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MOBILIZATION OF ADSORBED CADMIUM AND LEAD IN AQUIFER MATERIAL BY BACTERIAL EXTRACELLULAR POLYMERS

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Abstract—The mobility of cationic trace metals, such as Pb and Cd, in porous media can be severely limited by their adsorption at the solid/solution interface. The transport of metals can be enhanced by complexation with a ligand of "carrier" that (i) is soluble in water and does not strongly sorb to surfaces, (ii) has a high metal binding affinity and (iii) is not readily altered in soil by chemical or biological reactions. Extracellular polymers of bacterial origin are plausible carriers for metals in soil or aquifer systems. Bacterial extracellular polymers occur naturally in groundwaters and some have well established metal binding properties. In this study, extracellular polymers from 13 bacterial strains, including five subsurface isolates, were screened for their ability to mobilize Pb and Cd adsorbed to an aquifer sand. Batch adsorption isotherms were employed to screen polymers for their effect on metal phase distribution. All of the extracellular polymers tested reduced the linear distribution coeffients of Cd and Pb. Reductions in metal adsorption by over 90% were achieved at an extracellular polymer concentration of 10.6 mg l^{-1} The sorption isotherm of a selected extracellular polymer indicated that it had a low affinity for the sand sorbent and suggested that the polymer would be mobile in the porous sand medium. The distribution coefficient of the polymer for the sand was not effected by the presence Cd at low concentrations. Independently determined distribution constants for Cd and extracellular polymer with the sand and the binding constant for Cd to polymer yielded reasonable estimates of the observed distribution of Cd in the presence of the extracellular polymer. Column experiments performed with Cd in the presence and absence of the selected extracellular polymer confirmed that application of polymer solutions can enhance metal mobility in porous media.

Key words—aquifer material, adsorption, bacteria, cadmium, complexation, extracellular polymer, facilitated transport, lead

INTRODUCTION

Many superfund sites are contaminated with toxic trace metals. The mobility of heavy metals in soil systems is, in general, severely limited by virtue of the strong adsorption reactions between metal ions and the surface of soil particles (Gardiner, 1974; Hem, 1976; Lewis, 1977; Siccama and Smith, 1978). The retarded transport and slow desorption kinetics of adsorbed trace metals can constitute the rate limiting step in metal removal processes such as soil washing. Cost effective methods for metal removal require that adsorbed or precipitated metals be rapidly transferred to the aqueous phase where they are susceptible to advective transport. Factors that can increase the mobility of trace metals are of interest in both treatment process design and in risk assessment. An additional consideration in soil remediation efforts is that substances that are applied to soils to enhance metal mobility must also be environmentally innocuous for the application to receive serious consideration.

Phenomena that result in contaminant transport at rates greater than those determined from consideration of groundwater hydrodynamic flow and the adsorption of the contaminant to the stationary porous medium are referred to as "facilitated transport" (McCarthy and Zachara, 1989). Dissolved macromolecules and colloidal particles constitute two agents or "carriers" that can potentially act to enhance metal transport. To be effective, a carrier must bind trace metals and have relatively high mobility in porous media. Dissolved macromolecules found in groundwater include humic substances and bacterial extracellular polymers (Chudoba *et al.*, 1986; McCarthy *et al.*, 1993). Dissolved macromolecules of bacterial origin appear to have promise for enhancing metal transport, since they have been shown to be effective metal binding agents (Corpe, 1975; Brown and Lester, 1980; Brown and Lester,

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1982; Rudd *et al.*, 1984; Mittleman and Geesey, 1985; Lion *et al.*, 1988; Kellems and Lion, 1989.

The adsorption of a dissolved trace metal to a mineral surface in the presence of a bacterial extracellular polymer can be considered as a three component system in which metals, polymer and the surface interact through adsorption and complexation reactions. Figure 1 provides a schematic representation of how extracellular polymers may act to influence trace metal adsorption to the soil surface. Acidic polysaccharides may sorb to the surfaces of soil medium to produce an organic coating that may bind dissolved trace metals, while dissolved extracellular polymers may act as metal binding ligands in the aqueous phase. The concentration of trace metals in solution may increase or decrease depending upon the relative affinities of extracellular polymers for binding metals and for sorption to the soil surface.

As suggested by Figure 1, the sorption of an extracellular polymer on the soil surface can change the surface properties of the soil and the phase distribution of trace metals. Surface complexation between organic ligands and mineral surfaces may occur by ligand exchange with surface hydroxyl groups, by co-adsorption of metal ions and organic ligands, or by sorption of hydrophobic ligand moieties to soil organic matter. Because of the complex nature of bacterial extracellular polymers, van der Waals forces, hydrophobic expulsion, electrostatic attraction, and surface complexation may all contribute to their distribution between the solid and aqueous phases. This distribution is anticipated to be pH dependent, since protonation/deprotonation will alter the charge of both the extracellular polymer and the mineral surface.

The titration curves for metal binding by macrocolecular ligands, including humic acids and bacterial extracellular polymers, are generally featureless without sharply defined equivalence points, indicating the presence of multiple binding sites (Gamble *et al.*, 1980; Perdue *et al.*, 1980; Kellems and Lion, 1989). Models that have been used to describe metal–polymer interactions include those that invoke multiple discrete binding sites (Rudd *et al.*, 1984), a continuous distribution of binding sites (Kellems and Lion, 1989), or are based on Donnan equilibrium (Jang *et al.*, 1989).

