Metal and radionuclide bioremediation: issues, considerations and potentials
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Recent demonstrations of the removal and immobilization of inorganic contaminants by microbial transformations, sorption and mineralization show the potential of both natural and engineered microbes as bioremediation tools. Demonstrations of microbe-mediated mineral formation in biofilms implicate this mode of microbial life in geological evolution and remediation of inorganic contaminants.

Introduction

Bioremediation of metals and radionuclides may seem a misnomer because no process can degrade and thus eliminate inorganic elements. Consequently, relatively little attention has been paid to the understanding of microbe–metal and microbe–radionuclide interactions and how they can be exploited to assure environmental problems. Remedial goals can be achieved by: first, the precipitation and thus immobilization of inorganic contaminants; second, the concentration and thus reduction in volume of contaminated matrices; and third, the compartmentalization of metals to a part of the environment in which their harm is reduced. Recent awareness of vast metal and radionuclide contamination of subsurface soils, the bioimmobilization of which may be the only feasible means to protect public health from the eventual contamination of groundwater, has motivated research on the interactions of microbes with metals and radionuclides. This research, at the interface between microbiology, geochemistry, geology and molecular biology, benefits from developments in nanoscale characterization of microbial communities in their natural habitats [1] and the characterization of cell-associated metal complexes [2]. As a result, an understanding of how microbes affect partitioning of metals between gaseous, solid and liquid phases in situ is emerging to provide a scientific basis for metal and radionuclide bioremediation.

The distribution and diversity of microbes inhabiting contaminated sites and of the genes that code for phenotypes facilitating metal–microbe interactions are critical elements in metal and radionuclide bioremediation. Such microorganisms are ubiquitous in the environment, and their frequency is often increased in contaminated soils [3] and water [4*]. Both a familiarity with the physiology of the active populations and an understanding of how beneficial reactions are controlled in situ are needed for successful remedial strategies. Biotransformation of indigenous flora may be successful if active organisms are widely distributed, whereas microbes with rare metabolic capabilities whose ecophysiology is compatible with in situ conditions may be suited for bioaugmentation approaches.

Recent excellent books and reviews have addressed metal–microbe interactions [5••], the effect of microbes on metal mobilization [6], biomineralization [7••] and microbial metal homeostasis [8]. In this review, we discuss issues pertinent to the applications of microbial transformations, sorption, and mineralization of metals and radionuclides to bioremediation. However, a closer look at these phenomena indicates that this distinction is artificial because each metal–microbe interaction may involve a combination of the three. Recombinant organisms, some with promising bioremediation activities [9••], are described, although their application must first address safety concerns with the release of recombinant organisms. Such organisms, however, could be useful in treatments of contaminated affluents where containment is feasible.

Metal transformations
The transformation of metals by microorganisms serves various biological functions. Microbes reduce metals in anaerobic respiration, resulting in detoxification and precipitation if the reduced forms are less toxic and less soluble [6]. Yet, in the case of mercury, a metal for which no biological function exists, reduction evolved specifically as a detoxification mechanism [4*]. Iron, sulfur and manganese are oxidized by chemolithotrophs [10], and arsenite is oxidized by chemolithoautotrophs [11]. Both microbes obtain energy from these processes. Magnetotactic bacteria use both reduction and oxidation of iron during the intracellular formation of magnetite [12•]. In addition, many microbiologically induced metal transformations, including reduction/oxidation and alkylation/dealkylation, serve no known biological function. They occur via processes whose primary functions are unrelated to the transformed metals but nevertheless alter their solubility, mobility and toxicity and thus may be employed in bioremediation strategies.

Pure cultures of microbes that reduce arsenate and selenium [13], chromate [14], and iron and manganese [15] belong to diverse phylogenetic groups spanning the entire bacterial domain. Few reports identify microbes that carry...
out transformation in situ. Snoeyenbos-West et al. [16] sequenced and analyzed 16S rRNA genes that were obtained from DNA extracts of sandy aquifer sediments to show that Geobacter spp. and Geothrix spp., but not Shewanella spp., were enriched by the addition of electron donors and electron shuttles to stimulate iron reduction.

