COLLOID FORMATION AS AN APPROACH TO REMEDIATE TOXIC WASTES CONTAINING CHROMIUM AND LEAD

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ABSTRACT

We have explored the use of bacteria to remediate soil and aquatic sites containing toxic levels of Pb II or Cr VI. Bacterial isolates from metal-containing sites are capable of detoxifying water containing up to 10 mM Pb II. This activity is a two step process with an initial binding of Pb II to the cells followed by production of a black Pb-containing colloid. Numerous bacteria will reduce Cr VI to Cr III and some isolates have been found to bind Cr III to the bacterial cell. Colloids consisting of Cr III would result from the formation of chromium hydroxide or from binding to bacteria. The bacterial metabolism of Pb II and Cr III converts the biologically toxic and chemically reactive metal to compounds of reduced toxicity and modified chemical activity. We propose a system which can employ bacteria for the bioremediation of toxic sites containing lead or chromium.

INTRODUCTION

Microorganisms have been exposed to a variety of toxic compounds throughout evolutionary time and many of these microscopic forms of life have developed systems which enable them to grow in the presence of toxic elements. One mechanism of resistance to toxic metals is the reduction to a less toxic form. Bacterial transformation of Hg II to Hg^{o}, U VI to U IV, and Se VI or Se IV to Se have been well documented (1). The bacterial transformation of soluble Pb II to a black lead colloid has been described (2,3) and the reduction of Cr VI to Cr III has been extensively examined (4). We have numerous bacterial strains which will grow in high levels of numerous toxic metals including Cr VI, Cr III, and Pb II. These bacteria are being used to establish a system for bioremediation of toxic metal sites.

THE METAL-SOIL INTERFACE

Lead is one of the least mobile of the heavy metal contaminants of soil. Phosphates probably control lead solubility in soils because lead phosphates are highly insoluble and phosphate is present at about 10 times the concentration of lead ions (5). Below pH 9, only Pb II and $PB(OH)^{-}$ contribute significantly to total lead in solution. According to Lindsey (5), the log Ko of the following reaction is -8.5 at pH from 5.5 to 7.5. As the pH

Reaction 1: Pb-soil ------> Pb + Soil

drops below 6, solubility of lead is increased because the phosphate is depressed by iron and aluminum ions (5). The hydrous oxide contents under acid soil conditions also help predict lead solubility (6). Lead carbonates are highly insoluble which helps to explain why free lime content is useful in predicting lead solubility (6) and liming produces the plant uptake of lead (7). Also, high soil pH promotes Pb- organic complexes (8) and increasing organic content increases soil sorption (9). Lead solubility could increase in high chloride soils because $PbCl_2$ is relatively soluble (5,10) and chloride complexes are also soluble (11).

In general, the best predictors of lead mobility in soil are the texture, surface area, hydroxide content, and free lime content (6). Neither cation exchange capacity nor pH are good indicators of lead mobility (6,12). Soil solution levels vary little with pH from 3.9 to 7.6 (13) and typical levels are 0.05 $mg \cdot l^{-1}$. Surface horizons generally have more lead than deeper horizons, particularly in poluted areas (10,14).

Chromium exists in soil primarily in the +3 oxidation state (Cr III and CrO_2^{-}), and to a much lesser extent, the +6 oxidation state ($Cr_2O_7^{2-}$ and CrO_4^{2-}). Cr III exists primarily within minerals or forms mixed oxides of iron and chromium. Cr III compounds are very stable in soil effectively taking all chromium out of solution except under very acid conditions. At pH 5.5, there is essentially complete precipitation of Cr III (15). Thus, Cr III is immobile except under very acid soil conditions. This lack of mobility/solubility may account for inadequate supplies of chromium for animals and humans which consume plants grown on soils low in chromium (8).

Although Cr VI is slightly mobile under both acid (pH < 5.5) and alkaline (pH > 8.0) soil conditions, Cr VI is quickly reduced to immobile Cr III and effectively taken out of solution (16). Reduction to Cr III takes place under both aerobic and anaerobic conditions and is stimulated by additions of organic matter. This stimulation implies that soil microorganisms are directly involved in the reduction process. The conversion of Cr VI to Cr III is important because soluble Cr

IV is toxic to *plants* and animals (8). Cr IV is also readily reduced to Cr III in soil, but Cr IV is only found as a temporary artifact of soil pollution. Cr III can be oxidized to Cr IV in the presence of oxidized manganese (17). Liming, phosphorus addition, and organic matter amendment are effective in reducing chromium toxicity in Cr- polluted soils.

