ENGINEERING THE IMMOBILIZATION OF COUPLABLE ORGANICS IN SOILS AND THE SUBSURFACE

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ABSTRACT

Phenolic contaminants can become irreversibly bound to organic matrices in soils and sediments. The bound chemicals have been shown to possess attenuated environmental mobilities and reduced bioavailabilities. The purpose of this ongoing study is to a) evaluate the factors affecting binding of phenols to natural soils, sediments and aquifer material, and b) utilize this information to develop innovative engineering applications of oxidative coupling for the remediation of contaminated soils and subsurface materials. Phenol, *o*-cresol, and 2,4,5-trichlorophenol are being studied as target contaminants and their binding is being investigated on a shale and two near-surface soils. Model system studies are being conducted concurrently to evaluate the effects of pH and enzyme concentration on polymerization of aqueous phenols. Finally, knowledge gained from the natural and model experimental systems is being utilized to develop preliminary engineered approaches such as permeable reactive barriers designed to intercept contaminant plumes in the subsurface and transform the migrating contaminant into soil-bound residues.

Key words: *binding, oxidative coupling, aqueous phenols, reactive barriers*

INTRODUCTION

Remediation of soils, sediments, and aquifer materials contaminated with phenolic contaminants has in the past focused on restoration of the affected media to clean "background" levels. In most cases, however, the goal of complete removal of the pollutant has remained unrealistic and unachievable. The extended times required to approach completely clean remediation endpoints often impose practical limitations on the technical and economic feasibility of total cleanup by any current remediation technology. These realities compel a reconsideration of remediation strategies and objectives to those that address reduction and elimination of risks rather than absolute removal of specific chemical substances from the affected environmental media.

In the case of hydroxylated aromatics, the contaminants can be fixed or "trapped" within soil matrices as a result of enzyme and transition metal-oxide catalyzed processes that imitate humus formation. There is increasing evidence that such chemical processes can significantly affect the fate of phenolic contaminants in soils and sediments, potentially altering the associated health-risks from these xenobiotics (Wang et al., 1986; Voudrais and Reinhard, 1986; Nannipieri and Bollag, 1991; Bollag, 1992; Gianfreda and Bollag, 1994).

Chemical oxidation of the contaminant can occur in soil as a result of sorption followed by charge- or electron-transfer and the formation of intermediate reactive species such as free radicals. Several investigators have demonstrated the ability of iron and manganese oxides to oxidize mono- and polyphenols to polymeric species (Voudrais and Reinhard, 1986; McBride, 1987; Stone, 1987). Soils are also known to contain large background concentrations of enzymes such as

peroxidases, laccases, and polyphenol oxidases that are capable of catalyzing biochemical reactions resulting in the polymerization of hydroxylated aromatic compounds. Sjobald et al. (1976) were among the first to demonstrate the ability of a soil fungus-derived phenoloxidase to polymerize aromatic chemicals. Klibanov and coworkers utilized horseradish-derived peroxidase to investigate the polymerization of aromatic amines and phenols in aqueous systems (Klibanov and Morris, 1981; Klibanov et al., 1983). Hatcher et al. (1993) utilized a standard ^{13}C nuclear magnetic resonance technique to demonstrate covalent bond formation during the horseradish peroxidase-(HRP) catalyzed bonding between 2,4-dichlorophenol (DCP) and a peat humic acid. In more recent studies, Dec and Bollag (1994, 1995) documented the fortuitous dechlorination of DCP during extracellular laccase and HRP-mediated oxidative polymerization of the chlorinated phenol. Such dehalogenation reactions are of significance since the removal of the chlorine often results in a reduction in the toxicity of the product.

