Biosurfactant-facilitated Remediation of Metal-contaminated Soils

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Bioremediation of metal-contaminated wastestreams has been successfully demonstrated. Normally, whole cells or microbial exopolymers are used to concentrate and/or precipitate metals in the wastestream to aid in metal removal. Analogous remediation of metal-contaminated soils is more complex because microbial cells or large exopolymers do not move freely through the soil. The use of microbially produced surfactants (biosurfactants) is an alternative with potential for remediation of metal-contaminated soils. The distinct advantage of biosurfactants over whole cells or exopolymers is their small size, generally biosurfactant molecular weights are less than 1500. A second advantage is that biosurfactants have a wide variety of chemical structures that may show different metal selectivities and thus, metal removal efficiencies. A review of the literature shows that complexation capacities of several bacterial exopolymers was similar to the complexation capacity of a rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* ATCC 9027. — Environ Health Perspect 103(Suppl 1):59–62 (1995)

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Introduction

Remediation of soil contaminated with potentially toxic metal cations such as Pb^{2+}, Zn^{2+}, Cr^{3+}, Cd^{2+}, and Hg^{2+} has traditionally involved the excavation and transport of contaminated soil to hazardous waste sites for landfilling. Due to the great expense of traditional remediation, and recent U.S. Environmental Protection Agency (U.S. EPA) regulations that require pretreatment prior to landfilling (*1*), alternate cost-effective remedial techniques are needed. This has led to increasing interest in the application of microorganisms and microbial products to *in situ* remediation of metal-contaminated surface and subsurface soils.

Technologies using microorganisms and microbial products to remove metals have been successfully applied to wastestreams such as sewage sludge, industrial effluents, and mine water. Approaches used in these systems exploit microbial–metal interactions to concentrate and separate metals from the wastestream. These interactions, which are described in several recent reviews (*2–6*), include metal binding to the cell surface or within the cell

wall, translocation of the metal into the cell, volatilization of the metal as a result of a biotransformation reaction, and the formation of metal precipitates by reaction with extracellular polymers or microbially produced anions such as sulphide or phosphate (*3*).

In situ bioremediation of metal-contaminated soils presents a more complex separation problem due to the presence of soil. The soil surface area as well as the mineral and organic matter composition of the soil will determine the amount of metal sorbed. Metal sorption occurs through one of three mechanisms: cation-exchange, metal-ligand complexation to soil, or metal complexation with soil organic matter (*7*). Sorption by any of these mechanisms effectively limits the availability of metals for removal by flushing. Another complicating factor is the selectivity of a soil for a metal. In many instances soils are contaminated with a mixture of metals, and the relative affinity of the soil for any given metal in this mixture varies. Selectivity is both a function of ionic radius, for instance the sorption of $Hg^{2+} > Cd^{2+} > Zn^{2+}$, and of electron configuration, e.g., $Cu^{2+} > Ni^{2+} > Co^{2+} > Fe^{2+} > Mn^{2+}$ (*7*). Thus, the difficulty of removing specific metals from soil may vary.

Movement of metals in soils during soil flushing is also limited by the natural heterogeneities that occur in soil texture, structure, and organic matter content. These heterogeneities result in the development of complex networks of soil pores. Soil pores vary greatly in size ranging from less than 2 μm, approximately the size of a bacterial cell, to as large as 0.2 mm in diameter

(*8*). The smallest pores can act as a filter for metal-containing microorganisms and large colloidal–metal complexes and prevent transport of the metal through the soil. Movement of metals can also be retarded by the diffusion of metals into immobile zones created by small soil pores. The presence of metals in immobile zones can lead to extensive tailing, prolonging the flushing required to remove metals from the soil.

Discussion

Biosurfactants

One biologic technique that has potential for removal of metals from soil is the use of microbially produced surfactants (biosurfactants). Biosurfactants have the potential to impact the major factors that cause the removal of heavy metals from soil to be so difficult, namely, sorption, rate-limited mass transfer, and resistance to aqueous-phase transport. Biosurfactants are produced by plants, animals, and many different microorganisms (*9*). When considering approaches to remediation of contaminated sites, there are several apparent advantages to the use of biosurfactants rather than synthetic ones; they are biodegradable, they may be cost-effective, and it may be possible to produce them *in situ* at contaminated sites.