Magee *et al.* (1991) developed a three-component model for the description of the influence of dissolved organic matter on the sorptive distribution of hydrophobic organic pollutants in porous media. As a first approximation, this model can be adapted to consider the effect of an organic ligand (that sorbs to the solid water interface) upon metal ion distribution in soils. Use of the model requires that both extracellular polymer (ligand) and metal adsorption to the soil be amenable to description by a linear distribution coefficient, that polymer-bound metal and free-dissolved extracellular polymer have the same sorptive distribution coefficient, and that extracellular polymer binding of metal ions can be approximated by a single equilibrium constant. Given these assumptions, the three component model can be used to estimate the retardation factor (R^*) of metals based on the independently measured metal binding and sorptive distribution coefficients as:

$$R^* = \frac{1 + K_d^{\text{polymer}}[\text{polymer}] + K_d^s \rho/\theta}{1 + \dfrac{K_d^{\text{polymer}}[\text{polymer}]}{1 + (K_{\text{polymer}}^s \rho/\theta)}}, \qquad (1)$$

where R^* is the retardation factor of the pollutant and is equal to the pore water velocity of the bulk aqueous phase divided by the effective net velocity of the pollutant (including both carrier-bound metal and free metal ion in the aqueous phase); K_d^{polymer} is the metal stability constant with the extracellular polymer; [polymer] is the aqueous concentration of bacterial extracellular polymer; K_d^s is the metal distribution coefficient with the stationary soil phase; K_{polymer}^s is the distribution coefficient of the extracellu-

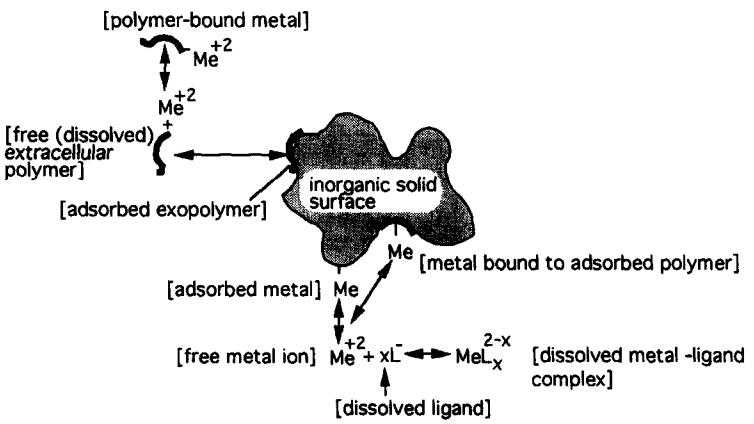

Fig. 1. Schematic of trace metal reactions with an inorganic surface as modified by the presence of a bacterial polymer

lar polymer with the stationary phase; ρ is the bulk density of the soil; and θ is the soil porosity. A high value of R^* indicates that the pollutant will move very slowly in a porous medium relative to the bulk aqueous phase. R^* reflects the temporal first moment of the break through curve (BTC) of contaminant and may be measured by a miscible displacement column experiment (Magee *et al.*, 1991). and has been shown to be independent of whether desorption kinetics influence the shape of the BTC (Valocchi, 1985).

Prior research has shown that bacterial growth can influence the phase distribution of metals with soil systems (Chanmugathas and Bollag, 1987a, b, 1988); however, the specific role of extracellular polymers has not been examined. Although the literature demonstrates that bacterial extracellular polymers can serve as a dissolved ligand that bind trace metals by homogeneous reactions in the aqueous phase (see discussion above), there has been no prior demonstration that extracellular polymers can effectively act to desorb metals that are bound to mineral surfaces or that metal-polymer complexes are mobile in a porous medium. It is not clear, for example, that extracellular polymers would not sorb to the surface of a porous medium and act to retard metal transport. In this study, extracellular polymers from thirteen bacterial isolates were screened for their ability to mobilize the adsorbed trace metals Cd and Pb. The assumptions and the utility of the modified three component retardation model of Magee *et al.* (1991) were experimentally tested for a selected extracellular polymer through the use of batch surorption isotherms and column transport experiments.

MATERIALS AND METHODS

The bacterial strains tested in this experiment are listed in Table 1. An isolated colony of stock culture was transferred aseptically with a flamed wire loop from an agar plate to a tube containing 20 ml of a phosphate buffered mineral salts medium containing 0.5% glucose and 0.036% each of casamino acids and yeast extract (Dohse, 1992). The liquid culture was placed on a rotary shaker (at 25°C) until the medium become turbid ($\approx$24 h). Five milliliters of the turbid culture broth was then added to 150 ml of fresh growth medium in a 500 ml Erlenmeyer flask and placed on a rotary shaker at 25°C. The optical density of the suspension was measured at 1–2 h intervals until the stationary phase of growth was attained (as defined by a constant culture OD). The extracellular polymers then were harvested from the medium as described below.

Several studies have been published regarding the effect of bacterial growth phase on extracellular polymer production (Pavoni *et al.*, 1972; Unz and Farrah, 1976; Williams and Wimpenney, 1977; Mian *et al.*, 1978; Uhlinger and White., 1983; Hsieh *et al.*, 1990). All bacterial extracellular polymers used in this study were harvested at the early stationary growth phase to maintain consistent culture conditions. Extracellular polymers produced by bacteria can be distinguished as freely released polymer and the cell-bound (capsular) polymer. Both extracellular polymer types were considered as potential candidates for the trace metal mobilization.