Recent studies have attributed irreversible binding of hydroxylated aromatics observed during sorption-desorption experiments with natural soils to enzyme and transition metal oxide catalyzed oxidative coupling of the contaminant to soil organic matter (Bhandari et al., 1996; 1997; Burgos et al. 1996). Bhandari et al. (1996, 1997) examined the impact of dissolved oxygen on sorption of phenol and chlorinated phenols to two sandy surface soils. Burgos et al. (1996) demonstrated that PAH metabolites such as a-naphthol are capable of becoming irreversibly bound to soils as a result of oxidative coupling reactions. Other researchers have investigated the incorporation of pesticides into soils and have attributed a significant portion of the binding to oxidative polymerization reactions occurring between the xenobiotics and soil organic matter (Barriuso and Koskinen, 1996).

The work presented here represents preliminary results from a multi-pronged investigation of transition metal-oxide and enzyme-catalyzed binding of phenolic contaminants to natural geosorbents. Data obtained from model and natural system studies will be used to design engineered remediation systems that exploit the oxidative coupling properties of hydroxylated aromatics.

EXPERIMENTAL APPROACH

The target chemicals used in this study included uniformly labeled ^{14}C-phenol, ^{14}C-*o*-cresol, and ^{14}C-1,2,5-trichlorophenol (Sigma Chemicals). Known volumes of the radiolabled target sorbate were added to a non-radioactive solution of the corresponding chemical. The solution was prepared in a pH 7 buffer with an ionic strength of 20 mM. The non-labeled phenols were added from concentrated stock solutions prepared in methanol. To avoid cosolvency effects due to methanol, the volume of stock solution added was kept below 0.1% by volume.

Model System Experiments

Horseradish peroxidase (Sigma Chemicals) was the enzyme utilized in the model (aqueous) system studies. A solution of known HRP activity was prepared by dissolving the corresponding

mass of enzyme in distilled-deionized (dd) water. A solution of hydrogen peroxide, the necessary cofactor, was also prepared in dd water. Enzyme assays were conducted in 8-mL glass centrifuge tubes closed with phenolic caps and teflon-lined silicone septa. A 100 mM solution of the non-labeled target chemical prepared in a phosphate buffer with an ionic strength of 20 mM was dispensed into replicate centrifuge tubes. This was followed by the sequential addition of known volumes of enzyme solution and H2O2. Control tubes received phenol, phenol+enzyme, or phenol+H2O2 only. The tubes were shaken for two minutes, centrifuged, and the supernatant sampled for unreacted phenol. The unreacted phenol was quantified using high performance liquid chromatography (HPLC) and the amount polymerized determined by a mass balance.

Natural System Experiments

Natural system experiments were conducted using the three target chemicals and three geosorbents. The geosorbents studied included a shale, a forest soil, and a grassland soil. These sorbents were associated with organic matter (OM) contents of 12.6%, 5.9%, and 2.5%, respectively. While the soil organic matter (SOM) associated with forest and grassland soils was characterized as young, amorphous OM with a high oxygen/carbon ratio, the shale contained OM that was diagenetically aged, condensed, and had a low O/C ratio.

All sorbents were air dried, crushed, sieved through a 2-mm sieve, split into smaller fractions, and sterilized before use in the sorption-desorption studies. Gamma-irradiation was selected as the method of sterilization because earlier work had shown this technique to have the least effect on sorption of organic contaminants to soils and sediments (Bhandari and Weber, 1998). Known masses of soils were added to the centrifuge tubes described earlier. The target phenol solution amended with its ^{14}C-isotope was added to replicate tubes. The tubes were capped and shaken at 25°C for periods ranging from 7 to 14 days (adsorption). The tubes were then centrifuged and the supernatant sampled for radioactivity. A liquid-scintillation counter (LSC) was used to quantify the disintegrations per minute (dpm) in the aqueous phase. The remaining supernatant was removed, the tubes refilled with fresh buffer, and replaced in the environmental shaker for reequilibration (desorption). The tubes were reequilibrated for a duration identical to the adsorption period, centrifuged, and sampled for the aqueous radioactivity. Thereafter, fresh buffer was added, the tubes reequilibrated, and resampled daily until the radioactivity in the supernatant was reduced to below-detection limit.