The reactions described by Fergusson (18) would appear to be important for chromium and lead binding with soil. Clays have an outer sheath of hydroxyl groups which may serve to absorb heavy metals (see Reaction 2). Additionally, the clay surface may be negatively charged contributing to the absorption of metal ions (see Reaction 3).

Reaction 2: $\text{Clay-(OH)}_n + M^{n+} <\text{-----}> \text{(Clay-O)}_n\text{-M} + nH^+$

Reaction 3. $\text{Clay}^{n-} + M^{n+} <\text{-----}> \text{Clay-M}$

From this discussion it is obvious that inorganic lead and chromium are immobile and that their mobility, solubility, and availability is generally not related to total metal present. In analyzing soil for the availability of plant essential metals (iron, zinc, manganese, and copper), chelates are often used (19,20). The most commonly used chelates are ethylenediaminetetraacetic acid (EDTA) and diethylenetriamine- pentaacetic acid (DPTA). The DTPA soil treatment has also been developed to assess pollution by heavy metals of soils in Australia (8). The addition of DTPA to soil contaminated with lead is known to increase lead uptake by plants (7). Other extractants have been used to remove organically bound chromium (17). Fulvic acid, a soil organic acid with metal binding capacity of 1 meq/gm, binds Pb II with log K values of 5.3 and 10.2 (21).

Thus, organic compounds, and in particular chelating organic compounds, would appear to be most important in the mobility of lead and chromium in soils. Examples of solvents used to clean up metals are decalin or diisopropylbenzene while metal-complexing agents would include polyphosphates, butyl phosphates, EDTA, DTPA, nitrilotriacetic acid (NTA), N- hydroxyethylenediaminetriacetic acid (HEDTA), and dihexyl-N,N- diethylcarbamyl methylene phosphate (DHDECMP). Naturally occurring complex organic acids in soils which are excellent bi- and polydentate chelating ligands for metals would include: benzoic, 3-hydroxy-5 methylbenzoic, p-hydroxybenxoic, protocatechuic, vanillic, and gallic (22). Therefore, in detoxification of soils containing lead or chromium we conclude the approach would have to be a "pump and treat" process which involves metal chelators in the treatment phase.

LEAD AND CHROMIUM IN THE AQUATIC ENVIRONMENT

The solubility of metals in water is highly dependent on the pH, alkalinity, and amount of organic matter in solution. The following characteristics have been reviewed for water (21): Seawater has a pH of 8.2, alkalinity/mM of 2.3, and organic level at 3.4 x 10-8M; high alkalinity river water has a pH of 8.4-9.0, alkalinity/mM of 1.2, and organic level of 10-6M; low alkalinity river water has a pH of 6.0-7.3, alkalinity/mM of 0.1-0.2, and organic level of 10-6M. In seawater and high alkalinity waters, Pb II and Cr III would readily precipitate and not remain in solution. The residence time for lead in seawater has been calculated to be 340 years which is low in comparison with the 92,000-500,000 years for cadmium (23). Therefore, we can consider the clean up of lead and chromium in the sediments of the aquatic environment to require chelation and solubilization similar to contaminated soil.

MEASUREMENT OF METAL TRANSFORMATIONS

In previous reports (3,4), we have reported that bacteria isolated form the soil are capable of completing transformation of Pb II to an insoluble material presumed to be elemental lead. We also have a series of bacteria which transform Cr VI to Cr III. Through routine laboratory techniques we have measured the rate of metal transformation and have determined that 250 mg of bacterial cells in 1 liter of water will transform Pb II to Pb colloid, Cr VI to Cr III and the binding of Cr III to bacteria at a rate of 0.1 mM metal/24 hr. This information we have incorporated into the bioremediation process which we propose.

BIOREMEDIATION PROCESS

We propose the use of either of two processes for the removal of Pb II and Cr VI form solutions. Both of these have a biological component and a physical unit for operation. One is termed the "batch transformation process" which is modeled after the complete-mix process for activated sludge treatment and the second is "immobilized cell transformation" which is based on the attached growth phase of the trickling filter treatment in the wastewater management process.

As shown in Fig. 1A, the system of batch transformation employs a large reactor where inflow at A would contain lead or chromium. To initiate the process, there would be the addition of bacteria of desired type and nutrients to support the growth of the bacteria. An assumption in the development of a kinetic model is that waste stabilization by the microorganisms occurs only in the reactor unit. This provides a conservative model.