Extracellular and capsular polymers

The procedures for extraction of extracellular polymers were adapted from techniques used by Corpe (1970) as described by Kellems and Lion (1989). The culture broth (at stationary growth phase) was transferred into 250 ml centrifuge bottles and centrifuged for 25 min at 9600*G* (5°C) to separate the cell mass from dissolved extracellular polymers. The supernatant was transferred into clean centrifuge bottles and mixed with an equal volume of 5°C acetone. On addition of acetone, the extracellular polymer precipitated, forming a white floc that was subsequently removed by centrifugation at 870*G* (5°C) for 15 min. Prior to the addition of the acetone, a small amount of supernatant containing extracellular polymer was tested for precipitation. In cases where no visible polymer precipitate was observed, the supernatant was directly subjected to dialysis without addition of acetone. The concentrated extracellular polymer precipitate or solution containing dissolved extracellular polymer was placed in 8000 molecular weight cut off (MWCO) dialysis tubing, and dialyzed against 18.9 MΩ cm^{-1} distilled-deionized water with constant mixing. Sufficient exchanges of water (8–10) were made over a 48 h period to remove excess solvents and low molecular weight impurities. The concentration of total organic carbon (TOC) of the dialyzing water was measured using an OI Corporation Model 700 TOC Analyzer to test for completion of the dialysis purification step. The absence of a TOC increase in the external solution was taken as evidence that the dialysis was complete. The dialyzed extracellular polymer was frozen, freeze dried, and stored at 4°C prior to its use in experiments.

Table 1. Bacterial strains tested

Culture	Gram reaction	Catalase	Oxidase	Source
Arthrobacter globiformis	+	+	ND*	ATCC† 8010
Arthrobacter sp. 9G4D	+	+	ND	W. Ghiorse, Cornell Univ.
Acinetobacter calcaceticus	−	+	−	ATCC 31012
Pseudomonas cepacia 249-100	−	+	+	T. Lessie, Univ. of Mass.
Pseudomonas flurescens	−	+	+	ATCC 13524
Pseudomonas putida G7	−	+	+	W. Ghiorse, Cornell Univ.
Pseudomonas A-100	−	+	+	M. Alexander, Cornell Univ.
Zoogloea sp. U106	−	+	+	R. Unz, Penn St. Univ.
Soil isolates from urban manufactured gas plant sites				
9702-M4	−	−	+	C. Thomas, Cornell Univ.‡
9704-M1	−	+	−	C. Thomas, Cornell Univ.‡
9710-M3	+	+	−	C. Thomas, Cornell Univ.‡
9712-M1	+	+	−	C. Thomas, Cornell Univ.‡
B6-2	−	ND	ND	C. Thomas, Cornell Univ.

*ND = not determined.
†ATCC = American Type Culture Collection.
‡Madsen *et al.* (1992) provide a description of the site.

Cell bound (capsular) extracellular polymers were extracted by EDTA using the procedure described by Brown and Lester (1980) as modified by Hsieh *et al.* (1990). Because a metal chelating agent (EDTA) was used to release capsular polymers from cells, failure to remove this reagent in the subsequent dialysis step would result in elevated apparent metal binding properties of the extracted extracellular polymer. Two tests were performed to investigate possible artifacts resulting from the extraction process for capsular polymer. In one test ^{14}C-labeled EDTA was used to extract capsular polymer. After dialysis, the capsular polymer was analyzed by liquid scintillation counting (Beckman model LS 9800) to test for ^{14}C-EDTA that was not removed by dialysis. The second test investigated the possible effect of EDTA on the characteristics of extracted extracellular polymers. An EDTA solution was added to the centrifugate from a culture broth containing dissolved extracellular polymers. This extracellular polymer-EDTA mixture was subjected to dialysis and freeze-dried as described above. The EDTA-treated extracellular exopolymer was then compared to the same polymer without EDTA treatment with regard to its ability to alter the adsorption of Pb.

Metal adsorption isotherms

A low-carbon aquifer sand was used as the porous medium (adsorbent) in this study. The sand was obtained from a quarry in Newfield, NY. Physical/chemical characteristics of the sand are shown in Table 2. Adsorption isotherms for Pb and Cd on the sand were carried out in the presence and absence of bacterial polymer to screen for the effect of extracellular polymer on the metal distribution coefficient. The metal concentrations used were restricted to a range in which the adsorption isotherm was linear. The protocol for adsorption isotherm experiments is described below.

Glass centrifuge tubes (pretreated with dichlorodimethysilane to reduce metal sorption to the glass surface) were prepared to contain 30 ml of a 5 mM $CaSO_4$ electrolyte, 300 mg (i.e. 10 g l^{-1}) of the aquifer sand, variable amounts of Pb^{210} or Cd^{109} (to give a specific activity of $\approx 1\ \mu Ci\ g^{-1}$ of Pb or Cd), and variable amounts of $Pb(NO_3)_2$ or $Cd(NO_3)_2$ (to give Pb or Cd concentrations ranging from 10^{-9} to 2×10^{-8} M). Radiolabeled trace metals were employed to provide a analytical method that was sensitive to low metal concentrations and free from matrix interference or contaminant artifacts. Since experiments were conducted in range in which isotherms were shown to be linear, measurement of any stable metal concentration contributed by the sand was not required. The suspensions were equilibrated for 24 h at 25°C while being tumbled on a rotor. Preliminary experiments indicated that the 24 h equilibration time was sufficient to ensure a constant solution composition. The adsorption isotherm was terminated by centrifuging the tubes at 1000*g* (25°C) for 20 min. The concentration of Pb^{210} in the supernatant was determined by liquid scintillation counting of the Pb^{210} beta decay (Beckman, model LS 9800). One milliliter of the supernatant was added to 10 ml of scintillation cocktail and counted for a time interval sufficient to give a

Table 2. Porous medium characterization

Specification	Particle size	Percentage (w/w)	Total %
Particle size analysis			
Very coarse sand	2–1 mm	1.1	
Coarse sand	1–0.5 mm	11.7	
Medium sand	0.5–0.25 mm	22.5	
Fine sand	0.25–0.1 mm	42.2	
Very fine sand	0.1–0.053 mm	13.2	93.7
Coarse silt	0.053–0.02 mm	4.3	
Medium silt	0.02–0.005 mm	0.0	
Fine silt	0.005–0.002 mm	2.0	6.3
Coarse clay	0.002–0.0002 mm	0.0	
Fine clay	less than 0.0002 mm	0.0	0.0
Bet suface area*	3.3 $m^2\ g^{-1}$		
Organic carbon content†	0.91%		

