toxicity probably binding U within the polyphosphates[35].

4. Biomineralization/bioprecipitation:

Heavy metal contaminants, in contrast to organic pollutants, cannot be destroyed. However, microbial metabolism can convert, mineralize, and immobilize them. The metal speciation and distribution are impacted by biomineralization, which alters their bioavailability and toxicity by metal-ligand complexation. The process of metals precipitating with various ligands like sulfides, phosphates, carbonates or hydroxides produced by microbes at the surface of the cells due to localized conditions is known as biomineralization. Bioremediation through biomineralization is a promising, cost-effective and environment-friendly process of removing heavy metal pollutants.

4.1. Metal precipitation as carbonates:

Urease belongs to the hydrolase class and produces ammonia and carbonic acid by hydrolyzing urea. During equilibration in water, these form bicarbonate, ammonia and hydroxide ions. The hydroxide ions cause an increase in pH that leads to the formation of carbonate ions by shifting the equilibrium of bicarbonate. These carbonate ions are used for the precipitation of heavy metals and radionuclides[36]. In addition to producing the urease enzyme, ureolytic bacteria offer nucleation sites for carbonate crystals. Microbial induced carbonate precipitation (MICP) exhibited by the urease-producing bacterium *Terrabacter tumescens* was beneficial at immobilizing metals like cadmium, copper, nickel, lead, cobalt, and zinc as carbonates[37].

4.2. Metal precipitation as sulphides:

Sulphate-reducing bacteria (SRB) use sulphate as electron acceptor terminally for anaerobic respiration and reduce sulphate to sulphide. Metal reduction occurs concomitantly with sulphides generating metal sulphide precipitates. The resulting metal suphides are sparingly soluble depending on their solubility product constants. The removal of various metals like Cd, Co, Cr, Cu, Mn, Ni and Zn in simulated acid leachate of soil by consortia of SRB cultures has been reported[38].

4.3. Metal precipitation as phosphates:

4.3.1. Phosphatase mediated precipitation:

The phosphate required for biomineralization resulting in the formation of metal-phosphate complexation can be obtained from mineral or organic phosphate via phosphatase enzymes. As phosphorous is an essential element whose soluble form is scarce in particular settings such as soil and water, various organic phosphate sources are required. 30-50% of all phosphorus is often found in organic forms. The phosphatases release Pi in the close vicinity of the cell that provide the sites for nucleation for biocrystallization[39]. The complexation and metal precipitation are facilitated by the interaction of the released phosphate ions with metal. Phosphatases catalyze the hydrolysis of phosphate esters under acidic, neutral and alkaline conditions depending on their optimal pH or localization i.e. membrane or extracellular. Various organic phosphate sources like glycerol-2-phosphate[40,41,10], glycerol-3-phosphate, phytate and tributyl phosphate have been reported to be utilized for phosphatase mediated bioprecipitation of metals.

The bioremediation of heavy metals such as Zn, Cu, Pb and Cd was demonstrated through microbial induced phosphate precipitation (MIPP) using phosphate mineralizing bacteria and glycerophosphate[42]. Similarly, an indigenous bacterial strain *Burkholderia ambifaria*, QY14,

