Trace Metal Mobilization in Soil by Bacterial Polymers

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Enhanced transport of trace metal in porous media can occur in the presence of a ligand or “carrier” that has a high affinity for binding the pollutant, is dispersed and mobile in the soil environment, is recalcitrant with respect to microbial degradation, and is acceptable to the public. These aspects of the facilitated transport to trace metals are discussed with respect to a naturally occurring carrier: extracellular polymers of bacterial origin. The literature is reviewed regarding the production and composition of bacterial extracellular polymers, the processes relevant to the facilitated transport of trace metals in soil by bacterial polymers, and potential for transformation of polymers in soils by microbial degradation. Model calculations of contaminant retardation are presented for the case of polymer-mediated transport of cadmium in a sandy aquifer material. The available information suggests that extracellular polymers can bind metal ions and are mobile in the soil environment. Extracellular polymers also appear to be relatively slowly degraded by soil microorganisms. These properties and the supporting model calculations indicate that extracellular polymers of bacterial origin merit consideration as agents that may be applied to contaminated soils to enhance trace metal mobility. — Environ Health Perspect 103:Suppl 1:53–58 (1995)

Key words: trace metals, Pb, Cd, extracellular polymers, soil, enhanced transport

Introduction and Background

Many metallic elements play an essential role in the function of living organisms. However, overabundance of essential trace elements (i.e., Zn, Cu) and nonessential metals (i.e., Cd, Hg, or Pb) can result in chronic or acute (at high concentrations) toxicity. In addition, trace metals are often concentrated as they progress through the aquatic food chain. The nonessential metals, mercury, lead, and cadmium are of particular concern, both because of their toxicity and because significant amounts of these metals have been released through anthropogenic activities into the global environment. Our research has focused on the biogeochemistry of lead and cadmium. These metals display strong adsorption in contaminated soils at Superfund sites, and, consequently, their removal can be extremely difficult.

Adsorption reactions at the solid–water interface play a dominant role in regulating the concentrations and transport of most trace elements in soil systems. Dissolved macromolecules or colloidal particles that are dispersed in groundwater may bind trace metals, allowing the pollutants to be transported with these much more mobile “carriers” (1). This process is termed “facilitated transport.” Colloidal particles with diameter less than 10 μm in soil systems include: clays, microorganisms, and biological debris (2). Dissolved macromolecules in soil systems include humic substances and bacterial polymers (3). The engineered application of carriers that can facilitate the transport of heavy metals may have potential application in the remediation of contaminated sites. In addition, since many carriers exist naturally in soil and groundwater, knowledge of the effect of these particles and dissolved molecules on metal mobility can greatly improve risk assessment.

There are at least four criteria to consider when choosing a candidate carrier to enhance the mobilization of heavy metal contaminants: a) the carrier must have high affinity for the pollutant; b) the carrier must be dispersed and mobile in the soil environment (i.e., it must be stable [resisting aggregation] and must not be susceptible to filtration [of colloids] or adsorption [of dissolved molecules] in its passage through porous media); c) if it is organic, the carrier must have low biodegradability to prevent clogging of injection wells and to ensure that it persists sufficiently long to permit transport and removal of carrier-bound metal; and d) the use of the carrier must be acceptable to the public. These aspects of the facilitated transport of trace metal are discussed below with respect to one potential carrier: extracellular polymers of bacterial origin.

The chemical reactions between microorganisms and metals have been reviewed by Ford and Mitchell (4) and include: a) intracellular accumulation of metals (5,6); b) association of metals with the cell wall (7); c) metal–siderophore interactions (8); d) extracellular mobilization/immobilization of metals by bacterial metabolic products (9,10); e) extracellular polymer–metals interactions (11–13); and f) transformation and volatization of metals (14). The applications of processes a, b, and f involve the use of whole bacterial cells, and, therefore, may be difficult to maintain and operate in soils (15).

Processes c and e appear to have promise for application to engineered environmental remediation, since organic ligands of cellular origin (specifically siderophores and extracellular polymers) can be harvested from a bacterial culture grown under well-defined and optimum conditions and then introduced into the soil.