Elements	Concentration (mg kg^{-1})
Elemental analysis‡	
Available nutrients	
P	2.1
K	13.0
Mg	192.2
Ca	18 400
Fe	26.5
Al	20.0
Mn	49.2
NO_3^-	ND§
Acid-soluble cations	
Cd	0.74
Cu	13.52
Ni	11.83
Mn	288
Co	0.26
Zn	32.2
Pb	9.05
Cr	6.20

*Determined using N_2, analysis performed using a Quantasorb Sorption System (QuantaChrome Corp., NY).
†Analysis performed by the Analytical Laboratories, Department of Soil Crop and Atmospheric Science, Cornell University using a modification of the Walkley Black Method (Allison, 1965).
‡Performed by Cornell University Nutrient Analysis Laboratories.
§ND = below limit of detection.

95% confidence level of ±2%. Counts were corrected for background and monitored for quench. The concentration of Cd^{109} in the supernatant was determined (±2%) by crystal scintillation counting of the Cd^{109} γ decay (Packard, Auto-Gamma model 5650). The amount of Pb (or Cd) adsorbed was determined by subtracting the final amount of Pb (or Cd) in solution phase from the total amount of Pb (or Cd) added. The distribution coefficient was determined by fitting of the data to a linear isotherm:

$$\Gamma = K_d C_s, \quad (2)$$

where Γ is the amount of Pb (or Cd) adsorbed per g of the sand; K_d is the trace metal distribution coefficient; and C_s is the equilibrium concentration of Pb (or Cd) in the solution phase.

The conditions for Cd and Pb adsorption isotherms in the presence of extracellular polymers were the same as described above; however, candidate polymers were included in the reactors at a concentration of 10.6 mg l^{-1}. Pb (or Cd) was allowed to equilibrate with the sand for 24 h before addition of the extracellular polymer. After addition of the extracellular polymer, an additional 24 h were allowed for the polymer, Pb (or Cd), and sand system to reach equilibrium. The "apparent" distribution coefficient of Pb (or Cd) in the presence of extracellular polymer was determined as described above. If the three component model is valid, then the measured metal distribution coefficient in the composite (metal, extracellular polymer, sand) system will vary as a function of the extracellular polymer concentration as well as the distribution coefficient for the polymer with the sand and the binding constant for the extracellular polymer with the metal. At constant extracellular polymer concentration and pH, low values of the apparent distribution coefficient would indicate that the extracellular polymer had a relatively low sorption to the sand surface and a significant ability to complex Cd or Pb.

Extracellular polymer sorption isotherms

The bacterial extracellular polymer from soil isolate 9702-M4 was selected and tested for sorption to the sand medium. The sorption isotherm was performed under conditions comparable to those for the trace metal isotherms. The concentration of extracellular polymer in the solution was determined by TOC analysis using an OI Corporation Model 700 TOC Analyzer, Results for dissolved extracellular polymer were corrected for the amount of organic carbon released from the sand in control samples without extracellular polymer. Sorption experiments with extracellular polymers were conducted using a fixed sorbent concentration (10 g of sand l^{-1}) and polymer concentrations ranging from 5 to 60 mg l^{-1}. Cd concentrations ranging from 1×10^{-9} to 1.3×10^{-8} M were included in one isotherm to evaluate the effect of the presence of low metal concentrations on extracellular polymer sorption.

Polymer–metal binding constant

At constant pH and ionic strength, the binding of a trace metal to ligand can be quantified in terms of a conditional stability constant:

$$K = [ML]/[M^{2+}][L], \quad (3)$$

where $[M^{2+}]$ is the concentration of the free metal ion, [L] is the ligand concentration; and [ML] is the concentration of the metal ligand complex.

Since metal, ligand (i.e. extracellular polymer), and metal–ligand complex form a homogeneous system, analytic methods that distinguish between free metal ions and those that are complexed are needed to measure the metal–polymer complexation (Benes and Steinnes, 1974; Goncalves *et al.*, 1985; Kellems and Lion, 1989). For this study, Cd binding to a selected bacterial extracellular polymer was determined using an ion selective electrode (ISE). A solid state cadmium ISE (Orion, Model 94-48) was used in conjunction with a double-junction reference electrode (Orion, Model 90-02). Titration of extracellular polymer solution by Cd(II) took place in a silanized-beaker at 25°C. All ISE titrations were conducted in an 0.01 M $NaNO_3$ electrolyte to provide a constant ionic strength. The test solution was constantly bubbled with NaOH scrubbed N_2 gas to minimize Cd complexation by carbonate ions. The pH of the titration was controlled at 7.50 ± 0.05 by addition of 0.01 N HNO_3 and NaOH. Incremental additions of a Cd stock [1×10^{-2} M $Cd(NO_3)_2$] were added to solution to make up Cd solutions of desired concentrations for calibration of the ISE. An extracellular polymer solution (66 mg l^{-1}, or 20.66 mg C l^{-1}) was then titrated with Cd. The concentration of Cd^{2+} was determined by the electrode potential from the calibration curve and the amount of Cd bound to the polymer was determined by subtracting the amount of free metal from the amount of Cd added.

Column experiments

Miscible displacement experiments were performed to confirm the effect of extracellular polymer on the transport of Cd. The glass column was silanated to reduce possible wall adsorption of Cd and was acid-washed prior each experiment. Teflon® tubing and connectors were used wherever possible. Preliminary experiments were conducted on the column system with a packing of Teflon® beads and gave 95% Cd recovery.