In general, surfactants are amphoteric molecules consisting of a nonpolar tail and a polar/ionic head. In aqueous solution, surfactants reduce surface tension by accumulating at interfaces and facilitating the formation of emulsions between liquids of different polarities. At low concentration,

surfactants are present as individual molecules. However, as the concentration of the surfactant is increased, a concentration is reached where no further change in interfacial properties takes place. The amount of surfactant needed to reach this concentration is called the critical micelle concentration (CMC). At the CMC, surfactant molecules aggregate to form structures such as bilayers, vesicles, or micelles. The type and size of aggregate formed depends on the surfactant structure and on the solution pH (10). Micelles are the smallest basic structure formed, generally less than 5 nm in diameter. A micelle is composed of a monolayer of surfactant molecules where the polar heads are oriented toward the surrounding aqueous solution and the nonpolar tails are oriented toward the hydrophobic center of the micelle. Vesicle structures are next in size and range from 10 nm to more than 500 nm in diameter. Vesicles are composed of surfactant bilayers, which are similar in structure to biological membranes. In aqueous solution, the polar surfactant heads of a bilayer face the outside while the nonpolar tails are sandwiched between the heads. Thus, the environment both inside and outside a vesicle is hydrophilic (aqueous) while the environment within the bilayer, composed of the nonpolar surfactant tails, is hydrophobic. Bilayers can also exist as flexible sheets or planar bilayers which are the largest of the basic surfactant structures. A bilayer sheet is essentially unlimited in size. If the solution on both sides of the bilayer is the same, the properties and behavior of the two bilayer surfaces will be identical. However, if the bilayer is at an interface, e.g., air–water or liquid–liquid, the bilayer may develop asymmetric properties. Typically, CMCs of biosurfactants range from 1 to 200 mg/l (11).

Biosurfactants are produced by many different bacterial genera. The chemical structure of biosurfactants varies widely, but all biosurfactants described thus far in the literature are anionic or nonionic. Biosurfactants can be classified into several broad groups: glycolipids, lipopeptides, lipopolysaccharides, phospholipids, and fatty acids/neutral lipids (11–13). The largest and best-studied group of biosurfactants are the glycolipids, which include the sophorose-, rhamnose-, trehalose-, sucrose-, and fructose-lipids. Both biosurfactant yield and composition are affected by growth conditions including carbon source, culture medium nutrients (e.g., nitrogen, phosphate, iron), temperature, pH, and agitation (14,15). In addition,

there are species level differences in the chemical structure of biosurfactants. For instance, the rhamnolipids produced by various *Pseudomonas* sp. differ both in the number of rhamnose molecules (1 to 2) and the length of the lipid moiety. Biosurfactant molecular weights range from approximately 500 to 1500 mw, although some exceptions exist, e.g., *Pseudomonas* strains growing on hexadecane have been reported to produce protein-containing surface-active substances with molecular weights of up to 14,300 (11).

Biosurfactants in Remediation

To date, interest has focused principally on the use of surfactants to remove organic contaminants from soil. Studies of organic contaminants have shown that both biological (16–18) and synthetic (19–29) surfactants can enhance either the chemical removal or the biodegradative removal of organic contaminants from soil. While these studies indicate the potential for use of surfactants to facilitate the removal of metals from soil, the literature contains very little actual information concerning surfactant removal of metal contaminants.

The goal of the use of surfactants for both organics and metals is similar; increase the apparent water solubility of the contaminant of interest to facilitate the removal by biodegradation or flushing. However, it should be noted that there are some key differences between metal-contaminated and organic-contaminated soils that must be considered. The most obvious difference is that unlike organics, metals cannot be biodegraded. In some cases metals may be transformed but transformation often only increases metal toxicity (e.g., $Hg^{++} \rightarrow CH_3-Hg^+$). A second difference to be considered between organic and metal contaminants is that organics of the most concern are neutral molecules, while metals are most often found as cationic species. Thus, since contaminant sorption depends on the chemical properties of both the soil and the contaminant, the choice of surfactant used for contaminant complexation will be important.

The addition of a biosurfactant may promote desorption of heavy metals from solid phases in two ways. The first is through complexation of the free form of the metal residing in solution. This decreases the solution-phase activity of the metal and, therefore, promotes desorption according to Le Chatelier's principle. The second is that under conditions of reduced interfacial tension, biosurfactants will accumulate at the solid-solution interface. This

may allow direct contact between the biosurfactant and the sorbed metal. The potential for biosurfactant-mediated desorption of metals is indicated by a study by Blakeburn and Scamehorn (30). In this study, a positively charged surfactant (cetylpyridinium chloride) was used to regenerate activated carbon beds saturated with a negatively charged organic solute (4-*tert*-butylphenol). The regeneration process involved desorption of the organic solute by a surfactant solution, followed by removal of the surfactant–organic solution. Analagously, the removal of cationic metals from soil would employ anionic biosurfactants to desorb cationic metals for subsequent removal by flushing.