Bacteria commonly produce extracellular polymers, which are major constituents of the cell capsule, slime layer, or glycocalyx. Some researchers use the term capsular polymer to refer to polymer that is tightly bound to the cell and characterize slime as
**Analysis**

Polymers have been employed to produce and harvest exopolymers. The extracellular polymers of many bacteria have been well characterized. Williams and Wimpenny reported that the exopolymer of a *Pseudomonas* strain consisted of 75% hexose, 4% acetate, and 6% pyruvic acid. The composition was independent of the carbon substrate fed to the cells (26). The exopolymer of *Zoogloea ramigera* consists of glucose and galactose, with glucose as the predominant sugar; these constituents were also independent of the carbon source (27). However, Uhlinger and White found that the proportions and absolute amounts of uronic acids in the exopolymer of *Pseudomonas atlantica* increased as the rate of synthesis increased. This exopolymer consisted of glucose, xylose, mannose, galactose, and uronic acids (28). Hsieh et al. found that the freely released extracellular polymer and the cell-bound (capsule) polymer of *P. atlantica* did not differ substantially in molecular weight or gross chemical composition. The molecular weight of both these polymers was found to be 700,000 (19). Another extropolymer from a *Pseudomonas* strain was reported to consist of 46% glucose, 30% thamnose, 21% uronic acid, and 3% o-acetyl groups (29). A mixed culture from the activated sludge process was reported to produce polymers that consisted of polysaccharide, protein, RNA, and DNA (30).

Studies of the effect of bacterial growth phase on exopolymer production have reached varying conclusions. Williams and Wimpenny found that extracellular polymer production by a strain of *Pseudomonas* was initiated late in the exponential growth phase and reached a maximum in the stationary phase (26). Similarly, Uhlinger and White reported that maximum production of extracellular polymer occurred during the stationary phase when the physiologic stress of the cells, as measured by the adenylate energy charge, was greatest (28). Pavoni et al. also found that maximum exopolymer production by a mixed culture of activated sludge organisms occurred during the late stationary phase of growth (30). However, Mian et al. found that the maximum rate of extracellular polymer synthesis by a *Pseudomonas* was achieved during the exponential growth phase (20). Several researchers have observed exopolymer production in both the exponential and stationary growth phases (19,31).

The above results indicate that the growth rate of a culture may affect extracellular polymer production. Saunders and Dickinson investigated the influence of mean cell residence time ($\Theta_c$) on exopolymer formation by a mixed culture growing on domestic wastewater. The amount of polymer observed decreased as $\Theta_c$ increased, indicating either reduced exopolymer production, increased biodegradation of polymer, or increased adsorption of polymer onto suspended solids (32). In a pure culture of *Pseudomonas aeruginosa* under conditions of carbon limitation, Robinson et al. observed that extracellular polymer concentration increased with decreasing dilution rates (increasing $\Theta_c$) despite constant or decreasing cell populations (33). However, Mian et al. found that both cell concentration and polymer concentration of *P. aeruginosa* were independent of dilution rate under conditions of nitrogen limitation (20). Both researchers discovered that the specific rate of exopolymer production increased with increasing dilution rate. The range of dilution rates used by Mian et al. was smaller than the range used by Robinson et al., which may explain the discrepancy between the two results. Mian et al. also studied the production of exopolymer under conditions of carbon limitation and found that polysaccharide was produced at a lower rate. Other researchers have also found that extracellular polymer production was favored when a nutrient other than carbon was limiting. Production of polymer was highest in phosphorus- or nitrogen-limited cells, lower in sulfur-limited, and even lower in potassium-limited cells (22).

Whether or not the production of extracellular polymer is advantageous to a microorganism is a matter of considerable debate. Jarman and Pace (34) found that at high specific rates of exopolymer synthesis, the ATP demand for this synthesis was a significant portion of the total ATP demand. The appearance of nonmucoid mutants in a continuous culture of *P. aeruginosa* was thought to indicate a selective advantage of nonpolymer producers over polymer-producing cells (20). However, it was also argued that production of extracellular polymer can provide a microorganism with advantages. Exopolymers assist in attachment to surfaces. Surface attachment, in turn, may provide access for cells to higher (adsorbed) substrate concentration and protection from predation (35,36). Attachment between cells is facilitated by polysaccharide production, and can be important in symbiotic relationships (37).