The sand-packed glass column was prepared using the following steps: The tare weight of the column was determined and the column was filled with sand while gently tapping the sides. When filled, the column was weighed to measure the dry weight of sand. The column was saturated with electrolyte solution (5 mM $CaSO_4$, ≈ 6 pore volumes). The saturated column was weighed and the pore volume of the column was estimated by the weight difference of the saturated and dry column after correction for the dead volume. Pore volume was confirmed by analysis of the break through curve (BTC) of a chloride tracer pulse. A non-linear least squares fit to the advection-dispersion equation (Parker and van Genuchten, 1984) was used to estimate the pore water velocity and the dispersion coefficient from the Cl^- BTC.

The physical characteristics for the column experiment were: pore water velocity = 0.23 cm min^{-1}, dispersion coefficient = 0.12 cm^2 min^{-1}, bulk density = 1.86 g cm^{-3}, porosity = 0.43, column length = 3.5 cm, column inner diameter = 0.92 cm, and the Peclet number ($Pe = VL/D$) was 6.9. A dual-piston high performance liquid chromatography pump (Waters model 590) was employed and provided precise control of the low column flow that was employed (Q = 0.065 ml min^{-1}). A 26-h pulse (104 pore volumes) of Cd solution (1.5×10^{-8} M in 5 mM $CaSO_4$, with Cd^{109} as a tracer) was added to the column. The column input was then switched to deliver an extracellular polymer solution (5 mM $CaSO_4$ with 150 mg l^{-1} of polymer from bacterial isolate 9702-M4). The column effluent was directed to a fraction collector and the activity of Cd^{109} in the effluent was determined. The pH of the column effluent was monitored periodically and remained reasonably constant (pH 7.20 ± 0.20). A parallel experiment was performed using a Cd input of the same duration followed by 5 mM $CaSO_4$ without bacterial extracellular polymer to compare the transport of Cd in the sand with and without the presence of a mobile phase containing extracellular polymer.

After the termination of each column experiment, the column was dissected to obtain the concentration distribution profile of adsorbed Cd along the column. Small incremental amounts of sand were collected and weighed to determine an incremental distance (by proportion to the

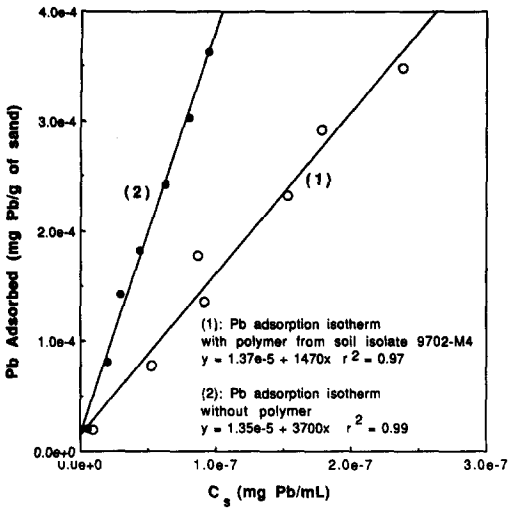

Fig. 2. Pb Adsorption isotherms to an aquifer sand in the presence and absence of the extracellular polymer produced by a bacterium isolated from soil.

total column length and the total weight of the packed sand). The adsorbed Cd^{109} was extracted by HNO_3 (22 N) and the Cd^{109} activity of the extract was determined by crystal scintillation counting.

RESULTS AND DISCUSSION

The extracellular polymers from 13 bacterial strains were studied for their effects on Pb adsorption isotherms with the aquifer sand. As an example, Fig. 2 shows Pb adsorption isotherms in the absence of extracellular polymer and in the presence of the 10.6 mg l^{-1} of the extracellular polymer produced by soil isolate 9702-M4. The Pb distribution coefficients are equal to the slopes of the respective linear isotherms. The presence of the extracellular polymer from isolate 9702-M4 resulted in a 60% reduction in the metal distribution coefficient. The pH of the isotherms was buffered by the soil suspension and was 7.58 ± 0.22 without polymer and 7.54 ± 0.12 in the presence of the polymer from isolate 9702 – M4. The reductions of the Pb distribution coefficients with the extracellular polymers of the other isolates tested are summarized together with isotherm pHs in Table 3. The pH of the Pb isotherm without polymer (7.58 ± 0.22) was reasonably close to those of the Pb isotherms with most of the extracellular polymers that were screened, except for isolate B6.2 (pH 6.97 ± 0.33).

The reduction of Pb distribution coefficients may have been caused, in part, by a shift in pH, since a lower pH would favor the desorption of Pb. However, the combined effect of lower pH and extracellular polymer from isolate B6-2 resulted in only a 17.4% reduction in the distribution coefficient. Far greater reductions in Pb adsorption occurred with other polymers at higher pH values. Therefore, the observed decreases in the Pb distribution coefficients in the presence of extracellular polymers from isolates other than B6-2 are primarily attributed to polymer complexation of metal rather than to a variation in pH.

Three of the extracellular polymers were also tested for their capability to release adsorbed Cd. Figure 3 illus trates the adsorption of Cd on the sand in the presence and the absence of 10.6 mg l^{-1} of the extracellular polymer from soil isolate 9702-M4. The reduction of the Cd distribution coefficients with the three extracellular polymers tested are summarized in Table 3.