Konopka et al. [3] based their identification of the bacteria as the domain selected for in lead-contaminated soils on fatty-acid-methyl-esterase analysis. Surprisingly, little is known about transformation of metals and radionuclides by archaea, although metal-contaminated environments may be considered to be extreme environments.

Whereas many transformations have been shown in culture, the potential for application depends on various factors pertinent to the specific catalytic capabilities and physiology of the organism, on environmental conditions and the nature of the contamination problem, and on societal and economic considerations. For example, many aerobic and anaerobic bacteria reduce Cr(VI) to the less toxic and less soluble Cr(III) by transferring electrons from various substrates using cytochrome-based respiratory chains and reductases. This activity is inhibited by Cr(VI) and other metals and phenolic compounds, compromising applications. On the other hand, the demonstrated coupling of Cr(VI) to the oxidation of organic contaminants in pure or co-cultures could facilitate applications in mixed waste effluents and contaminated sites [17•].

Hydrogen-dependent reduction of the radionuclide Tc(VII) carried out by periplasmic hydrogenases in Escherichia coli, Desulfovibrio desulfuricans [17•,18] and Geobacter sulfurreducens [19••] results in the precipitation of Tc-oxides in the periplasm. This process is insensitive to O₂, lyophilization and prolonged storage under N₂, making it particularly attractive for nuclear waste decontamination [17•]. However, the recently discovered precipitation of TcO₂ on the surface of magnetite formed during the acetate-driven reduction of Fe(III) to Fe(II) by G. sulfurreducens, which is enhanced by the electron shuttle anthaquinone-2,6-disulfonate, is a more promising process for the immobilization of Tc(VII) in groundwater aquifers because of the abundance of Fe(III), Geobacter sp. [19••] and natural electron shuttles in aquifer sediments (see also Update).

Whereas the products of Set(VI), As(V) [13,20], Cr(VI) [14,21] and Tc(VII) [17•,22•] reduction remain in the contaminated matrix, the reduction of Hg(II) results in volatile Hg(0) and a loss to the atmosphere. With the exception of a recently described Fe(II)-dependent reduction by an inner membrane cytochrome c oxidase in Thiobacillus ferrooxidans [23•], bacteria resistant to µM concentrations of Hg compounds reduce Hg(II) to Hg(0) by an NADPH-dependent mercury oxido-reductase (MR) that is encoded by the merA gene. Resistance to organomercury is due to organomercury lyase (OL), the merB gene product that, together with MR, degrades organomercury compounds to Hg(0) and a reduced organic moiety. merA, merB and genes specifying transport and regulatory functions are organized in the inducible mer operon [4•,24]. The mer-induced removal of Hg(II) is the most advanced application of metal transformations in bioremediation. Engineered and natural isolates have been successfully applied to remove mercury from wastewater [25,26,27••]. A packed bed bioreactor reduced the concentration of mercury in chemical wastewater from as high as 10 mg/L to below 50 µg/L, retaining 97% of the mercury load [27••] as Hg(0) droplets within the bed matrix [28•]. However, because mercury resistance is ubiquitous among bacteria [29], wastewater flora may be the best inoculant for such treatments. For example, Wagner-Döbler et al. [28•] reported the presence of a mixed culture consisting of 13 bacterial isolates in bioreactors that had been initially inoculated with a pure culture of an Hg(II)-reducing strain. By DNA sequencing, southern hybridization and restriction fragment polymorphism, 21 mer loci have been described in Gram-negative bacteria isolated from the primate gastrointestinal tract. These loci reveal that the operon and its individual genes posses a mosaic structure, and that their evolution is driven by frequent horizontal gene transfer and recombination events [30]. To the best of our knowledge, such studies have not been extended to mercury-resistant strains from water and soil.