**Production and Composition of Bacterial Extracellular Polymers**

Extracellular polymers are composed mainly of polysaccharides. Protein has also been detected in exopolymers. Extracellular polysaccharides usually are acidic in nature. Bacterial exopolymers typically contain between 5 to 25% uronic acids. Acidic functional groups include ketal-linked pyruvulated sugars, sialic acids, and carboxylate groups. These anionic polymers readily bind metals (24). At low pH values, the presence of adsorbed extracellular polymers may enhance the adsorption of metals to surfaces. At higher pH values, dissolved bacterial polymers may bind to trace metals in the aqueous phase, thus reducing the adsorption of metals onto the soil (23,25).

This review covers the production and composition of bacterial extracellular polymers, relevant processes in the facilitated transport of trace metals in soil by bacterial polymers, the potential for transformation of polymers in soils by microbial degradation, and preliminary model calculations for facilitated transport of cadmium by exopolymers. For additional information, Sutherland (22) provides an excellent review of the biosynthesis of extracellular polymers, and Lion et al. (23) discuss metal–polymer interactions.
In addition, exopolymers may enhance nutrient uptake, interact with cations (including trace metals), and provide protection against lytic agents (37). Jones found that the presence of toxic metals in seawater caused marine microorganisms to produce large quantities of extracellular organic matter that may interact with the metal ions and reduce the toxicity of the environment (38).

**Binding of Trace Metals by Extracellular Polymers**

The binding of metals to exopolymers (that may be considered, for purposes of discussion, as organic ligands) is based on the formation of coordination compounds. The metal acts as a Lewis acid (i.e., an electron acceptor), and the ligand acts as a Lewis base (i.e., an electron donor). The strength of the bond between a particular metal and ligand is measured by the degree of association and is often defined in terms of a conditional stability constant, \( K = [ML]/[M][L] \) at constant pH and ionic strength, where \([M]\) is the metal concentration, \([L]\) is the ligand concentration, and \([ML]\) is the concentration of the complex. Determination of the stability constant of a metal–ligand complex requires analytic methods that can distinguish between free metal ions and those that are complexed. Several techniques can be used to determine the concentration of free ions in the presence of complexing agents: dialysis in situ (39), membrane filtration, voltammetric methods (40), and ion-selective electrode measurement (12).

“Chelation” takes place when a ligand forms coordinate bonds with a metal through more than one pair of shared electrons, thus forming a ring structure. A chelate complex may be bidentate, tridentate, etc., depending on the number of bonds in the structure. Due to the formation of additional bonds, chelate complexes are relatively stable and have high stability constants. Because bacterial polymers are large molecules, chelation might take place when trace metals are bound to the polymers. The formation of a metal–ligand coordinative bond is based on the theory of hard and soft acids and bases. The degree of softness or hardness refers to the electron mobility or polarizability of a species. Hard acids tend to form strong bonds with hard bases and soft acids with soft bases (41). The principle donor atoms of exopolymers are nitrogen, oxygen, and sulfur. For \( Pb^{2+} \) and \( Cd^{2+} \), \( Cd^{2+} \) forms stable complexes with \( N \) and \( S \), and \( Pb^{2+} \) forms stable complexes with \( O, N, \) and \( S \) (42).

Unlike the metal titration curves for simple organic ligands, the titration curves for metal binding by complex ligand mixtures generally are featureless, with no sharply defined equivalence points (12,43,44). Titration curves such as these indicate the presence of multiple binding sites. Therefore, a more sophisticated model is usually necessary to describe the binding of metal ions to extracellular polymers. One approach has been to treat extracellular polymers as having multiple binding sites (45). In discrete multiligand models, the fraction of ligand sites occupied by a metal cation, \( \bar{v} \), is given by:

\[
\bar{v} = \sum \frac{K'_i [M]}{1 + K'_i [M]} \left( \frac{C_i}{C_L} \right)
\]

where \( K'_i \) is the conditional stability constant, \( C_i \) is the concentration of the \( i \)th binding site, and \( C_L \) is the concentration of available binding sites. It has been assumed that the \( K'_i \) values for discrete, individual ligands can also be assumed to represent average stability constants for distinct classes of sites in a macromolecular ligand, such as an extracellular polymer. Some example applications of this approach may be found in the work of Rudd et al. (13) and Kellemes and Lion (12).