It is interesting to note the difference in the effect of the extracellular polymer of *Ps. putida* on the adsorption isotherms of Cd vs. Pb. The extracellular polymer of *Ps. putida* reduced the distribution coefficient of Pb by approximately 12%; however, this same extracellular polymer reduced the distribution coefficient of Cd by more than 90%. *Ps. putida* is tolerant to cadmium, and accumulates the metal when grown in its presence (Higham and Sadler,

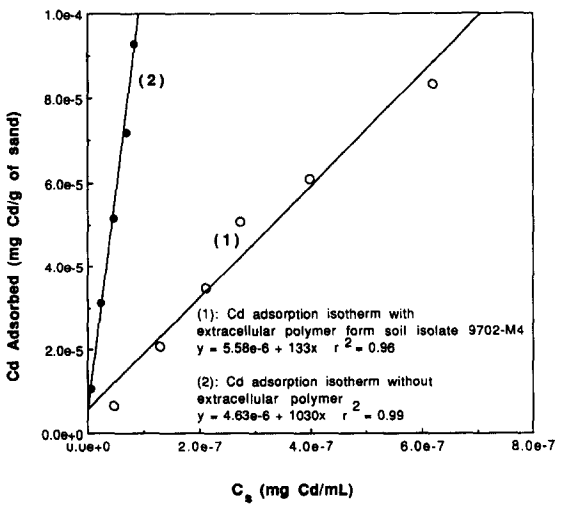

Fig. 3. Cd adsorption isotherms to an aquifer sand in the presence and absence of the extracellular polymer produced by a bacterium isolated from soil.

Table 3. Summary of the effect of bacterial extracellular polymers metal distribution coefficients*

Organism	Reduction in Pb distribution coef.	Isotherm pH	Reduction in Cd distribution coef.	Isotherm pH
Acinetobacter calcoaceticus	63.1%	7.45 ± 0.12		
Arthrobacter 9G4D	91.1%	7.50 ± 0.25		
Arthrobacter globiformis	85.5%	7.31 ± 0.08		
Pseudomonas A-100	71.5%	7.37 ± 0.18		
Pseudomonas cepacia 249-100	85.0%	7.35 ± 0.16	94.4%	7.30 ± 0.13
Pseudomonas fluorescens	91.8%	7.30 ± 0.08		
Pseudomonas putida	12.0%	7.46 ± 0.08	92.8%	7.51 ± 0.15
Zoogloea sp. U106	68.7%	7.54 ± 0.18		
Soil isolate B6-2	17.4%	6.97 ± 0.33		
Soil isolate 9702-M4	60.0%	7.54 ± 0.12	87.0%	7.49 ± 0.24
Soil isolate 9704-M1	56.9%	7.36 ± 0.15		
Soil isolate 9710-M3	87.9%	7.44 ± 0.09		
Soil isolate 9712-M1	77.2%	7.15 ± 0.04		

*All results are for a polymer concentration of 10.6 mg l^{-1}.

1984). The cadmium specific selectivity of the *Ps. putida* extracellular polymer indicates that it may have potential for use in applications that involve the extraction and purification of Cd.

The evaluation of the EDTA extraction procedure for capsular extracellular polymer using ^{14}C-labeled EDTA revealed that a significant fraction of added EDTA (1.08 g EDTA/g of polymer, or ≈5.6% of EDTA added) remained associated with the capsular polymer despite extensive dialysis against distilled-deionized water. Comparison of Pb adsorption isotherms in the presence of EDTA-treated and untreated extracellular polymer from soil isolate 9712-M1 revealed that EDTA treatment improved the apparent metal binding characteristics of the extracellular polymer. The EDTA-treated extracellular polymer released higher concentrations of adsorbed Pb, and displayed virtually the same effect on Pb adsorption as did the capsular polymer (obtained by EDTA extraction) from isolate 9712-M1. It was apparent that in the EDTA extraction of capsular extracellular polymer, some EDTA remained with the polymer after dialysis. The results in this report are for extracellular polymers that were obtained in the absence of EDTA treatment.

Although the adsorption isotherms of Cd and Pb in the presence of many of the extracellular polymers that were tested indicated a significant reduction of metal distribution coefficient, this result did not explicitly prove that a given extracellular polymer would result in improved advective transport of metal in the porous medium. Confirmatory column transport experiments were carried out with a selected polymer and Cd metal to verify the polymers ability to enhance metal mobility. The extracellular polymer from soil isolate 9702-M4 was used in these experiments. As shown in Table 3, the presence of 10.6 mg l^{-1} of the extracellular polymer from isolate 9702-M4 reduced the distribution coefficient for Pb and Cd in the isotherm studies by 60 and 87%, respectively. In separate studies this extracellular polymer has also been shown to also be effective at solubilizing sorbed polynuclear aromatic hydrocarbons and to be resistent to degradation by mixed cultures of bacteria (Dohse and Lion, 1994).

Two alternative mechanisms may be responsible for the observed reduction in the metal distribution coefficients by extracellular polymer: (1) significant sorption of the polymer on the sand occurred and changed the surface properties by occupying the metal binding sites, and/or (2) the sorption of extracellular polymer on the sand was negligible and metal complexation by the dissolved extracellular polymer occurred. To act as an effective carrier in the facilitated transport of a metal, the extracellular polymer must be dispersed and mobile in the soil environment [i.e. it must be stable in the aqueous phase (resisting aggregation and subsequent removal by filtration) and must not be surface active or strongly sorbed.] Sorption isotherms of the extracellular polymers from isolate 9702-M4 on the sand were used to provide an indication of the mobility of the polymer in the porous medium. The sorption isotherms for the extracellular polymer onto the aquifer sand in the presence and absence of Cd are shown in Fig. 4. In the absence of added Cd, the distribution coefficient of extracellular polymer on sand was 3.46 ml g^{-1}. The extracellular polymer distribution coefficient in the presence of Cd was 3.05 ml g^{-1}. Since sorption of the extracellular polymer was relatively weak, the polymer sorption isotherm results indicate that the reduction in Cd distribution coefficients in the presence of the extracellular polymer resulted from release of the adsorbed metal by complexation with