A study by Beveridge and Pickering (31) examined the effect of a range of synthetic cationic, anionic, and neutral surfactants on the sorption of metals by clays. The cationic surfactants used were found to reduce the sorption of Cu, Pb, Cd, and Zn by montmorillonite, probably through competition by the cationic surfactant for negative sites on the clay surface (cation exchange). In contrast, cationic surfactants had little effect on sorption of metals by illite or kaolinite, which was attributed to a smaller influence of ion exchange due to the surface properties of these clays. Surprisingly, the anionic surfactants tested seemed to increase the sorption of the metals in this test system. The authors suggest that this may have been due to formation of metal–surfactant species, which precipitated from solution or sorbed to the clay surfaces.

Although it is well-known that microbial cells can complex metals from solution, there is little information in the literature concerning the use of biosurfactants to complex metals. Other microbial products such as bacterial and algal exopolysaccharides have been shown to bind a variety of metals. Emulsan, produced by *Acinetobacter* RAG-1 was found to bind up to 240 µg uranium (UO_2^{2+})/mg emulsan (32). Similarly, a *Pseudomonas* exopolysaccharide bound up to 96 µg uranium/mg exopolymer (33). A study of cadmium complexation by an *Arthrobacter* exopolysaccharide showed that cadmium binding (3.3 µg/mg exopolymer) was significantly less than that of uranium (34). A *Klebsiella* exopolysaccharide bound comparable amounts of cadmium (11 µg/mg exopolymer) as well as copper (22 µg/mg exopolymer) (35). A study of several marine *Pseudomonas* sp. exopolysaccharides showed complexation of copper, iron, lead, nickel, and zinc (36). When

studied separately, the affinity of the *Pseudomonas* exopolysaccharides for the metals generally followed the order: lead >> copper = iron > zinc > nickel. Interestingly, complexation capacity was species specific in this study with up to one order of magnitude difference in metal complexation between different *Pseudomonas* sp. In summary, metal complexation with exopolymers seems to exhibit both metal selectivity and metal complexation capacities that are species specific.

Exopolysaccharides differ from biosurfactants in that they are large (c.f., molecular weight of emulsan is 9.8×10^5), and have minimal surface activity, although they exhibit strong affinities for oil–water interfaces (37). Biosurfactants may offer an advantage over exopolysaccharides in the remediation of soils because of their comparatively small size. The potential for use of biosurfactants in removal of metals from soils is indicated by a study of biosurfactant complexation of cadmium (38). The bio-

surfactant used in this study was a monorhamnolipid produced by *Pseudomonas aeruginosa* ATCC 9027. This work showed that 92% of the Cd^{2+} in a 0.5 mM solution of $Cd(NO_3)_2$ was complexed by a 5-mM solution of rhamnolipid, a complexation of 22 µg/mg rhamnolipid. This value is comparable to the cadmium complexation capacities reported for *Arthrobacter* (34) and *Klebsiella* (35) exopolymers (3.3 and 11 µg/mg exopolymer, respectively). Cadmium complexation by rhamnolipid was stable from pH 6.0 to 7.0. Cryo-electron microscopy of the rhamnolipid structures formed in the presence of cadmium shows vesicles ranging in size from 10 to 300 nm in diameter, with a size distribution as follows: 71% of the vesicles were in the 10 to 50 nm range, 26% of the vesicles in the 51 to 250 range, and 3% of the vesicles were > 250 nm in diameter (39). The small size of these metal–rhamnolipid vesicles and the absence of metal precipitates in the metal–rhamnolipid mixtures should

facilitate the movement of metal–rhamnolipid vesicles in a complex system like soil.

While the use of microorganisms and microbial products, e.g., biosurfactants, in bioremediation of metal-contaminated soils shows promise, the development of remedial technologies will require further study in several areas. For instance, soils contain numerous cations that may compete with metal contaminants for biosurfactant complexation sites. Therefore, the selectivity of biosurfactants for metals both in solution and in soil systems must be investigated. There is also relatively little information about biosurfactant structure and structure sizes, or the effect of biosurfactant-metal interactions on these structures. Clearly, biosurfactant structure size and charge will affect movement of biosurfactant-metal complexes through the soil. In addition, structure size and charge will affect the access of biosurfactants to soil pores and therefore, the interaction of biosurfactant with sorbed metals.

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