The in situ bioremediation of mercury is more challenging than end-of-pipe solutions, because mercury that persists in the environment is associated with more complex matrices and is usually in sulfide forms [31]. Here, the mer-specified pathway, comprising only one or two well-characterized enzyme activities, is an attractive choice for proof-of-concept demonstrations. Most remarkably, cloning of a mer operon in Deinococcus radiodurans, the most radiation-resistant organism known, resulted in Hg(II) reduction in the presence of 6000 rad/hr [32•]. Strains were constructed with the prospect of remediating United States Department of Energy subsurface sites containing radionuclides, metals and organic contaminants [33]. The bacterial mer system was used to engineer several species of plants for phytoremediation of inorganic mercury [34,35] and organomercury [36,37••]. These plants emit Hg(0) into the air. The atmospheric deposition of Hg(II), following the oxidation of Hg(0) in the atmosphere, remains a major source of Hg in the environment [38], raising concerns that the strategy will contribute to Hg(0) emissions. Although calculations suggest that plant-enhanced emissions would have little effect on the global atmospheric Hg(0) pool [34], public perception may present a serious hurdle for the implementation of this mer-based phytoremediation strategy.

**Biosorption**

Biosorption is the passive sequestration of metals and radionuclides by interactions with live or dead biological matter and is, at present, the most practical and widely used approach for the bioremediation of metals and radionuclides. Biosorption is an effective treatment of wastewater [39••], but its potential in soil is less advanced [40]. Ion exchange, adsorption, microprecipitation, and electrostatic and hydrophobic interactions facilitate biosorption. Whereas
mechanisms of metal binding by individual cellular organelles and chemical moieties are known [39**], sorption of metals to intact cells or microbial biomass is governed by a multiplicity of mechanisms and interactions and thus not always fully understood. Langley and Beveridge [41*], who attempted to understand the role of carboxyls in the binding of metal cations to O-side chains of lipopolysaccharide (LPS), concluded that metals bound most likely to phosphoryl groups in the core–lipid A of LPS, and that the negatively charged side chains influenced binding to Gram-negative bacteria by affecting cell hydrophobicity. Likewise, the kinetics of metal binding to fungal biomass suggested that “heterogeneous nonequivalent interactions are made complex by the multiplicity of binding sites, charges, accessibilities and the properties of bound metals” [42].

Sorption of metals to cells is likely to play a critical role in all microbe–metal interactions. Interactions with specific groups on the surface of the cell may enhance or inhibit metal transport and, thus, transformation and biomineralization. For example, the precipitation of uranyl phosphate by *Citrobacter* sp. is initiated by an electrostatic interaction with phosphate groups in LPS. This interaction provides nucleation sites for mineral formation and initially protects an outer membrane phosphatase to allow for the subsequent release of phosphate and further crystallization [43**].

Elegant strategies have used recombinant DNA technology to produce specialized strains for the removal of metals by sorption. Most of these strategies equipped the bacterial cell surface with metal-binding polypeptides by fusing binding domains to outer-membrane-anchored proteins. Binding domains included polyhistidines [44], synthetic phytocelatines [45], randomly generated polypeptides [46*,47] and metallothioneins (MTs) [9**,48]. Whereas all these constructs showed an increase in metal binding, the MTs were the only ones to be tested in microcosms. This construct consisted of a protein fusion between the mouse MT and the autotransporter β-domain of the IgA protease from *Neisseria gonorrhoeae* placed under the control of inducible promoters in mini-transposon delivery systems [9**]. Expression of this fusion directed the MT to the cell surface, facilitating a threefold increase in binding of Cd(II) by several recombinants of Gram-negative bacteria [9**,48]. This modest increase in binding capacity was sufficient to improve growth of the tobacco plant *Nicotiana benthamiana* (a fivefold increase in biomass content, and an increase by 3.5 times in chlorophyll content) in soil contaminated with 150 µmol Cd(II) per kilogram of soil that was inoculated with 10⁸ cells per gram of an MTβ recombinant of the soil bacterium *Ralstonia metallidurans* CH34 (formerly *Ralstonia eutropha*) [9**]. The concept of using mini-transposons, a tool facilitating the mobility and maintenance of recombinant DNA, to equip microorganisms that are adapted to the target environment with bioremediation capabilities should find many applications in *in situ* bioremediation, provided that concerns regarding their containment are assuaged.