Another approach to the description of metal binding by extracellular polymers would be to assume a statistical distribution model of multiple binding sites (46). Perdue and Lytle have assumed a normal distribution of each class of binding site strengths giving:

\[
\bar{v} = \frac{1}{\sigma \sqrt{2\pi}} \int_0^\infty \left\{ \frac{(M)10^{\text{p}K'}}{[1 + (M)10^{\text{p}K'}]} \right\} \exp\left(-\frac{1}{2} \left[ \frac{\mu - \log K'}{\sigma} \right]^2 \right) d \log K'
\]

where \( \sigma \) is the standard deviation for the distribution of \( pK' \) values, \( \mu \) is the mean \( pK' \) value for the mixture of ligands, and \( (M) \) is the concentration of unbound metal. The polymer–metal titration curve can be modeled by fitting with the values of \( \mu \) and \( \sigma \) for the multiligand mixture. Kellemes and Lion have used this approach to model Pb binding to the extracellular polymer produced by *P. atlantica*. Interestingly, the mean \( K \) values in a bimodal distribution agreed well with the \( K \) values used to fit a discrete two-site model (12). More sophisticated statistical distribution models, instead of the normal distribution model, can also be considered, if necessary, to describe the shape of the metal–ligand titration curve.

Jang et al. classify an aqueous polymer solution as consisting of two phases: a gel phase that contains the polymer and any trapped liquid, and a bulk solution phase. A polymer subphase was defined within the gel phase as the polymer chain and a small aqueous region surrounding the polymer in which a strong electrostatic attractive force was present. Cations are attracted by the electrostatic force of the polymer an, therefore an elevated concentration of cations exists at the boundary and within the polymer subphase. Donnan equilibrium is used to describe the distribution of cations between the phases, as follows:

\[
p\bar{H} - p\text{H} = p\text{Na} - p\text{Na} = n [p\text{Na} - p\text{Na}]
\]

where \( H, Na, \) and \( M \) are the activities of hydrogen, sodium, and metal ions, respectively; and \( n \) is the valence of the metal (the overbar represents the polymer subphase) (47). The polymer subphase was considered the reaction zone for protonation-deprotonation and metal binding for use in a model that determined the stability constants for copper and alginic acid interactions (48).

**Polymer Mobility**

Four fundamental mechanisms are responsible for the sorption of organic compounds: van der Waals forces, hydrophobic expulsion, electrostatic attraction, and surface complexionation. Hydrophobic expulsion along with van der Waals forces are dominant for the sorption of nonionic hydrophobic compounds onto soil organic matter (49). Electrostatic interactions are expected to contribute to adsorption of ionicizeable organics such as extracellular polymers on polar ionizable mineral and organic surfaces. This interaction is expected to be pH dependent, since protonation/deprotonation of both the ligand and the mineral surface will change the charge distribution. Surface complexion between organic ligands and mineral surfaces may occur by ligad exchange with hydroxyl groups on the surface or by ternary surface co-adsorption of metal ions and organic ligands (50). Since they contain a multiplicity of binding sites, the interactions of extracellular polymers with mineral surfaces may be complex and involve multiple bonds. If the polymer–surface interactions are of sufficient number and energy, the adsorption of polymer may become "irreversible." The extent of
migration of extracellular polymer and associated pollutants in groundwater may therefore be determined in part by their sorption to the stationary aquifer media. A study of extracellular polymer mobility in an aquifer sand has indicated that extracellular polymer is relatively mobile (51) (Figure 1). The mass recovery in this experiment was about 90%, indicating that some polymer may have been adsorbed irreversibly. The polymer retardation factor, determined from the temporal first moment of the breakthrough curve, was 1.5. This retardation factor of exopolymer in an aquifer sand is orders of magnitude smaller than the retardation factors of trace metals \( (R = 19,000 \text{ for } \text{Pb} \text{ and } 4700 \text{ for } \text{Cd}) \). Therefore, the experimental results suggest that extracellular polymers can be used as carriers in the facilitated transport of trace metal pollutants. The generality of this conclusion has not as yet been established for a range of polymers and soil types.

**Biodegradation of Extracellular Polymers**

The degradation of exopolymers in the environment must be understood before the effect of these polymers on metals mobility can be predicted. Unfortunately, little is known about the rate or mechanisms of polymer degradation.

Several researchers have found that microorganisms do not degrade their own exopolysaccharide (20,26). However, there is also evidence that bacteria can degrade the exopolymers of other bacterial species. Marine microorganisms capable of growing on the capsules of *Flavobacterium*, *Azotobacter*, *Rhizobium*, and *Arthrobacter* have been isolated from enrichment cultures (53).