isolated from Cd-contaminated farmland soil could also precipitate Cd utilizing sodium glycerophosphate as substrate[43]. Different environmental isolates *Bacillus* sp. and *Rahnella* sp. from the US DOE Oak Ridge site[44], *Serratia* sp. and *Chryseobacterium* sp. PMSZPI from the Domiasiat site in Meghalaya, India[41,10] competently precipitated uranium from contaminated solutions. The phosphatases efficiently precipitated uranium under anaerobic conditions[45] or in the presence of gamma radiation[41]. The Pi released from organophosphate donors via phosphatase enzyme precipitates U as uranyl phosphate mineral like meta-autunite that was confirmed by X-ray diffraction (XRD) analysis. The localization of these precipitates depending on the pH of the experiment and corresponding phosphatase (acid or alkaline) activity was visualized by transmission electron microscopy (TEM)[41,10]. Sometimes, the naturally occurring bacteria cannot be used in certain conditions like high ionizing radiation or radioactive wastes with highly acidic or alkaline pH. In such conditions, genetically engineered microorganisms (GEMs) expressing foreign genes from naturally occurring strains exhibiting the requisite attributes are generated using recombinant DNA technology. In similar lines, suitable hosts have been used for efficiently overexpressing microbial phosphatases and these GEMs can be used for heavy metal bioremediation with greater efficiency. *Escherichia coli* overexpressing *Citrobacter* PhoN demonstrated 2.5 times more uranium precipitation as compared to *Citrobacter* wild type constitutively expressing the PhoN[46]. *E. coli*, *Citrobacter* and *Pseudomonas* have shown effective phosphatase mediated precipitation but these are radiosensitive organisms and cannot be employed in radioactive wastes for metal precipitation. Recombinant strain of radiation resistant *Deinococcus radiodurans*, overexpressing phosphatases from *Sphingomonas* sp. and *Salmonella* sp. have been utilized successfully for precipitating uranium under alkaline or acidic conditions and high doses of ionizing radiation[40,47,48].

4.3.2. Polyphosphate mediated precipitation:

Inorganic polyphosphates in microbes can be employed for bioremediation of uranium from contaminated aquatic milieus. Filamentous marine cyanobacterium *A. torulosa* on long term exposure (120 h) to U in phosphate deficient medium exhibited extensive cell lysis, akinete formation, bleaching and hydrolysis of polyphosphates resulting in the release of phosphate consequently precipitating 88% of 100 μM U as meta-autunite mineral. The cells showed regeneration on germination of akinetes owing to the induction of alkaline phosphatase in U free environment[49].

5. Bioreduction:

The process of bioreduction refers to the biological reduction of metals to their lower redox states. This is a crucial process as the oxidized form of metals can be harmful to the environment due to its solubility. By utilizing biological systems, the bioreduction process helps to stabilize the metal and reduce its toxicity. Microorganisms play a crucial role in the geochemistry of subsurfaces in ecological habitats by utilizing metals or metalloids as terminal electron acceptors, effectively immobilizing them in nature. Given its versatility for bioremediation, microbial reduction has been extensively studied. The metal reduction in cellular metabolism plays several functional roles, including dissimilatory reduction for energy generation, assimilatory reduction for biosynthesis, or detoxification, and nonspecific reduction. It is important to note that certain contaminant metals and metalloids can be less

soluble or more volatile in their reduced state compared to their oxidized state. Metal reduction can be a beneficial method to help decontamination of metal from waste streams[4].