Another approach for the construction of recombinant bacteria with enhanced metal accumulation combines specific metal transporters with MTs that are located in the cytoplasm. *E. coli* constructs bound 20 times more Hg(II) [49*] and five times more Ni(II) [50] with transporters encoded by merTP (from *Serratia marcescens*) and nixA (from *Helicobacter pylori*), respectively. The Hg(II)-accumulating strain was subsequently shown to efficiently remove mercury from wastewater in hollow fiber bioreactors [51]. Moreover, affinity to Hg(II) exceeded that of chelating agents, suggesting the feasibility of a combined treatment by which metals released by chelators are subsequently sequestered by the recombinant strain. To date, none of these constructs has been used to remove metals from contaminated environmental material.

**Biomineralization**

Biomineralization is the formation of insoluble metal precipitates by interactions with microbial metabolic products. The realization of the importance of this process to mineral formation and geochemical evolution, a topic outside the scope of this manuscript, is one of the most exciting recent developments in research on metal–microbe interactions [8,52*,53**]. Microbial precipitation of metals and radionuclides as minerals of sulfide [53**,54,55], hydroxide [41*,52*], phosphate [53**] and carbonate [56] have potential and documented applications in bioremediation.

Polycrystalline NaUO₂PO₄ accumulates in and around the cell wall of *Citrobacter* sp. N14 by sorption to LPS and the activity of an outer membrane acid-phosphatase (PhoN), whose primary function may be to buffer the vicinity of the organism in low pH environments [43**]. Mineral formation drives two opposing gradients in the outer membrane, an incoming one of UO₂²⁻ and an outgoing one of PO₄, resulting in total removal of uranium from solution and binding of 1.1 mg NaUO₂PO₄ per milligram of cell (dry weight). A recombinant version of this mechanism was prepared by Keasling et al. [57], who cloned the gene encoding polyphosphate kinase (ppk) in *Pseudomonas aeruginosa* and showed that a shift from growth in the presence of high PO₄ to growth in low PO₄ resulted in the precipitation of a complex containing both phosphorus and uranium on the cell surface.

The formation of sphalerite (ZnS) by aerotolerant *Desulfobacteriaceae* in a natural biofilm concentrated Zn by a factor of 10⁶ from groundwater [53**], and precipitation of metal sulfides by sulfate-reducing bacteria (SRB) constituted the second phase of a combined sulfur oxidation/reduction biotreatment for soil decontamination [58*]. An engineered sulfide formation by *E. coli* expressing the thiosulfate reductase (*pshABC*) from *Salmonella enterica* serovar Typhimurium removed Cd(II) from solutions and formed a dark intracellular precipitate containing both cadmium and sulfur [59*]. An aerobic sulfide reduction system was engineered by Wang et al. [60*], who combined serine acetyl transferase with a highly active cysteine desulhydrase from *Treponema denticola*. A
recombinant *E. coli* strain removed Cd(II) from a 100 μM solution with efficiency exceeding 99% [60•]. The utility of these recombinant systems to treat contaminated environments or effluents remains to be demonstrated.

The underlying concept of precipitating metals as sulfides is the notion that their insolubility results in immobilization and reduced toxicity. However, the toxicity of metal sulfides may warrant investigation, as similar resistance levels to Cd(II) were observed with the *phsABC E. coli* recombinant and its DH5α parent (although the former removed significantly more cadmium from solution than the latter) [59•]. Immobilization of metal sulfides may be inappropriate under some circumstances. An example is the environmental production of methylmercury, the most toxic and most readily accumulated form of mercury [38]. Strong evidence suggests that Hg(II), the substrate for methylation, enters methylating SRB by diffusion as soluble HgS0 forms [61,62•] that are favored in the presence of polysulfides at high pH [63]. Thus, immobilizing mercury as HgS cannot be considered to be an environmentally safe practice. Another issue with the formation of metal sulfides is the lack of specificity that might limit availability of essential metals. SRB must have met this challenge during their evolution, possibly by the production of metal-binding metabolites that successfully compete with S2− for essential but not toxic metals [64]. The molecular basis for such selectivity is an interesting topic for research.