Attached-growth biological treatment processes are usually employed to recover organic matter found in the wastewater; however, here we propose it is to remove toxic metals. Attached growth processes may include trickling filters, roughing filters, rotating biological contractors, and fixed-film nitrification reactors. The system described in Fig. 1B, contains a trickling-type filter as a primary component in the bioremediation effort. In operation the contact bed is filled with wastewater from the top and the wastewater is allowed to remain in contact with the medium for a short time. The bed is trained and allowed to rest before the cycle is repeated.

CALCULATIONS

The calculation of the mean hydrolic retention time for the system, 0s, is defined as follows:

$$Os = Vt/Q = \frac{Vr + Vs}{Q}$$

The mean cell-residence time ,0c, is defined as the mass of organisms in the reactor divided by the mass of organisms removed form the system each day and is given by the following expression:

$$Oc = \frac{VrX}{QwX + QeXe}$$

An explanation of the components in the equations are as follows: 0s, mean hydrolic retention time; 0c, mean cell residence time; Q, influent flow rate; Qw, flow rate of liquid containing bacteria; Qe, flow rate from separation unit; Xe,

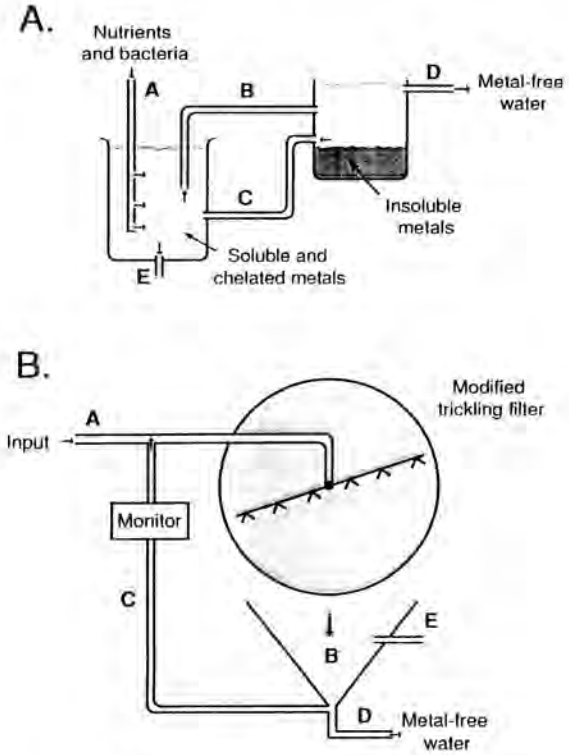

Fig. 1. Models for bioremediation of metals. A. Batch- transformation process. B. Immobilized-cell transformation.

bacterial concentration in effluent from solids separation unit; Vr, volume of reactor; Vs, volume of settling tank; Vt, retention time. Values used in the calculations are as indicated: BOD5, 250 mg/l cells in influent and 20 mg/l cells in effluent; temperature is 20°C, cell residence time is 10 days; flow rate in Fig. 1A, 5 Mgal/day influent; flow rate in Fig. 1B, 2 Mgal/day influent; filter depth in Fig. 1B, 6 feet.

From the calculations, the batch-transformation process would need to be 1.4 Mgal. It would have a hydrolic retention time of 6.7 hours. The immobilized-cell transformation would have a volume of $16.8 \times 10^3 ft^3$ and a hydrolic loading value of 1.5 gal/ft^2-min for the first filter. The second filter would have a volume of $47.5 \times 10^3 ft^3$ with a hydrolic loading value of 0.5 gal/ft^2-min. The efficiencies of these processes were projected to be 87 to 97%.

DISCUSSION

We had as our design premise the use of 2-5 Mgal/day for industrial wastewater. This water could come directly from industry or could be from a process in which metals are solubilized from soil. Although colloids are considered to persist indefinitely in suspension, the colloids referred here as associated with lead or chromium do not conform to that definition. There is considerable aggregation associated with the cellular transformation events which results in rapid removal of the chromium or lead components from solution.

Bioremediation of metals is markedly different from biotransformation of organic molecules. With bioremediation of organics, the final result is the conversion of the carbon compounds into CO_2 or cell mass. Metals, however, will remain in the immediate environment unless methylation processes occur. Volatile metal compounds are not commonly produced under aerobic operations. Complete removal of metals from soil or aquatic environments is an essential component of environmental restoration. The value of the bacterial process is the conversion of a toxic, chemically reactive metal to a form which is biologically less active and chemically less reactive.

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