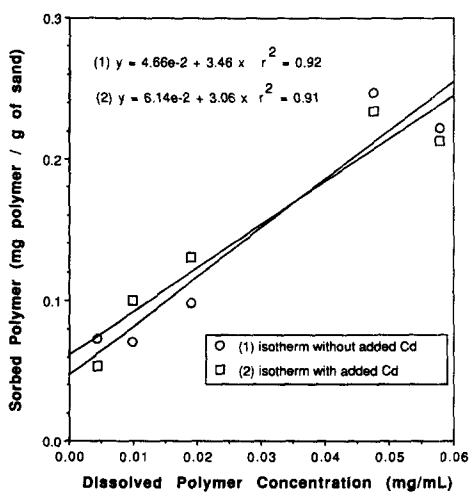

Fig. 4. Polymer sorption isotherms to an aquifer sand in the presence and absence of Cd metal

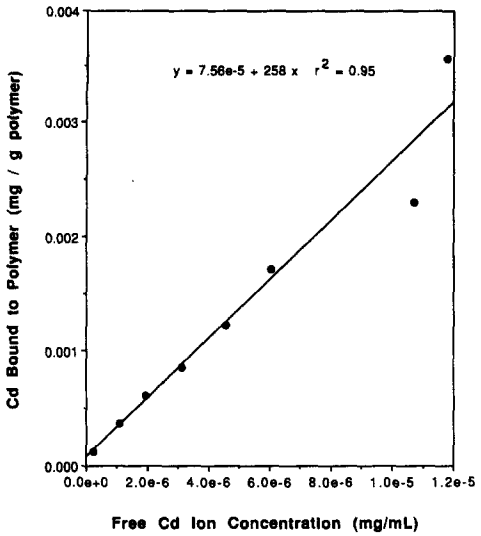

Fig. 5. Titration of the extracellular polymer from soil isolate 9702-M4 with Cd.

dissolved polymer (vs. modification of the sand surface by polymer). The extracellular polymer sorption results also suggest that the presence of low levels of Cd had no significant effect on the sorption of 9702-M4 exopolymer and indicate that the mobility of the extracellular polymer will not change when it forms a soluble complex with Cd. Finally, the isotherm results confirm a condition for the application of the three-component model of Magee *et al.* (1991) to estimate of the facilitated transport of trace metal by extracellular polymer. Use of the model implicitly assumes that complexation of trace metals will not alter the sorption of extracellular polymer onto the soil medium.

The extracellular polymer from soil isolate 9702-M4 was titrated with Cd to evaluate the stability constant of the metal polymer complex (Fig. 5). If the extracellular polymer was present in excess, then the linear slope of the plot serves as a measure of the "overall" stability constant. The value obtained was approximately 2.6×10^5 ml g^{-1} of polymer. Prior analyses of bacterial extracellular polymers suggest that they behave as a ligand with multiple metal binding sites (Rudd *et al.*, 1984; Kellems and Lion, 1989). A Scatchard plot of the titration data indicated the presence of a small fraction of strong binding sites. However, a more detailed characterization of extracellular polymer–metal interactions was not required to predict the effect of dissolved polymer on metal transport.

Based on the sorption isotherm and titration results, the extracellular polymer of soil isolate 9702-M4 displayed the ability to complex Cd, had a relatively low sorption to the sand surface, and released adsorbed Cd and Pb from the sand. These characteristics suggest that the extracellular polymer could facilitate metal transport. Experiments were conducted to verify the ability of the polymer to function as a carrier in the advective transport of Cd through a column of the sand. A 26-h pulse (104.5 pore volumes) of Cd solution (1.5×10^8 M, in 5 mM $CaSO_4$) was added to the column, followed by the extracellular polymer solution with concentration of approximately 150 mg l^{-1} (46.95 mg C l^{-1} in 5 mM $CaSO_4$). A control experiment was also carried out on a fresh sand column in which the Cd input was followed by application of 5 mM $CaSO_4$ without addition of extracellular polymer. The resulting Cd breakthrough curves are compared in Fig. 6. Prior to the introduction of the extracellular polymer solution, the column effluent did not contain Cd after 104 pore volumes of Cd input was added. However, after switching to the extracellular polymer solution and addition of approximately 10 pore volumes of polymer solution, a significant Cd concentration ($\approx$13% of the input Cd level) was measured in the effluent. The Cd concentration in the effluent subsequently decreased to about 3% of the input concentration and remained approximately the constant until the experiment was terminated. The total recovery of the Cd input mass over the duration of the experiment was $\approx$57%. In the case where the 5 mM $CaSO_4$ electrolyte (control, without polymer) was used to remove the adsorbed Cd, no significant amount of Cd was eluted from the column even after addition of over 1000 pore volumes of electrolyte solution (see Fig. 6).

After the termination of each column experiment, the sand column was dissected and the concentration of adsorbed Cd was determined as a function of position. The Cd concentration–distance profile in the sand column is shown in Fig. 7 for the cases where extracellular polymer was present and absent. Comparison of the spatial distribution of Cd for these two cases confirms that Cd was mobilized by the extracellular polymer solution.