The sorbent was then subjected to solvent extraction with a mixture of methanol and dichloromethane. Solvent extraction was repeated 5 to 7 times until the radioactivity in the extract was reduced to below-detection limit. Thereafter the sorbent was air dried and combusted in a biological material oxidizer. The $^{14}CO2$ evolved was captured in a special scintillation cocktail and the activity quantified on a LSC.

These experiments were repeated for all sorbent-target chemical combinations. Sorption and desorption data were fit to the Freundlich isotherm model.

RESULTS AND DISCUSSION

Model System Studies (Enzyme Assays)

Polymerization of trichlorophenol (TCP) by HRP was investigated in aqueous systems. Effects of pH and enzyme concentration were studied. Preliminary experiments showed that the oxidative coupling reactions catalyzed by HRP were rapid and complete within two minutes. Moreover, it was also shown that the minimum H2O2 concentration necessary for optimum polymerization was equal (equimolar) to the concentration of phenol in the system. At higher concentrations, H2O2 appeared to chemically oxidize the target chemical. Figure 1 illustrates the effects of pH and enzyme concentration on TCP remaining. The production of polymers was visually observed by the yellow coloration of the solution. Polymer production was confirmed using HPLC.

The data shown in Figure 1 illustrates that at higher pHs (pH 7 and 8), the enzyme concentration had a significant effect on TCP removal or polymerization. TCP removal at the HRP concentration of 1.0 units/mL was twice as large as compared to the enzyme concentration of 0.25 U/mL. As pH was lowered, the TCP removal efficiency increased and the effect of enzyme concentration on TCP removal diminished. Complete removal of TCP was observed at pH 4 for all enzyme concentrations. Since 1,2,5-TCP is an ionogenic chemical with a pKa of 6.9, it exists predominantly in its ionized form above pH 6.9 and in its protonated form below this pH. Figure 1 clearly illustrates that it is the protonated form of the phenol that readily participates in the HRP catalyzed oxidative coupling reaction. At pH > pKa, TCP removal is greater for higher enzyme concentrations. Removal of TCP by polymerization changes the equilibrium distribution of the ionized and protonated phenol. As TCP is removed by polymerization, the solution re-equilibrates and some ionized TCP is converted to its protonated form. A higher enzyme concentration allows for greater chances of contact between the enzyme and the small number of protonated TCP molecules, therefore affecting the reaction rate and extent.

Natural System Studies (Adsorption-Desorption)

The natural system experiments looked at sorption, desorption, hysteresis, and extractability of the target chemicals in contact with the shale, forest, and grassland soils. Figure 2 illustrates the Freundlich isotherm fits for adsorption-desorption data in the case of phenol and forest soil. The *n* value for a Freundlich fit of the adsorption data was 0.77. The *n* values for phenol sorption on grassland soil and shale were 0.78 and 0.80, respectively (data not shown). Values of *n* less than one are indicative of non-linear sorption processes that occur on a heterogeneous sorption domain such as SOM. Non-linear sorption results when the interaction between a sorbate and a sorbent is not purely partitioning but a combination of partitioning and site-specific interactions. For phenol,

the younger and more reactive OM associated with the forest soil manifested greater sorption non-linearity as compared to the older, less reactive, kerogen type OM associated with the shale. This illustrates that unlike phenanthrene (Huang et al., 1997), the fate of phenols in soils and sediments is greatly influenced by the younger OM domains that are rich in reactive hydroxyl, carboxyl, and phenolic groups and can play large roles in site-specific chemical processes.

Data illustrated in Figure 2 shows significant sorption-desorption hysteresis for phenol on forest soil. Hysteresis was measured using the Hysteresis Index (*HI*) defined by Huang and Weber (1997) as:

$$HI = \frac{q_e^d - q_e^a}{q_e^a}\Big|T, C_e$$

where qe^a and qe^d are solid-phase solute concentrations for the adsorption and desorption experiments, respectively, and *T* and *Ce* specify conditions of constant temperature and residual aqueous-phase concentration. A *HI* of 1.0 is indicative of no hysteresis. *HI* for the case shown in Figure 2 was 0.69.