The intricate process of iron biogeochemical cycling has been extensively studied, with a specific focus on the conversion between its oxidized ferric and reduced ferrous states, which are facilitated by microorganisms. In anaerobic metabolism, Fe(III) plays the crucial role of a terminal electron acceptor, whereas Fe(II) acts as an electron donor in both aerobic and anaerobic processes. Fe (II) is an identified reductant that mediated the indirect reduction of the toxic Cr (VI) and Tc (VII) to non-toxic Cr (III) and Tc (IV) respectively. Some of the most well-studied bacteria for reduction include *Desulfovibro, Shewanella* and *Geobacter* genus, species of these groups decontaminate a wide range of heavy metals including U[50]. Uranium is typically present in a mobile form as uranium (VI) carbonate complexes. While U(VI) is soluble in most water bodies, U(IV) is insoluble. Thus, microbial reduction can be utilized to de-contaminate waters and waste streams by uranium precipitation. Certain microorganisms, such as *Geobacter metallireducens* and *Shewanella putrefaciens* can enzymatically reduce U(VI). These microorganisms utilize the oxidation of $–CH_3COO-$ or H_2 to facilitate their growth by coupling it with U(VI) reduction. Additionally, various *Desulfovibrio* species can reduce soluble uranium, but this metabolism cannot be utilized to support growth[51]. The reduction of U(VI) to U(IV) involves utilizing electrons from organic metabolism to convert the oxidized U(VI) into the reduced U(IV). This two-electron reduction process has been extensively studied and has been observed to proceed via U(V) intermediates during U(VI) reduction by *Shewanella oneidensis MR-1*. During in situ U(VI) bioremediation, *Geobacter* is predominately observed in the anaerobic microbial community. Due to the robust growth of *Desulfovibrio* species and its retention of reduction capabilities post-storage, these are focused on practical ex-situ uses. In the laboratory remediation set-ups, both *G. metallireducens* and *D. desulfuricans* rapidly precipitated high concentrations (1 mM) of U(VI) to U(IV) extracellularly forming uraninite (UO_2). These precipitates settled rapidly at the bottom of reaction vessels that could be separated by filtration. Microbial reduction is very effective in removing uranium over other methods of ion exchange and biosorption. Microbes could efficiently precipitate uranium from uranium carbonate complexes, resulting in the retrieval of pure and concentrated U. Certain species use organic compounds as electron acceptor for uranium reduction, thereby simultaneously reducing organic contaminants. This method provides a promising opportunity for on-site remediation of uranium in groundwater aquifers[50]. The biggest drawback of bioreduction is the reoxidation of absymally soluble U(IV) following remobilization as soluble U(VI) is detrimental to the accomplishment of long term remediation and hence it has not been taken up by our laboratory.

6. Conclusion:

Anthropogenic activities have resulted in an increase in heavy metals and radionuclides in the environment that pose serious health hazards. It is, therefore, important to understand the survival strategies of the microbes in combating metal stress. Fig. 1 summarizes the various mechanisms for metal detoxification. Bioremediation is an eco-friendly and cost-effective solution. The lab-scale efforts discussed in this chapter provide a promising solution to develop future technologies to mitigate metal/uranium contamination from the environment.

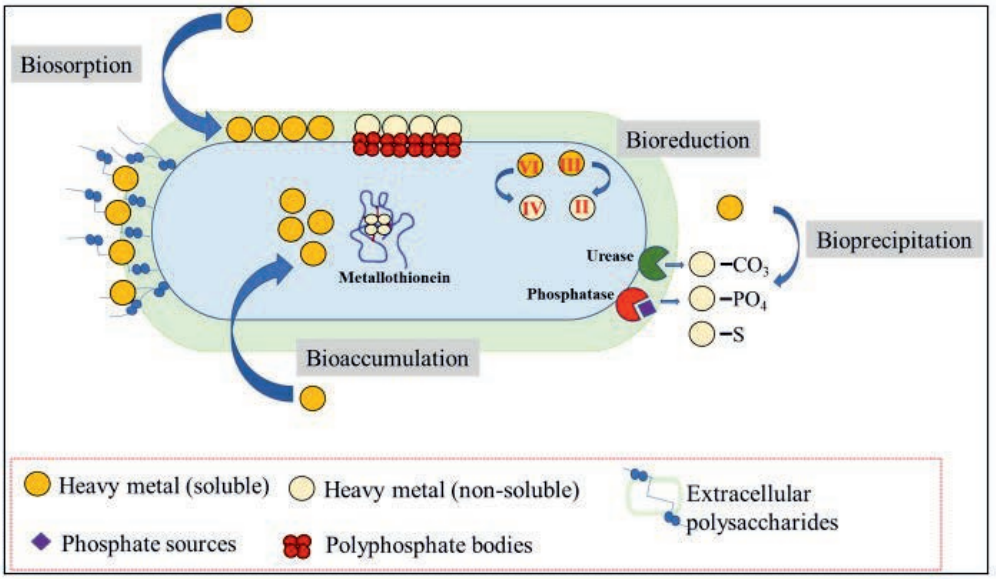

Fig. 1: Schematic illustrating the bioremediation mechanisms

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