It is interesting to note that, in the presence of extracellular polymer, the shape of the Cd BTC showed a sharpened front and extensive tailing. This nonideal (asymmetric) behavior may result from

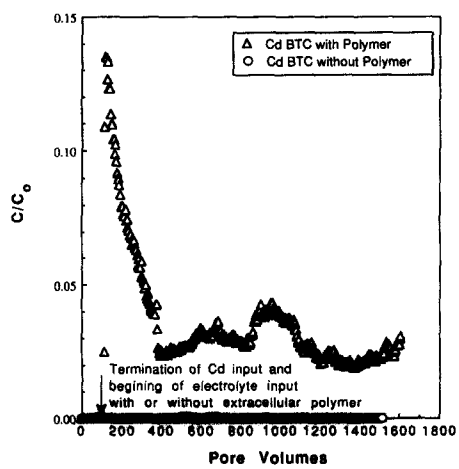

Fig. 6. Column breakthrough curves for Cd in the presence and absence of extracellular polymer for soil isolate 9702-M4

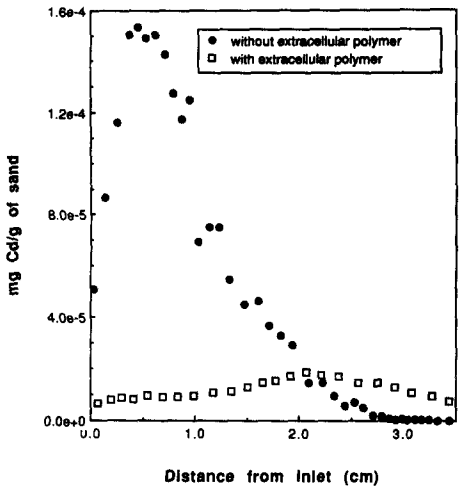

Fig. 7. Spatial distribution of adsorbed Cd after elution with solutions containing 5 mM $CaSO_4$ with and without extracellular polymer from soil isolate 9702-M4

several factors, including non-equilibrium conditions resulting from slow adsorption/desorption kinetics, diffusive mass transfer, and isotherm nonlinearity. The Peclet number ($Pe = VL/D$) in the column experiment was 6.9 (note, the pore water velocity ($V = 0.22$ cm min^{-1}) and dispersion coefficient ($D = 0.12$ cm^2 min^{-1}) were determined by a nonlinear least squares fit to the chloride BTC using the CXTFIT program developed by Parker and van Genuchten (1984). This magnitude of Pe, and the fact that the Cl^- BTC was symmetric, indicates that dispersion was not a dominant factor in determining the shape of the Cd BTC. The input Cd concentration to the column was in the linear range of the isotherm; hence, isotherm nonlinearity is not likely to have been responsible for the asymmetric BTC. Therefore, kinetic limitations in the desorption of Cd from the sand medium are considered to be the mechanism most responsible for the asymmetry of the Cd breakthrough curve. Although the Cd desorption kinetics may have been "slow" in the presence of the extracellular polymer they were negligible in the absence of extracellular polymer. As a result, addition of extracellular polymer to the column significantly enhanced Cd mobility.

The three-component model proposed by Magee *et al.* (1991) can be used to estimated the Cd retardation coefficient in the presence of extracellular polymer based on the independently determined values for the distribution and binding constants. As noted above, $K_d^{polymer} = 2.6\ 10^5$ ml g^{-1} from the measured value for stability constant of trace metal to extracellular polymer, $K_d^s = 1026$ ml g^{-1} from the measured distribution coefficient between Cd and sand, and $K_{polymer}^s = 3.46$ ml g^{-1} from the distribution coefficient between extracellular polymer and sand. For the column experiment, the extracellular polymer concentration was 150 mg l^{-1}, ρ_b was 1.86 g cm^{-3} and n was 0.44. The R^* thus calculated from equation (1) is 1250. For the column without extracellular polymer, the retardation coefficient may be estimated as $R = 1 + K_d^s\rho_b/n$, where ρ_b was 1.83 g cm^{-3} and θ was 0.48. The resulting retardation coefficient for Cd in the absence of extracellular polymer is 3920. The presence of 150 mg l^{-1} extracellular polymer was, therefore, anticipated to enhance Cd transport by approximately a factor of three. Since a BTC for Cd in the absence of extracellular polymer could not be obtained, this calculation can not be directly confirmed. However, the distribution coefficient of Cd in the column experiment with polymer present can be approximated from the Cd concentration of the effluent at the termination of the experiment (5.06×10^{-8} mg ml^{-1}), and the concentration of Cd absorbed on the soil at the end of the column (7.38×10^{-6} mg g^{-1} of sand, from dissection of the column). If the local equilibrium is assumed, the resulting distribution coefficient would be 146 ml g^{-1} of sand). This values agrees within a factor of two with the "effective" K_d calculated from the three component model [$K_d = (R^* - 1)\ \theta/\rho = 295$] and compares favorably to the value obtained from the Cd absorption isotherm at a lower extracellular polymer concentration (133.2 ml g^{-1} of sand).

CONCLUSIONS

The bacterial extracellular polymers tested under the conditions of batch isotherms showed a significant ability to release absorbed Cd and Pb. The sorption of the extracellular polymer from soil isolate 9702-M4 was low and unaltered by the presence of low amounts of Cd. Column experiments with Cd metal in the presence and absence of this extracellular polymer showed enhanced Cd transport with the extracellular polymer. The application of bacterial extracellular polymer for the purpose of mobilizing of trace metals appears promising. In this study, the high mobility of extracellular polymers coupled with their metal binding affinity allowed them to act as a "carrier" of Cd in a sand medium. The generality of this behavior must be demonstrated for other metals and other porous media. This research considered only a small subset of the spectrum of microbial extracellular polymers. Based on the very large number and diversity of microorganisms that produce extracellular polymers and the possibility that the properties of these extracellular polymers can be engineered through regulation of growth conditions, genetic manipulation, and selection of strains that produce effective metal-binding extracellular polymers, the effectiveness of polymer-induced metal mobilization can likely be improved well beyond that observed in this research. Finally, in the process of environmental restoration, there is merit to considering acceptability of treatment techniques to the public. In this regard it is helpful that bacterial extracellular polymers occur naturally in groundwaters, so that their use would not result in the introduction of a xenobiotic agent.

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