Figure 3 illustrates the removal of phenol from shale by sequential desorption, solvent extraction, and combustion. Results are expressed in terms of the percentage removal of phenol initially sorbed on the geosorbent (*qe*). A major portion of the sorbed phenol (65-85%) was removed during repetitive desorptions. The phenol resistant to desorption but readily extractable by solvent amounted to 20-35% of *qe*. Approximately 4-7% of the sorbed phenol was non-extractable and therefore irreversibly bound to the soil matrices. It is hypothesized that the solvent extractable portion of the target chemical constitutes a fraction that is physically sequestered in the organic matrix and is extracted when the solvent "swells" the SOM and releases the trapped chemical into the solvent. On the contrary, the non-extractable fraction of the target sorbate is believed to be strongly bound to the soil matrices, possibly as a result of transition metal oxide (or enzyme) catalyzed oxidative coupling. Both physical sequestration and binding are more significant at low initial aqueous concentrations of the sorbate and are indicative of site-specific processes.

A summary of results for phenol sorption on shale, forest soil, and grassland soil is presented in Table 1. This table also lists results from cresol and TCP sorption experiments conducted on the shale. It should be pointed out that since the solution pH was very close to the pKa of TCP, TCP existed in the system in both the protonated and ionized forms and therefore possibly behaved differently than the other sorbates.

All results presented in Table 1 are for an initial aqueous sorbate concentration of 1.0 mM. The amount of phenol sorbed on the geosorbents is clearly related to the OM content of the sor-

bent. The shale with an OM content of 12.6% had the highest *qe* of 5.83 mmol/g while the grassland soil that contained 2.5% SOM exhibited the smallest *qe* of 0.49 mmol/g. The amount of phenol sequestered (solvent extractable) was directly related to *qe* and the OM content of the sorbent. The bound fraction of the phenol, however, did not appear to be directly dependent on the SOM content. Whereas only 4% of the phenol initially sorbed on shale was non-extractable, 37% and 62% of the phenol was bound to the grassland and forest soils, respectively. This clearly indicates that unlike sorption or sequestration, the irreversible binding of phenol was greatly influenced by the type of organic matter rather than its amount. The younger, reactive organic matter associated with forest and grassland soils was able to participate in binding processes more readily than the older, kerogen type, unreactive organic matter associated with the shale. In the case of shale, the sorption, sequestration, and binding of target contaminants were controlled by the sorbates' hydrophobicity. TCP had the highest *qe* and phenol the smallest. For all sorbates, the irreversibly bound fraction on shale amounted to 4% of *qe*.

We compared the nondesorbable (sequestered + bound) fraction of the target chemical to the Hysteresis Index, proposed by Huang and Weber (1997) as a measure of contaminant sequestration. From Figure 4, it appears that there is a clear relationship between the nondesorbable fraction of the sorbate and its *HI* value. TCP did not fit the general trend manifested by other sorbate-sorbent combinations possibly because of its existence in the system as a combination of the protonated and ionized forms, therefore, resulting in varied interactions with the geosorbent. It appears that the Hysteresis Index can be used as a convenient measure to obtain a preliminary idea about a sorbent's capacity to sequester or bind phenolic contaminants.

Engineered System Studies

Ongoing studies are investigating methods to engineer oxidative coupling reactions *in situ* in order to arrest the migration of phenolic contaminants in contaminated soils and groundwater. Engineering applications of the technology being studied include permeable reactive barriers of immobilized enzymes or transition metal oxides, land farming by direct application of enzymes or metal oxides to near surface contaminated soils and sludges, and phytoremediation using peroxidase-exuding grasses to decontaminate phenol-contaminated soils. The knowledge obtained from model and natural system studies is being used to design robust engineering approaches that reduce risks from soils, sediments, and groundwater contaminated with hydroxylated aromatics.