Other investigators have indicated that, although many exopolymers are readily biodegradable, the rate is slow compared to that of simpler metabolites. The rate of polymer degradation varies with the type of microorganism that produced the polymer and the type of environment in which the polymer is degraded. The polymers produced by activated sludge organisms were found to have a BOD \(_5\) (in wastewater) to COD ratio of 0.10, implying a low level of biodegradability (30). Martin and Richards (54) studied the degradation (in soil) of the extracellular polysaccharides of *Chromobacterium violaceum* and two unidentified soil bacteria. The exopolymer of *C. violaceum* was more resistant to degradation; after 1 week, 10% of the polymer was mineralized to CO\(_2\) and after 8 weeks, 53% of the polymer had been degraded. The extracellular polymers of the soil bacteria were degraded more readily: 50% after 1 week and 70% after 4 weeks. The exopolymers (from *Arthrobacter viscosus*, *Azobacter indicus*, *Bacillus subtilis*, *C. violaceum*, and three *Pseudomonas* strains) were investigated by Martens and Frankenberger (55) and found to be degraded relatively quickly in soil. After 1 to 2 weeks, the monosaccharide concentration in the soil was not significantly different from that in the soil control, but only 60 to 75% of the carbon was recovered as CO\(_2\) after 8 weeks, indicating conversion of exopolymer to new biomass. Obayashi and Gaudy (56) studied the degradation (in wastewater) of the polysaccharides produced by *Arthrobacter viscosus*, *Azotobacter vinelandii*, *Xanthomonas campestris*, and *Zoogloea ramigera*. Unacclimated wastewater cultures were able to grow on each of these polysaccharides within 2 to 10 days. After enrichment, the wastewater was able to rapidly degrade the polysaccharides; 80 to 93% of the polymer COD was removed within 8 to 22 hr.

Dissolution of polymer may also be influenced by the sorption of polymers to soil particles, other organic matter, or metals. There has been little research in this area. Recently, Francis et al. (57) found that the tridentate citrate complexes with cadmium, copper, and lead were completely resistant to degradation, and that this resistance was caused by the chemical nature of the complex, not by the toxicity of the metal. It is possible that the biodegradability of extracellular polymers will be similarly altered when the polymer is complexed with various metals. Understanding of bacterial polymer degradation in metal-contaminated soils is necessary to fully determine the extent that facilitated transport of metals by exopolymers occurs in these environments.

**Model Calculations of Polymer-Facilitated Transport**

Magee et al. (58) developed a three-phase model to estimate the retardation of sorbed pollutants in the presence of a carrier that is also allowed to sorb to the stationary soil media. This model can be applied to metal pollutants under conditions in which the adsorption of metal to the porous media obeys a linear isotherm (or is governed by a single distribution coefficient). The model also assumes that metal binding by the polymer carrier can be described by a single stability constant and that sorption of the carrier to the porous media in the presence and absence of metal obeys the same linear isotherm. Given these assumptions, the retardation coefficient for the metal is given by (58):

\[
R' = \frac{1 + K'_{om}[\text{Carrier}]}{1 + K'_{om}[\text{Carrier}]/n}
\]

where [Carrier] is the concentration of the exopolymers, \( \rho_0 \) is the bulk density of the porous medium, \( n \) is the porosity, \( Ks \) are the distribution or binding coefficients, and the subscripts and superscripts \( d \), \( s \), and \( om \) designate the dissolved trace metal ions, the solid phase, and the organic ligand (exopolymers), respectively. The retardation factor \( (R') \) is defined as the ratio of the average transport velocity of pore water to that of a pollutant. Using this model, the transport of trace metals can be estimated with the knowledge of the binding constant of metal to polymer \( (K'_{om}) \), metal to soil \( (K'_{s}) \), and polymer to soil \( (K'_{om}) \). An ideal polymer for use in soil remediation should have a low polymer-to-soil surface binding constant \( (K'_{om} \) and a high trace metal-to-polymer binding constant \( (K'_{om}) \), since this combination results in a smaller retardation coefficient for the pollutant in the presence of the polymeric carrier. Figures 2 and 3 show calculated values for the ratio of the metal retardation coefficient in the presence of a carrier \( (R') \) relative to a baseline case in which the carrier concentration is zero \( (R) \). Two hypothetical cases are considered to illustrate the effect of individual binding constants on the retardation factor. Both cases assume that the trace metal has a distribution coefficient with the porous medium, \( K'_{d} \),