Biofilms are particularly conducive for biomining. Indeed, massive mineral formation has been described in both surfacial [65] and groundwater [53••] biofilms affected by acid mine drainage. Not only do biofilms support a large biomass, resulting in high microbial activities, but mineralization processes are promoted by limited mixing and the resulting high localized-solute concentrations, pH conditions and redox potential (Eh) (66•) in the vicinity of the cell. Biofilms of *P. aeruginosa* PAO1 [66•] bound significantly more Fe(III), Au(III) and La(III) than planktonic cells [41•]. The authors suggested that intracellular binding of Fe(III), larger crystals of reduced Au(III), and La(III) mineralization by increased cell hydrophobicity resulted from an altered mode of cellular growth and different local environments in the biofilm. The importance of the cell microenvironment in mineral formation is exemplified by *R. metallidurans* CH34. In this highly metal-tolerant bacterium [5••], the periplasm is an alkaline environment because of the depletion of protons, which are consumed by an antiporter, during toxic metal efflux. Respired CO2 is therefore converted to bicarbonate in the periplasm to form saturating concentrations of metal–bicarbonate, the precipitation of which results in up to five grams of precipitate per gram of biomass [56•].

Conclusions

Bioremediation of metals and radionuclides can be achieved by their immobilization, concentration and partitioning to an environmental compartment at which their hazard is reduced. The bacterial mer-mediated volatilization of mercury is the best example of how microbial transformations are exploited in wastewater treatments, and this process has a potential for *in situ* bioremediation as well. Biosorption to microbial biomass can be enhanced by using recombinant microbes that display metal-binding domains on their surface and accumulate metals intracellularly. The use of such strains requires efficient containment in the site of application. Mineralization by microbial metabolites immobilizes metals and radionuclides. Microbes in biofilms are especially active in mineralization because of the establishment of chemical gradients and physical conditions, in the cell envelope and the immediate vicinity of active cells, that are conducive to mineral formation.

Update

Work done most recently demonstrates that the reduction of iron hydr(oxides) by *Shewanella alga* indirectly stimulates Cr(VI) in a coupled two-step process [67], similar to that demonstrated for the immobilization of Tc(VII) [19•••]. When iron was limiting, Fe(III) was recycled upon reduction of Cr(VI) by Fe(II), allowing for continued biological iron reduction [67]. The stimulation of iron-reducing microorganisms may provide a method for the *in situ* immobilization and detoxification of Tc(VII) and Cr(VI) in subsurface environments containing both high and low levels of bioavailable Fe(III).

Acknowledgements

Financial support from the NABIR program of the US Department of Energy (DE-FG02-99ER62864) and the National Science Foundation (EAR-9910268) is appreciated.

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This study shows that negative charges on O-side chains of LPS in the Gram-negative outer membrane affect cation binding by influencing cell hydrophobicity and not by affecting electrostatic interactions.


This paper describes a mechanism for the precipitation of Na$_2$UO$_4$PO$_4$ by an initial nucleation step with phosphates in LPS followed by an acid-phosphatase-dependent polycrystal formation.


A description of fimbrial display systems for the exposure of synthetic polypeptides on the cell surface. The exposed polypeptide enhances metal adsorption, demonstrating an approach for the production of metal-sorbing biomass.


The phsABC gene was successfully expressed in E. coli, resulting in production of sulfide and the removal of Cd(II) most likely as cell-associated precipitate of CdS. This is a demonstration of an approach for the production of metal-sorbing biomass.


This paper contains a proof-of-concept demonstration of an aerobic sulfur reduction system, based on assimilatory SO$_4$ reduction, for the removal of metal sulfides.


The authors demonstrate an increased deposition of cytoplasmic crystals of Au(III) and cell-wall-associated La(III) in biofilm relative to crystal formation during planktonic growth, suggesting that physiology and physical-chemical conditions around cells in biofilms are conducive for metal mineralization.


This paper provides a description of the mechanism by which Ralstonia met-aluliduran CH34 deposits metal carbonates in the cell wall during growth in the presence of toxic metals.


Removal of metals from soils was achieved by a two-step process in which metals were initially leached by stimulating the activities of sulfur-oxidizing microbes, allowed by an anaerobic treatment during which metals were precipitated as sulfides by SRB.


The phsABC gene was successfully expressed in E. coli, resulting in production of sulfide and the removal of Cd(II) most likely as cell-associated precipitate of CdS. This is a demonstration of an approach for the production of metal-sorbing biomass.


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