CONCLUSIONS

The purpose of this paper was to present and discuss results from an ongoing study focused on engineering the immobilization of hydroxylated organics in soils and sediments. The three target-chemicals studied included phenol, o-cresol, and TCP and the three geosorbents investigated included a shale, a forest soil, and a grassland soil. Model system studies demonstrated that the

protonated form of phenol participates in enzyme-catalyzed oxidative coupling reactions. Phenol polymerization is therefore faster and more complete at pHs below the target-chemical's pKa. Natural system investigations demonstrated that sorption for all sorbate-sorbent combinations was non-linear. Except in the case of TCP, the Hysteresis Index was correlated to the nondesorbable portion of the sorbate. Finally, the younger and more reactive organic matter associated with the forest soil bound significantly greater phenol than the diagenetically reduced, more hydrophobic but less reactive, kerogen-type organic matter associated with the shale. Future studies will evaluate the possibility of engineering the *in situ* immobilization of phenolic contaminants in soils, sediments, and groundwater.

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Table 1. Desorbable, extractable and irreversibly bound fractions (in μmol/g) of phenol, cresol and tcp associated with forest soil, grassland soil and shale. Data presented is for initial aqueous an concentration of 1.0 mM.

	Sorbed	Desorbable	Extractable	Bound
Phenol/Forest	1.28	0.38	0.12	0.79
Phenol/Grassland	0.49	0.26	0.05	0.18
Phenol/Shale	5.83	4.88	1.28	0.23
Cresol/Shale	9.25	5.66	3.25	0.38
TCP/Shale	43.0	18.47	24.53	1.80

Figure 1. Horseradish peroxidase catalyzed oxidative coupling: effect of ph and enzyme concentration on TCP removal.

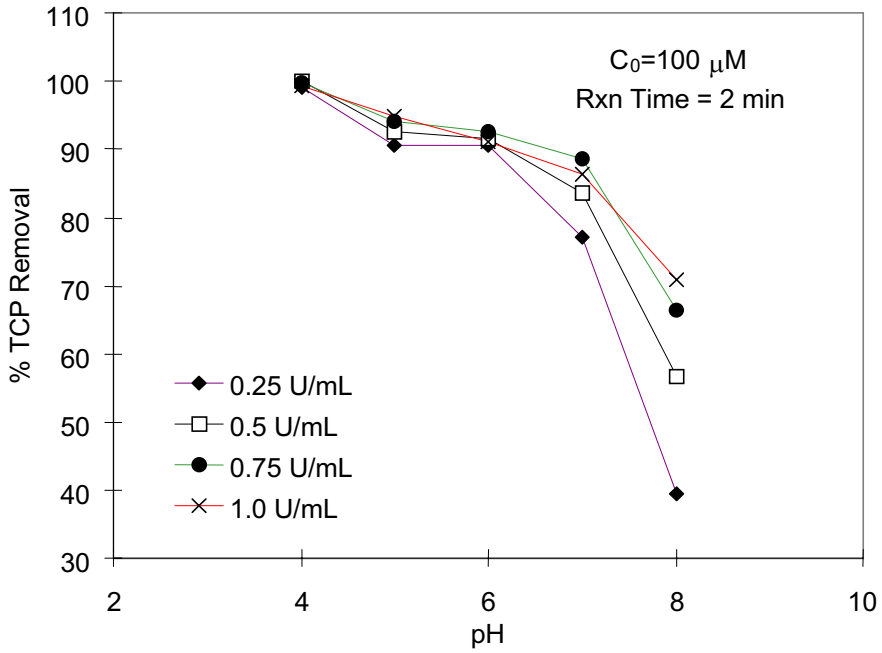

Figure 2. Freundlich isotherm fits of adsorption and desorption data for phenol and forest soil.