![Figure 1](http://example.com/figure1.png)

**Figure 1.** Transport of an extracellular polymer in an aquifer sand. The polymer was produced by a bacterium isolated from subsurface soil at a coal tar waste disposal site (52).
POLYMER MOBILIZATION OF METALS IN SOILS

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Figure 2. The ratio of the trace metal retardation factor in the presence of a carrier, \( R^* \), to that in its absence, \( R \), as a function of carrier–metal binding constant \( K_{d}^{m} \) assuming that the carrier distribution coefficient is inversely proportional to its metal binding affinity \( (r = K_{d}^{m} / K_{d}^{c} = \text{constant}) \).

Figure 3. The ratio of the trace metal retardation factor in the presence of a carrier, \( R^* \), to that in its absence, \( R \), as a function of carrier–metal binding constant \( K_{d}^{m} \) assuming that the carrier distribution coefficient is directly proportional to its metal binding affinity \( (r = K_{d}^{m} / K_{d}^{c} = \text{constant}) \).

compared to that measured for cadmium with an aquifer sand \( (K_{d}^{m} = 972 \text{ ml/g}) \) and H. Chen, unpublished results) and that the bulk density and porosity are comparable to that of an aquifer sand \( (r = 1.83 \text{ g/cm}^3 \) and \( \Theta = 0.38 \). For the first hypothetical case, it was assumed that the carrier distribution coefficient was inversely proportional to its metal binding affinity \( (i.e., \ r = (K_{d}^{m})^{-1} = (K_{d}^{c})^{-1} \) constant). This assumption might be valid in cases in which the binding sites for the polymer that interact with metals are also those that confer upon the polymer its hydrophilic properties (e.g., carboxyl and hydroxyl groups). A polymer with a high density of metal binding sites would be expected to be both mobile (low \( K_{d}^{m} \)) by virtue of its polar character and an excellent metal binding agent (high \( K_{d}^{m} \)).

Figure 2 shows that the effective metal retardation factor decreases with increasing \( K_{d}^{m} \) (and decreasing \( K_{d}^{c} \)). For the second hypothetical case, it was assumed that the carrier distribution coefficient declined in proportion to its metal binding affinity \( (i.e., \ r = K_{d}^{m} / K_{d}^{c} = \text{constant}) \). This assumption might be valid if binding to the carrier resulted in charge neutralization and sorption of the carrier–metal complex or if the carrier–metal binding sites were also responsible for adsorption of the polymeric carrier to the soil surface. In this case, the model calculations show that metal retardation at first decreases with increasing \( K_{d}^{m} \) under conditions in which the carrier is an effective metal-binding ligand and is still sufficiently mobile. However, when \( K_{d}^{m} \) is high enough, the carrier-bound metal is less mobile than the metal in the absence of the carrier and metal retardation is increased. In this limiting case, the retardation factor estimated from the three-phase model becomes \( R^* = (1 + K_{d}^{m} ho / n) \).

Preliminary results in our laboratories suggest that the first of the hypothetical cases described above is likely to prove most consistent with observed behavior.

Conclusion

Clearly, facilitated transport of metals by bacterial extracellular polymers should be considered in the arsenal of biotechnological approaches to Superfund site remediation. Additional research is still needed, however, to establish the generality of the effect of polymers upon metal mobility for a broad range of metals, polymers and soil types. Complete understanding of the facilitated transport of trace metals by naturally occurring bacterial extracellular polymers may permit the design of engineered systems for mobilizing metals from contaminated soils. It may also permit more accurate risk assessment at contaminated sites. Our recent work and that of other researchers shows that exopolymers have a high affinity for metal ions and are mobile in the soil environment. Extracellular polymers also appear to be relatively slowly degraded by soil microorganisms, allowing sufficient time for metal transport during "pump and treat" remediation. The addition of extracellular polymers to soils may prove to be acceptable to the public, since they occur naturally. Another advantage is that bacterial polymers can be produced in high yields under well-defined and optimum conditions before addition to the soil in remediation efforts.
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