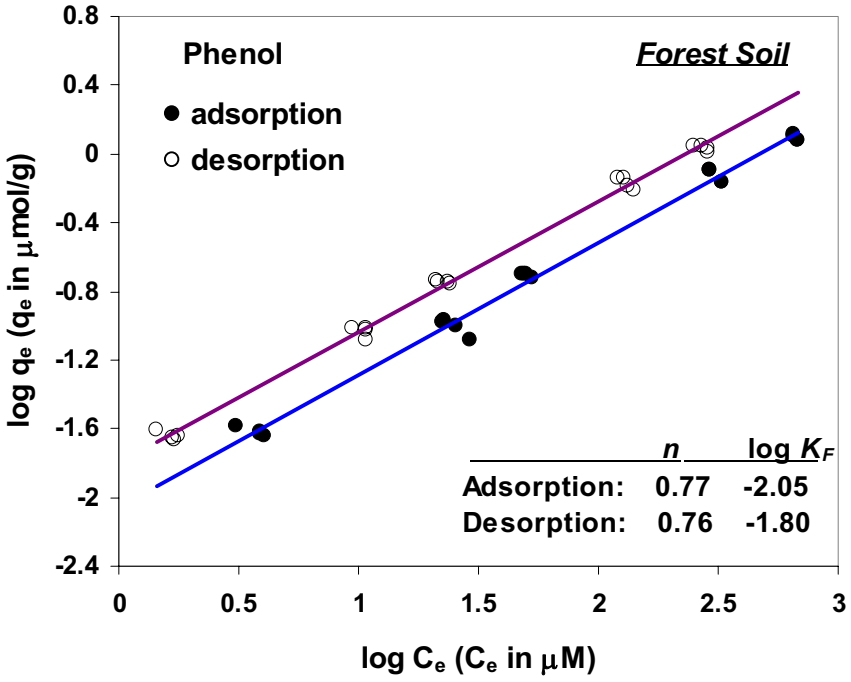

Figure 3. Recovery of ^{14}C-phenol from shale during desorption, extraction and combustion of the sorbent.

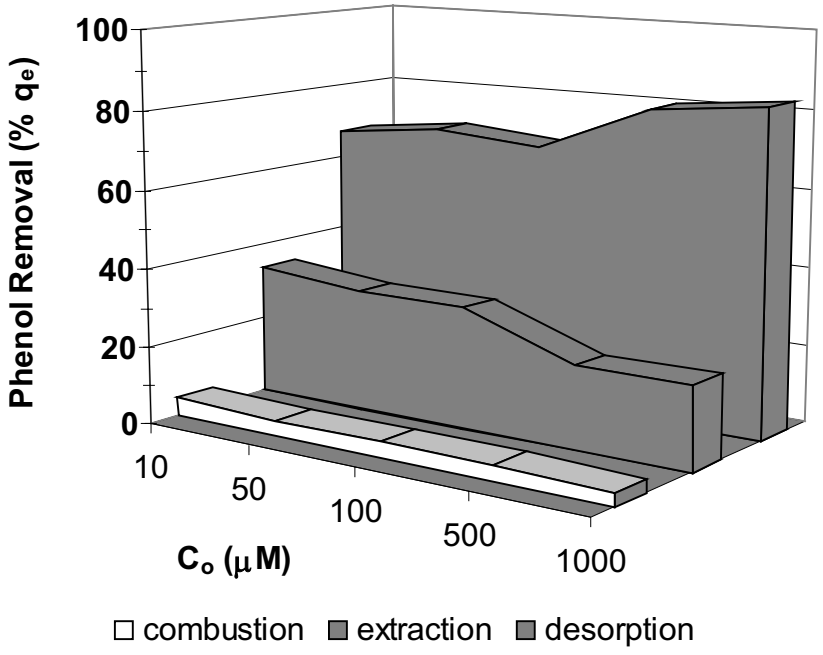

Figure 4. Correlation between Hysteresis Index and the nondesorbable fraction of the sorbate.